XV CONGRESS SIC - Lisbon

PROGRAM

25th to 27th MAY 2017
LISBON, PORTUGAL
Centro Cultural de Belém

Sociedade Ibérica de Citometría

JUNTA DIRECTIVA
DE LA SOCIEDAD IBÉRICA DE CITOMETRÍA | SIC

PRESIDENTE
José Enrique O'Connor

VICEPRESIDENTE
Maria Jorge Arroz

SECRETARIO-TESORERO
Julía Almeida Parra

VOCALES
Rui Gardner
María Teresa Molero Labarta
Martín Pérez Andrés
Lola Martínez

ORGANIZING COMITTEE
Catarina Martins

Alexandre Salvador
António Medina Almeida
Artur Paiva
João Loureiro
Lola Martinez
Luís Miguel Borrego
Margarida Lima
Margarida Saraiva
Natacha Gonçalves Sousa
Paulo Rodrigues-Santos
Rui Gardner

SCIENTIFIC COMITEE
Catarina Martins

Alexandre Salvador
António Medina Almeida
Artur Paiva
Bruno Costa Silva
João Farela Neves
João Loureiro
José Enrique O'Connor
Juana Ciudad
Julía Almeida Parra
Lola Martinez
Luís Miguel Borrego
Margarida Lima
Margarida Saraiva
Maria Jorge Arroz
María Teresa Molero Labarta
Mariona Pascal
Martín Pérez Andrés
Natacha Gonçalves Sousa
Paulo Rodrigues-Santos
Rui Gardner

WELCOME MESSAGE

Dear Colleagues and friends,

The real adventure of the XV SIC Congress started about one year ago for us, Organizing and Scientific Committees. It was in fact a great challenge that the SIC Board gave us!

Therefore, we settled a multidisciplinary Iberian network to build from scratch this important event for flow cytometrists, particularly in Portugal and Spain. The members of the Organizing and Scientific Committees worked very hard to choose the more relevant themes, to bring brilliant experts and researchers in diverse fields of Flow Cytometry, and at the same time, to address an interesting social program that honors the beautiful Lisbon.

We believe that we have put together all the ingredients for this to be a memorable Congress! We truly wish it will have future scientific repercussions in the professional pathways of all participants (clinicians, researchers, speakers, students, sponsors).

For now, it is wonderful to finally see you in the skyline of the Portuguese capital. We would really like to thank you for your presence in the XV SIC Congress, and we truly hope you enjoy attending it as much as we did organizing this meeting.

Welcome, and let's have Flow Cytometry shining in the sunny skies of Lisbon!

On behalf of the Organizing and Scientific Committees
of the XV SIC Congress,

Catarina Martins

XV CONGRESS SIC - Lisbon

WELCOME MESSAGE FROM THE SIC PRESIDENT

Dear Colleagues:

It is with great pleasure that I welcome you to the XV Congress of the Iberian Society of Cytometry (SIC). This scientific event, held in the splendid city of Lisbon, represents a new milestone for the SIC in its desire of being the home of Iberian cytometrists. I want to acknowledge all the friends in the Organization that have made possible this Congress, bringing together educational activities, state-of-the-art cytometry and an attractive social program.

I want to give a special welcome to our distinguished invited speakers, among them the two Presidents of the International Society for the Advance of Cytometry, Paul Wallace and Andrea Cossarizza. Their presence highlights the links of SIC with fraternal societies in the field of Cytometry.

Last but not least, my special welcome to our young attendants. The educational and scientific programs of the Congress, carefully elaborated to cover established and emerging applications, will offer to them a panoramic view of modern cytometry. But the best value of this Congress for the younger should be, undoubtedly, the interaction with their expert colleagues.

Thus, on behalf of the Junta Directiva, I wish to all of you a productive and pleasant time in Lisbon.

José-Enrique O'Connor
President of SIC

PRE-CONGRESS
WORKSHOP 1 - 2

25th MAY 2017

ROOM
Amadeo de Souza-Cardoso

09H00
13H00

WORKSHOP 1
MULTICOLOR FLOW CYTOMETRY - FROM BASICS TO TIPS AND TRICKS
FOR A SUCCESSFUL MULTICOLOR FLOW CYTOMETRY

Organization: *Rui Gardner, Alexandre Salvador, Lola Martinez*

Multicolor Flow Cytometry : Simple overview of how Flow Cytometers work; Instrument configuration – lasers and emission filters (optical layout); Understanding Fluorescence: Excitation and Emission Spectra (introducing Spectra Viewers); Spillover and Compensation.

Panel Design tips : Titrating your reagents – Stain Index; Choosing the right fluorochromes / Antibody combinations; Avoiding or correcting spillover; Tools for multicolor panel design: Fluorofinder, Spectra viewers, Spillover Spreading Matrix (SSM), Resolution Impact Matrix (RIM); Setting up voltages – which method?; Compensation controls – cells or beads?; Gating controls in multicolor Flow Cytometry (FMOs).

Practical exercise: Defining a good multicolor immunophenotyping panel.

Teachers:
Rui Gardner, MSKCC, New York, USA,
Alexandre Salvador, ESTeSL, Lisboa, Portugal
Lola Martinez, CNIO, Madrid, Spain

ROOM
Fernando Pessoa

09H30
13H00

WORKSHOP 2
DATA ANALYSIS IN LEUKEMIA AND LYMPHOMA IMMUNOPHENOTYPING

Organization: *Julia Almeida*
Sponsored by Cytognos S. L.

Theoretical-practical course, consisting of data analysis with the Infinicyt program, which will be carried out by students directly on their computers (in which the INFINICYT program), under the guidance and supervision of teachers.
Teachers:
Julia Almeida, Centro de Investigación del Cáncer, Salamanca, Spain
Juan Flores, Centro de Investigación del Cáncer, Salamanca, Spain
Quentin Lecrevisse, Centro de Investigación del Cáncer, Salamanca, Spain

PRE-CONGRESS WORKSHOP 3 - 4

PROGRAM SHORT VERSION

25th MAY 2017

ROOM
Maria Helena Vieira da Silva

09H00
13H00

WORKSHOP 3
CHARACTERIZATION OF RARE AND NEWLY DESCRIBED IMMUNE LYMPHOID SUBSETS IN HUMANS

Organization: Martin Perez-Andres

Selection of antibody clones for lymphocytes classification: IgH subclasses in B-cells. Identification and classification of NK subsets.Correlation between phenotype and function of T-cell subsets.Human Innate lymphoid cells (ILCs).

Teachers:
Martin Perez-Andres, CIC, IBMCC, USAL-CSIC, Salamanca, Spain
Elena Blanco Álvarez, CIC, IBMCC, USAL-CSIC, Salamanca, Spain Yenan Bryceson, Karolinska Institutet, Stockholm, Sweden Jenny Mjösberg, Karolinska Institutet, Stockholm, Sweden

ROOM
Sophia de Mello Breyner

09H30
13H00

WORKSHOP 4
VALIDATION OF EQUIPMENT, REAGENTS AND METHODS IN FLOW CYTOMETRY

Organization: *Juana Ciudad, Juan Flores, Catarina Martins*

Theoretical-practical course, consisting of application of validation protocols to equipment, reagents and methods used in routine flow cytometry laboratories. These approaches will be particularly directed to Accreditation requirements in laboratories starting or already under Accreditation processes.

Teachers:
Juana Ciudad, Centro de Investigación del Cáncer, Salamanca, Spain
Joana Caetano, Instituto Português de Oncologia,Lisboa, Portugal

CONGRESS PROGRAM SHORT VERSION

25th MAY 2017

	ROOM Sophia de Mello Breyner	ROOM Fernando Pessoa
09H00 13H00	PRE-CONGRESS WORKSHOPS	PRE-CONGRESS WORKSHOPS
14H45	OPENING CEREMONY	
15H00 16H00	OPENING SESSION Chair: *Rui Gardner* mRNA Assessment by Flow Cytometry using the Branched DNA Technique *Paul Wallace, Buffalo, NY, USA*	
16H00 16H30	COFFEE BREAK Poster Viewing	
16H30 18H30	PARALLEL SESSION Haematological malignancies of Rare Cells Sponsored by Takeda Chairs: Margarida Lima & António Medina Almeida Anaplastic Lymphomas *Jonathan Fromm, Seattle, Washington, USA* Hodking Lymphomas *Margarida Lima, Oporto, Portugal* Mast Cell Leukemia *Cristina Teodosio, Leiden, Netherlands*	PARALLEL SESSION Flow cytometry and Hypersensitivity: Allergy and Autoimmunity Chairs: *Luis Miguel Borrego & Mariona Pascal* Role of Flow cytometry in allergy *Edward F. Knol, Utrecht, Netherlands* Food Allergy: diagnosis and monitoring *Mariona Pascal, Barcelona, Spain* Drug Allergy and flow cytometry: diagnosis and monitoring Lina Mayorga, Malaga, Spain
18H30 19H00	COMMERCIAL SESSIONS Sponsored by Alexion	COMMERCIAL SESSIONS Sponsored by Merck
19H00 20H00	SIC General Meeting	
20H00 22H00	WELCOME COCKTAIL	

CONGRESS PROGRAM SHORT VERSION

26th MAY 2017

	ROOM Sophia de Mello Breyner	ROOM Fernando Pessoa
09H00 10H00	PLENARY SESSION Chairs: *Artur Paiva* Advances in the flow cytometry diagnosis and monitoring of hematological malignancies *Alberto Orfão, Salamanca, Spain*	
10H00 11H45	PARALLEL SESSION Functional Cytomics and emerging applications Chairs: *Enrique O'Connor & João Loureiro* Cytogenomic analyses show expanded genomes in the Pucciniales (rust fungi) and suggest ploidy variation along their life cycles *Pedro Talhinhas, Lisbon, Portugal* The mammalian spermatozoa: Is it time for flow spermetry? *Fernando Peña, Cáceres, Spain* Phenotype and Function of rare immune cells *Andrea Cossarizza, Modena, Italy*	PARALLEL SESSION Stem Cells and Transplantation Chairs: *Artur Paiva & Alexandre Salvador* Minimal residual disease and immune reconstitution after stem cell transplantation *Noemi Puig, Salamanca, Spain* (Re) Programing Haematopoiesis and Immunity *Filipe Pereira, Coimbra, Portugal* Immune reconstitution following Allogeneic Stem Cell Transplantation *João Lacerda / Maria Soares, Lisbon, Portugal*
11H45 12H15	COFFEE BREAK Poster Viewing	
12H15 14H00	PARALLEL SESSION Immunophenotype of T and NK cells in acute and chronic lymphoproliferative syndromes Chairs: *Teresa Molero & Maria Arroz* T-cell differentiation and its implications for the diagnosis, classification and monitoring of ALL T *Alberto Orfão, Salamanca, Spain* Immunophenotype of T lymphocytes in acute and chronic lymphoproliferative syndromes *Julia Almeida, Salamanca, Spain* Immunophenotype of NK lymphocytes in acute and chronic lymphoproliferative syndromes *Margarida Lima, Oporto,Portugal*	PARALLEL SESSION Vesicles, exosomes, microparticules Chairs: *Lola Martinez & Bruno Costa Silva* Education of bone marrow-derived cells during metastasis by tumor-secreted exosomes *Hector Peinado, Madrid, Spain* Past, Present and Future of Extracellular Vesicles Analysis by Flow Cytometry *Òscar Fornas, Barcelona, Spain* Immunosuppressive Exosomes Present in Human Ovarian Tumor Microenvironments Rapidly and Reversibly Arrest T Cell Activation *Paul Wallace, Buffalo, NY, USA*
14H00 15H15	WORKING LUNCH with Commercial Sessions	

CONT.

CONGRESS PROGRAM SHORT VERSION

26th MAY 2017

| CONT.

	ROOM Sophia de Mello Breyner	ROOM Fernando Pessoa
15H15 17H00	PARALLEL SESSION Advances in primmary immunodeficiencies screening and diagnosis by flow cytometry Chairs: *Martin Perez-Andrés & João Farela Neves* New standardized strategies in the orientation of lymphoid defects by flow cytometry: Combined Immunodeficiencies and Primary Antibody Deficiences analysis by FCMMartín *Perez Andrés, Salamanca, Spain* Flow cytometry in the identification of innate citotoxic dysfunctionYenan Bryceson, Stockholm, Sweden Mendelian Susceptibility to Mycobacterial Disease - Strategies for Diagnosis by Flow Cytometry *Júlia Vasconcelos, Oporto, Portugal*	PARALLEL SESSION Monitoring Immunotherapy for Cancer *Session with the collaboration of SPO & SEOM* Chairs: *Gabriela Sousa, Luis De la Cruz, Paulo Rodrigues-Santos* Introduction to Immunotherapy *Bruno Silva-Santos, Lisbon, Portugal* *Immunodynamics assays for* Cancer Immunotherapy *Paulo Rodrigues-Santos, Coimbra Portugal* Monitoring NK cell-based Immunotherapy in the elderly *Rafael Solana, Córdoba, Spain*
17H00 17H30	COFFEE BREAK Poster Viewing	
17H30 18H00	COMMERCIAL SESSION *Sponsored by Beckman Coulter*	COMMERCIAL SESSION
18H00 18H30	SIC Groups presentations GECLID presentation	
20H30 24H00	CONGRESS DINNER	

XV CONGRESS SIC - Lisbon

CONGRESS PROGRAM SHORT VERSION

27th MAY 2017

	ROOM Sophia de Mello Breyner	ROOM Fernando Pessoa
09H00 10H00	PLENARY SESSION Chairs: *Lola Martinez* Minimal Residual Disease detection in hemato logical malignancies: recent advances and future perspectives *Julia Almeida, Salamanca, Spain*	
10H00 11H00	PLENARY SESSION Accreditation of Flow Cytometry Laboratories Chairs: *Catarina Martins & Juana Ciudad* ISO 15189 and ISO 17025, standards for (clinical) laboratories *Catarina Martins, Lisbon, Portugal* IPAC / ENAC Undergoing Accreditation in Flow Cytometry Labs: Steps to Follow and Critical Points *Ulrich Sack, Leipzig, Germany* Flexible scope in flow cytometry: Experience of a laboratory accredited by UNE-EN-ISO 15189 *Juana Gil, Madrid, Spain*	
11H00 11H30	COFFEE BREAK Poster Viewing	
11H30 13H00	PLENARY SESSION Immunology (SPI) Chairs: *Margarida Saraiva & Manuel Vilanova* Analysing the impact of T cell subsets on brain cognitive function *Julie C. Ribot, Lisbon, Portugal* Choreographing Immunity and Tolerance Induction in the Thymus *Nuno Alves, Oporto, Portugal* Metabolic cues implicated on monocyte biology *Ricardo Silvestre, Libon, Portugal.* Cell competition in the thymus *Vera Martins, Lisboa, Portugal*	
13H00 13H15	CLOSING CEREMONY	

25th May 2017

Pre-Congress Workshops

09H00 - 13H00

Room Amadeo de Souza-Cardoso

WORKSHOP 1

MULTICOLOR FLOW CYTOMETRY – FROM BASICS TO TIPS AND TRICKS FOR A SUCCESSFUL MULTICOLOR FLOW EXPERIMENT"

ORGANIZATION
Rui Gardner, MSKCC, New York
Alexandre Salvador, ESTeSL, Lisboa
Lola Martinez, CNIO, Madrid

WORKSHOP CONTENTS
Multicolor Flow Cytometry:
Simple overview of how Flow Cytometers work;
Instrument configuration – lasers and emission filters (optical layout);
Understanding Fluorescence: Excitation and Emission Spectra (introducing Spectra Viewers);
Spillover and Compensation.
Panel Design tips:
Titrating your reagents – Stain Index;
Choosing the right fluorochromes/Antibody combinations;
Avoiding or correcting spillover;
Tools for multicolor panel design: Fluorofinder, Spectra viewers, Spillover Spreading Matrix (SSM), Resolution Impact Matrix (RIM);
Setting up voltages – which method?;
Compensation controls – cells or beads?;
Gating controls in multicolor Flow Cytometry (FMOs).
Practical exercise:
Defining a good multicolor immunophenotyping panel.

TEACHERS
Rui Gardner, MSKCC, New York
Alexandre Salvador, ESTeSL, Lisboa
Lola Martinez, CNIO, Madrid

Room Fernando Pessoa
WORKSHOP 2
"DATA ANALYSIS IN LEUKEMIA AND LYMPHOMA IMMUNOPHENOTYPING"

ORGANIZATION:
Julia Almeida, Centro de Investigación del Cáncer, Salamanca

WORKSHOP CONTENTS:
Theoretical-practical course, consisting of data analysis with the Infinicyt program, which will be carried out by students directly on their computers (in which the INFINICYT program), under the guidance and supervision of teachers.

TEACHERS
Julia Almeida, Centro de Investigación del Cáncer, Salamanca
Juan Flores, Centro de Investigación del Cáncer, Salamanca
Quentin Lecrevisse, Centro de Investigación del Cáncer, Salamanca

SPONSORSHIP
Cytognos S.L.

cytognos | Flow Cytometry Solutions

Room Maria Helena Vieira da Silva
WORKSHOP 3
“CHARACTERIZATION OF RARE AND NEWLY DESCRIBED IMMUNE LYMPHOID SUBSETS IN HUMANS"

ORGANIZATION
Martin Perez-Andres, IBMCC, USAL-CSIC, Salamanca, Spain

WORKSHOP CONTENTS
Selection of antibody clones for lymphocytes classification: IgH subclasses in B-cells.
Identification and classification of NK subsets.
Correlation between phenotype and function of T-cell subsets.
Human Innate lymphoid cells (ILCs).

TEACHERS
Martin Perez-Andres, CIC, IBMCC, USAL-CSIC, Salamanca, Spain
Elena Blanco Álvarez, CIC, IBMCC, USAL-CSIC, Salamanca, Spain
Yenan Bryceson, Karolinska Institutet, Stockholm, Sweden
Jenny Mjösberg, Karolinska Institutet, Stockholm, Sweden

Room Sophia de Mello Breyner
WORKSHOP 4
"VALIDATION OF EQUIPMENT, REAGENTS AND METHODS IN FLOW CYTOMETRY"

ORGANIZATION
Juana Ciudad, Servicio de Citometria, Universidad de Salamanca
Juan Flores, Centro de Investigación del Cáncer, Salamanca
Catarina Martins, NMS|FCM, Lisboa

WORKSHOP CONTENTS
Theoretical-practical course, consisting of application of validation protocols to equipment, reagents and methods used in routine flow cytometry laboratories. These approaches will be particularly directed to Accreditation requirements in laboratories starting or already under Accreditation processes.

TEACHERS
Juana Ciudad, Centro de Investigación del Cáncer, Salamanca
Joana Caetano, Instituto Português de Oncologia, Lisboa, Portugal

CONGRESS PROGRAM

25th May 2017

14H45 - 15H00 OPENING CEREMONY | Room Sophia de Mello Breyner

15H00 - 16H00 OPENING SESSION | Room Sophia de Mello Breyner

Chair: Rui Gardner

mRNA ASSESSMENT BY FLOW CYTOMETRY USING THE BRANCHED DNA TECHNIQUE

ISAC LECTURE

Paul Wallace
Department of Flow & Image Cytometry,
Roswell Park Cancer Institute, Buffalo, NY

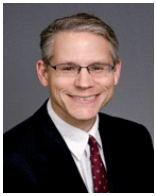
Abstract: Flow cytometry permits the simultaneous measurements of many biomarkers in individual cells from bulk populations. Until now analysis has been limited to primarily analysis of proteins and total DNA or highly abundant DNA sequences. Since most mRNA gene transcripts are present at very low quantities our ability to detect these by flow cytometry has been limited. The branched DNA technique amplifies the signal from a single mRNA species several thousand fold. This procedure allows for the determination of low copy number mRNA expression within a mixed population of cells and is compatible with antibody-based targeting of both extracellular and intracellular antigens. To show proof of concept, we used the technique to detect BCR and ABL transcripts in cell lines positive for the t(9;22) translocation. While, as expected, all cells had detectable BCR and ABL transcripts, cells with the t(9;22) translocation had quantitatively more BCR and ABL mRNA than those that were negative for the translocation. These results correlated with data obtained by RT-PCR. We have extended these investigations to the evaluation of human peripheral blood and the expression of CD8. We detected CD8 mRNA expression in both T cells and NK cells which correlated with protein expression. After stimulation with phorbol 12-myristate 13-acetate and ionomycin for up to 4 h, an increase in CD8 mRNA was detectable 30 minutes before increased CD8 protein was detected. These data were correlated with EOMES and T-bet protein expression to identify distinct subset of CD8 positive T and NK cells. Collectively, these results exemplify how the branched RNA flow cytometry labeling procedure can be applied to simultaneously assess mRNA and protein dynamics to gain insight into the regulation of gene transcription and translation in individual cells.

16H00 - 16H30 COFFEE BREAK and Poster Viewing

16H30 - 18H30 PARALLEL SESSION | Room Sophia de Mello Breyner

Haematological malignancies of Rare Cells
Chairs: Margarida Lima & António Medina Almeida

ANAPLASTIC LYMPHOMAS



Jonathan Fromm, MD, PhD
Laboratory Medicine and Associate Director,
Hematopathology Laboratory, University of Washington Medical Center (UWMC); Seattle, Washington, USA

Abstract: Anaplastic Large Cell Lymphoma (ALCL) is a large cell CD30+ lymphoma that is often systemic (usually nodal). However, a recently described subset of these lymphomas is associated with breast implants (breast implant ALCL, or BI-ALCL). The flow cytometric findings in systemic ALCL and BI-ALCL will be described.

Systemic ALCL can be characterized by the presence (ALK+ ALCL) or absence (ALK- ALCL) of anaplastic lymphoma kinase (ALK), a tyrosine kinase aberrantly expressed by these large cell lymphomas that harbors a translocation of ALK (on chromosome 2) with one of various partners. ALK+ ALCL makes up 70% of systemic ALCL cases and both lymphomas involve nodal and extranodal sites.

A number of studies have examined the flow cytometric findings in systemic ALCL (Juco et al (2003); Muzzafar et al. (2009); Kesler et al. (2007)). In general, the neoplastic cells of ALCL could frequently be detected and immunophenotyped by flow cytometry; specifically, the neoplastic population could be detected in 25 of 29 cases (86%) and 19 of 23 cases (83%) (Kesler et al and Muzzafar et al, respectively). Immunohistochemistry was frequently used to complement the flow cytometric results, including detection of the ALK protein by immunohistochemistry.

The immunophenotype observed in the ALCL cases (composite data from 3 above papers) is shown in the Table 1 (below).

These lymphomas expressed CD30 and CD45. Interestingly, although the neoplastic cells are of T cell origin, T cell antigen expression (CD2, CD3, CD4, CD5, CD7, and CD8) was often aberrantly lost, with only a subset of cases showing expression of a given T cell antigen. Expression of myeloid antigens (CD13 and CD15) was occasionally seen and B cell antigens (CD19 and CD20) were not expressed.

Our laboratory at the University of Washington often evaluates systemic ALCL with a standard T cell tube (for CD2, CD3, CD4, CD5, CD7, CD8, CD30, CD34, CD45, and CD56) and a tube for classical Hodgkin lymphoma (CHL; CD5, CD15, CD20, CD30, CD40, CD45, CD64, CD71, and CD95). Expression of CD30 and CD71 in the CHL tube is often quite helpful in identifying the neoplastic population of ALCL. The neoplastic cells of systemic ALCL lack expression of CD40 (or express very weak CD40), while the neoplastic Hodgkin and Reed-Sternberg cells of CHL always express intermediate to bright CD40 (Carbone A et al. (1995); Fromm et al. (2009)), a feature that allows these CD30+ lymphomas to be distinguished.

Breast implant ALCL (BI-ALCL) is a newly recognized type of CD30+, ALK- T cell lymphoma. These lymphoma are particularly uncommon with an incidence of 0.1-0.3 BI-ALCL cases/100,000 women with prostheses (Laurent et al. (2016)). As its name would imply, these lymphomas are associated with prior placement of breast prostheses; prognosis of these lymphomas differed depending on whether the neoplastic cells are invasive/infiltrative or not.

Our group published a characterization of the immunophenotype of BI-ALCL by flow cytometry. Surprisingly, the neoplastic cells showed immunophenotypic features more in keeping with CHL than ALCL (loss of T cell antigens) and expression of CD15, CD30, CD40, CD71, and CD95).

After this report, Montgomery-Goecker and co-workers (Montgomery-Goecker et al. (2015)) published their flow cytometric findings of two additional BI-ALCL cases. These two cases also showed loss of T cell antigens (no expression of CD3, CD5, CD7, CD8) except CD4, and uniform expression of CD30. Both cases were mainly negative for CD45. One of two of these cases showed expression of CD13 and CD33. Neoplastic cells represented 48% and 6.5% of the total events.

Despite the similarities, CHL and BI-ALCL can typically be distinguished as: 1) a diagnosis of CHL in a breast capsule tissue would be extremely uncommon; 2) while some CHL cases may have expression of CD40 at the level seen in these BI-ALCL cases, the majority of CHL cases have significantly brighter levels of CD40 expression; 3) in CHL, a plot of CD45 versus CD5 demonstrates either two populations (one with lower CD45 and no CD5 and a second with brighter CD45 and CD5 expression) or a diagonal relationship for CD45 and CD5 due to T cell rosetting (Fromm and Wood (2012); and 4) reactive CD4+ T cell populations with increased expression of CD7 and CD45 are seen in greater than 80% of CHL cases (Fromm et al (2010)). These immunophenotypic features will not be seen in ALCL.

In conclusion, both systemic and BI-ALCL can be immunophenotyped by flow cytometry. These T cell lymphomas show aberrant loss of T cell antigens and expression of CD30. In addition, BI-ALCL often shows immunophenotypic features of CHL but can be distinguished from that lymphoma by clinical information, the level of CD40 expression, the lack of T cell rosetting and lack of a CD4+ T cell population with increased CD7 and CD45 (that is characteristic of CHL)

Table 1: Composite Immunophenotype of Systemic ALCL as Determined by Flow Cytometry (from 3 studies)

Antigen	Expression#	Antigen	Expression
CD2	72.0 (36/50)	CD15	40 (8/20)
CD3	39.3 (24/61)	CD25	82.6 (19/23)
CD4	66.7 (38/57)	CD30	96.7 (58/60)
CD5	27.6 (16/58)	CD45	96.8 (61/63)
CD7	35.2 (19/54)	CD19	0 (0/63)
CD8	10.7 (6/56)	CD20	0 (0/63)
CD13	62.1 (18/29)		

#Percentage of cases positive (number cases positive/total number of cases)

HODKING LYMPHOMAS

Margarida Lima, MD, PhD
Head of the Laboratory of Cytometry, Hematology Department, Hospital de Santo António, Centro Hospitalar do Porto (HSA/CHP); Invited professor, Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto (ICBAS/UP); Oporto, Portugal

Abstract: According to the WHO classification of tumors of hematopoietic and lymphoid tissues, Hodgkin lymphoma (HL) is classified as two distinct entities, namely nodular lymphocyte predominant HL and classical Hodgkin lymphoma (CHL), the later comprising 4 subtypes (1). Traditionally, the diagnosis of CHL requires the morphologic identification of neoplastic Hodgkin and Reed-Sternberg (HRS) cells in a background of reactive cells consisting of lymphocytes, histiocytes, plasma cells, neutrophils and eosinophils. The suspicion is confirmed by immunocytochemistry, which typically reveals positivity of HRS cells for CD15 (3-fucosyl-N-acetyl-lactosamine), CD30 (member of the tumor necrosis factor receptor superfamily - TNFRSF8) and Pax-5 (transcription factor), and usually absence of expression of CD3, CD20, and CD45 (2,3). Other antigens that are known to be frequently expressed on HRS cells are CD40 (TNFRSF5), CD54 (ICAM-1), CD58 (LFA-3), CD71 (transferrin receptor), CD86 (ligand for the T cell costimulatory molecule, CD28), CD95 (FAS), and HLA-DR (MHC class II molecule).
Flow cytometry (FCM) of lymph node (LN) biopsies usually fails the diagnosis of CHL mainly for following reasons: the FCM protocols implemented in most clinical laboratories are focused on the diagnosis of non-Hodgkin lymphoma (NHL); HRS cells are rare cells, comprising less than 1% (and often less than 0.1%) of the cells within the LN affected by CHL; HRS are large cells (diameter up to 100 micrometers, comparatively to small lymphocytes that have up to 10 micrometers in diameter); and T cells form rosettes with HRS cells in cell preparations of CHL biopsies, giving rise to T cells-HRS cells aggregates.
In 2006, Fromm et al. demonstrated for the first time the feasibility of using multicolor FCM to identify the HRS cells. Using a 10-color protocol and a modified 4-laser BD LSRII flow cytometer, they were able to detect HRS cells by FCM in 89% of 27 LN involved by CHL and none of the LN without CHL (29 non-CHL neoplasms and 23 reactive LN) (4). The protocol used consisted in combining monoclonal antibodies (mAbs) specific for CD3 (ECD or PE-Cy7), CD15 (APC), CD19 (Alexa594), CD20 (PE-Cy7), CD30 (PE), CD40 (PE-CY5.5), CD45 (APC-Cy7), CD64 (FITC), CD71 (APC-A700), CD86 (PE), CD95 (APC), and HLA-DR (ECD) in 2 different tubes, and using DAPI to exclude nonviable cells. Three years later, the same group

proposed a single 9-color tube to identify HRS cells, using the same cytometer: CD5 (APC-Cy7), CD15 (APC), CD20 (PE-Cy7), CD30 (PE), CD40 (PE-Cy5.5), CD45 (PE-Texas red), CD64 (FITC), CD71 (APC-A700), and CD95 (PB) (5). They used this tube to study 279 blindly identified and 141 selected (suggestive of CHL) LN biopsies. Of the 53 CHL cases identified by histopathology (10 in the unselected group; 43 in the selected group), the FC sensitivity and specificity were of 89% and 100%, respectively. Latter on (2014), the same group validated a 6-color tube for the same purpose, using a similar cytometer: CD3 (APC-Cy7), CD20 (PE-Cy7), CD30 (PE), CD40 (PE-Cy5.5), CD64 (FITC), and CD95 (APC) (6). By analyzing 408 biopsies (including 55 CHL) they obtained a sensitivity and specificity of 85.4% and 99.7%, respectively. In general, the most common immunophenotype of the HRS cells was found to be CD45+low, CD15+, CD30+, CD40+, CD54+, CD58+, CD71+, CD86+, CD95+, CD123+, HLA-DR+, CD3-, CD5-, CD19-, CD20-, and CD64- (5-8). HRS-T cell rosettes give rise to a composite (CD15+, CD30+, CD40+, CD95+, CD3+, CD5+) immunophenotype, and interactions between HRS cells and T cells can be disrupted with blocking antibodies (e.g. anti-CD2 and anti-CD58) (5). The methods used for the preparation of LN cells and immunophenotyping of HRS cells were previously described in detail (7). To the best of our knowledge, to date, no other group has devoted itself to the study of the HRS cells by FCM. However, several studies have focused on the FCM analysis of the background of inflammatory cells observed in HL (9-15). Relevant alterations described included increased percentages of neutrophils and eosinophils, normal or increased CD4/CD8 ratios (9,10), increased expression of CD7 and other T cell antigens (11,12), increased proportions of CD4+, CD25+, CD152+, and FoxP3+ regulatory T cells (13), and higher fractions of CD4+CD26- T cells (14,15). Considering all these parameters together makes FCM even more useful of FCM for the diagnosis of HL (16).

Taking advantage of our large experience in the study of tissue biopsies by FCM (around 2.900 tissue biopsies studied from 2009 to 2016, mainly for the diagnosis of NHL), we have recently invested in the study of HL by FCM. First, biopsy samples are screening for NHL using three 8-color tubes (BD FACSCanto II, 200.000 events/tube): Lymphocyte screening tube (CD8+IgLambda-FITC/CD56+IgKappa-PE/CD5-PC5.5/CD10-PC7/CD3-APC/CD19-APC-H7/CD4+CD20-V450/CD45-KrO); B-cell tube (BCL2cyt-FITC/CD10-PE/CD38-PC5.5/HLA-DR-PC7/CD81-APC/CD19-APC-H7/CD20-V450/CD45-KrO); and T-cell tube (CD2-FITC/CD7-PE/TCR-GD-PC5.5/CD28-PC7/CD5-APC/CD3-APC-H7/CD4-V450/CD8-KrO). If no evidence of NHL is found, and there is sample available, additional tubes are performed to identify HRS cells, with at least 400.000 events being collected in each tube. After a period of testing of several combinations of mAbs, and based on the reports of Fromm et al., we are now using a HL protocol, consisting of two 8-color tubes in order to identify HRS cells: CD15-FITC/CD95-PE/CD40-PERCP-Cy5.5/HLA-DR-PC7/CD30-APC/CD3-APC-H7/CD20-V450/CD45-KrO and CD14-FITC/CD86-PE/CD40-PERCP-Cy5.5/HLA-DR-PC7/CD30-APC/CD3-APC-H7/CD81-V450/CD45-KrO.

Until now, our experience is based on the results obtained in 57 biopsies from patients suspected of having lymphoma. Fifty seven surgical biopsies (47 cases with negative screening for NHL and 10 cases with the diagnosis of NHL) from 57 patients (31 males and 25 females, median age of 47 years, ranging from 14 to 89 years) were evaluated for the presence of HRS cells using the HL protocol. Biopsy sites were as follows: LN – 52 cases (25 cervical, 10 inguinal, 7 axillary, 3 mediastinal, 2 intra-parotid, 5 non-specified); amygdala – 2 cases; and oropharynx or nasopharynx – 3 cases. Subsequent review of the histopathology reports revealed that these included biopsies from tissues affected by CHL (15 cases, all corresponding to LN), NHL (10 cases - B cell NHL 9 cases; T cell NHL 1 case), non-hematological tumors (9 cases), histiocytic disorders (2 cases - Langerhans cell histiocytosis and histiocytic lymphadenitis. All 15 HL consisted on CHL subtypes: nodular sclerosis (10 cases), mixed cellularity (1 case), lymphocyte rich (1 case) and lymphocyte depleted (0 cases); 3 cases are still waiting for sub-classification. The remaining 21 cases corresponded to reactive tissues.

As previously reported by Fromm et al. (5-8), we observed that HRS cells with a distinctive phenotype (CD45+low, CD15+, CD30+, CD40+high, CD86+, CD95+, and HLA-DR+high in most cases) can be readily identified in CHL samples (14/15 cases, 93.3%). The question is whether FCM is specific enough to distinguish CHL from reactive immunoblastic proliferations arising in the setting of infections, drug reactions, vaccines, malignancies, and autoimmune diseases. Indeed, in our experience, as in the experience of other authors (17), HRS-like cells were present in some reactive LN, especially those containing T and B cell derived immunoblasts. These cells often show CD30 expression and they may morphologically mimic HRS cells, sometimes leading to erroneous diagnoses of HL by histology. Positivity of the immunoblastic cells for B (e.g. CD20) or T (e.g. CD3) antigens, and negativity for CD15 is usually useful to distinguish them from true HRS cells (17). Finally, as also stated by Fromm et al., in cases where FCM is consistent with CHL, other related lymphoid neoplasms where HRS-like cells can be found still remain on the differential diagnosis; these include, among others, the so-called grey zone lymphomas with features intermediate between CHL and diffuse large B cell NHL (16) (18). However, in these situations, routine FCM panels are usually sufficient to establish the diagnosis of NHL.

MAST CELL LEUKEMIA

Cristina Teodósio, PhD
Department of Immunohematology and Blood Transfusion, Leiden University Medical Centre (LUMI);
Leiden, Netherlands

Authors: C Teodosio[1], A Mayado[2], M Jara-Acevedo[3], L Sanchez-Muñoz[4], I Alvarez-Twose[4], A García-Montero[1], A Matito[4], C Caldas[1], AF Henriques[4], A Lopez[1], JI Muñoz-González[1], N Dasilva-Freire[1], JI Sanchéz-Gallego[1], L Escribano[1], A Orfao[1], on behalf of the Spanish Network on Mastocytosis (REMA)

Institutions: Department of Immunohematology and Blood Transfusion, Leiden University Medical Centre, Leiden, The Netherlands.
[2]Servicio General de Citometría (NUCLEUS), Centro de Investigación del Cáncer (IBMCC-CSIC/USAL and IBSAL) and Departamento de Medicina, Universidad de Salamanca, Salamanca, Spain.
[3]DNA Sequencing Service (NUCLEUS), University of Salamanca, Research Biomedical Institute of Salamanca (IBSAL), Salamanca, Spain.
[4]Instituto de Estudios de Mastocitosis de Castilla La Mancha (CLMast), Hospital Virgen del Valle, Toledo, Spain.

Abstract: Mast cell leukemia is a rare disorder which represents the leukemic manifestation of Systemic Mastocytosis (SM). Therefore, MCL patients fulfil the diagnostic criteria for SM, including the detection of prominent multifocal clustering of mast cells (MCs) in the bone marrow (BM), which represents a major diagnostic criterion, and four minor criteria, including an atypical MC morphology, expression of CD2 and/or CD25 in MCs, presence of an activating mutation at codon 816 of *KIT* and serum tryptase 20ng/mL. As a SM variant, MCL patients fulfil one major and a minor or at least three minor criteria. MCs in MCL are usually immature, sometimes bi- or multi-lobed (promastocytes), however in some patients more mature forms may be predominant. From a immunophenotypic point of view, MCs in MCL usually express CD117, with low tryptase and FcRI levels, often expressing CD25 and CD30 but not CD34. Detection of CD2 is only restricted to limited number of cases and lack of both CD2 and CD25 can also be observed in some patients. Expression of CD52, HLA-DR and CD123 may also be observed.
Despite the rarity of the disease, MCL is known to have heterogeneous presentation. Based on the World Health Organization (WHO) criteria, MCL can be divided in a "leukemic" variant, with >10% MCs detected in peripheral blood (PB) and an "aleukemic" variant, with <10% PB infiltration. In both groups of patients MCs represent at least 20% of all nucleated cells in the BM, the primary diagnostic criterion for MCL, and the prognosis is poor. In addition to these variants, recent reports suggest that MCL may develop either as a *de novo* disease or secondary to e.g. aggressive systemic mastocytosis. Furthermore, in a few cases, the course of the disease may be less aggressive and more chronic, without signs and symptoms of organ damage ("C-findings"), showing a better overall prognosis than the acute variant of the disease. These observations have led to the recent proposal to discriminate between chronic MCL (without "C-findings") and acute MCL with "C-findings".
Differential diagnosis of MCL may represent a challenge, not only due to its heterogeneous presentation, but also because e.g. it may or not present with an associated haematological neoplasm (AHN), $CD25^+$ atypical MCs can also be found in BM of patients with chronic eosinophilic leukemia (CEL), discrimination between blasts maturing to MCs *vs.* basophil might be difficult, and blast with maturation to MC can be detected in 13% of AML. In this presentation we will review the diagnostic criteria for MCL, as well as the recently defined variants of the disease and the differential diagnosis from other myeloid neoplasms, mainly focusing on the utility of multiparameter flow cytometry in the diagnostic work-up of the disease.

SELECTED ORAL COMMUNICATIONS PRESENTATIONS
CO_2428 BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM. A CASE WITH DIFFERENT INMUNOPHENOTYPE AND CHROMOSOMAL ABERRATIONS
CO_2433 THE PROLIFERATION INDEX OF BONE MARROW CELLS MAY ASSIST THE DIFFERENTIAL DIAGNOSIS OF CHRONIC MYELOMONOCYTIC LEUKEMIA VS. MONOBLASTIC/ MONOCYTIC LEUKEMIAS

Session Sponsored
by Takeda

16H30 - 18H30 PARALLEL SESSION II | Room Fernando Pessoa

Flow cytometry and Hypersensitivity: Allergy and Autoimmunity
Chairs: Luis Miguel Borrego & Mariona Pascal

ROLE OF FLOW CYTOMETRY IN ALLERGY

Edward F. Knol, PhD, associate professor
University Medical Center Utrecht, Depts. Immunology and Dermatology/Allergology, Huispost F03.821
Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

Abstract: Development of flow cytometry, discovery of activation markers such as CD63 and unique markers identifying basophil granulocytes have led to the introduction of the so-called basophil activation test (BAT) in allergy research and diagnostics. BAT measures basophil responses to IgE-dependent and –independent stimuli on between 150 and 1000 basophil granulocytes in less than 0.1 ml fresh blood. CD63 is binding a non-basophil-specific epitope inside the granular membrane. Upon degranulation the CD63 epitope is exposed on the outside of basophils. The CD63 increase is related to release of histamine of basophils. In contrast to the measurement of histamine, the BAT can be easily introduced in research and diagnostic settings. BAT can be part of the diagnostic evaluation of patients with food-, insect venom-, and drug allergy and chronic urticaria. It may be helpful in determining the clinically relevant allergen. Basophil sensitivity may be used to monitor patients on allergen immunotherapy, anti-IgE treatment or in the natural resolution of allergy. BAT uses fewer resources and is more reproducible than challenge testing, especially in the diagnostic procedure of food allergy, in which double-blind placebo-controlled challenge tests are often still used. As it is less stressful for the patient and avoids severe allergic reactions, BAT ought to precede challenge testing.
The nature of basophil activation as an ex vivo challenge makes it a multifaceted and promising tool not only for the allergist, but only in a research setting. BAT is implemented to screen for allergenic activity of (modified) allergens. In addition, also in the basic research of basophil activation BAT has been helpful. In this presentation, an overview of the characteristics of the CD63 in basophils and its implementation in BAT will be discussed.

FOOD ALLERGY: DIAGNOSIS AND MONITORING

Mariona Pascal, PhD
Immunology Department, CDB, Hospital Clínic de Barcelona,
Barcelona, Spain

Abstract: Basophil activation test (BAT) is an in vitro test used for the diagnosis and monitoring of allergy to allergens such as foods, drugs, hymenoptera venoms and inhalants. Briefly, basophils of the patient are incubated with the suspected allergen, and if the patient is sensitised, they become activated. This activation can be assessed by the measurement of mediators released, but also with the activation markers expressed in the cell membrane. In food allergy diagnosis, Oral food challenge (OFC) is still the gold-standard; however, it is time- and resource-consuming with the risk of inducing an allergic reaction, indeed potentially severe. In several published studies, BAT has shown higher specificity than conventional specific IgE testing in the skin or in serum for diagnosing certain food allergies. Thus, BAT has emerged as a new diagnostic test for food allergy. Nevertheless, it is very important to note that the diagnostic utility of BAT is allergen-specific and needs to be validated for different allergen preparations and patient populations, as well as, the methodology adopted and the flow cytometry analysis, since all these can have a significant impact on the accuracy of the results obtained. Besides discrimination between food allergic and tolerant subjects, it can provide valuable information about the characteristics of food-induced reactions, p.e. the severity of the allergic symptoms and the dose of peanut protein at which patients may react during OFC have been reported, but further studies are required for other food allergies. Moreover, BAT can be useful in assessing the natural resolution of food allergies that are commonly outgrown over time (cow's milk and egg allergy), but also to distinguish different phenotypes of patients with these allergies (for instance, tolerance to extensively heated or unheated forms of these foods). In research studies, BAT has also been used to monitor the clinical response to immunomodulatory treatments for food allergy (peanut, cow's milk and egg), observing decreased basophil reactivity and sensitivity to the respective food allergens. In conclusion, BAT is a promising tool as a clinically useful test. However, further studies are required in order to enable a wider use of BAT in clinical practice.

DRUG ALLERGY AND FLOW CYTOMETRY: DIAGNOSIS AND MONITORING

Cristobalina Mayorga, PhD
Research Laboratory- Allergy Unit, University
Hospital of Málaga, IBIMA, Málaga, Spain

Abstract: Drug hypersensitivity reactions (DHRs) are those adverse reactions produced after the exposure to a drug at doses normally tolerated by non-hypersensitive subjects. They constitute an important drug safety problem as a result of their severity and unpredictability. DHR diagnosis can lead to the withdrawal of important and often non-substitutable treatments, the prescription of alternative drugs that can be more expensive and potentially toxic. All this can be an issue for the public health system, especially when the diagnosis is not fully confirmed. Diagnosis of DHRs is complex, generally based on a detailed clinical history, skin testing (ST) and drug provocation testing (DPT). However, clinical history can be unreliable and ST have not optimal sensitivity for some drugs. This leaves DPT as the gold standard for diagnosing DHR, however, it is not risk-free, needs to be done in a specialised setting by trained personnel, is time consuming and cannot be performed for severe reactions. Therefore, there is a need to use in vitro testing to ensure accurate diagnosis, both to improve patient safety and to reduce health system costs.
The methods used for the diagnosis of DHRs depend on the mechanism involved and the kinetic of the reaction. IgE- or T-cell-mediated.

In immediate or IgE-mediated DHRs, the determination of drug-specific IgE antibodies has been performed by immunoassays. However, it is available for a limited number of drugs and with a low sensitivity. In the last decades, basophil activation tests (BAT) using flow cytometry has allowed the detection of activated basophils using specific markers, despite the low percentage of peripheral basophils (<1% of total leukocytes). Moreover, BAT does not need to use drugs conjugated with a carrier molecule, allowing its use in a wide variety of drugs and is particularly useful for evaluating hypersensitivity reactions to drugs for which there is no other in vitro test available.
The evaluation of cell-mediated DHR is more complex, as they include heterogeneous clinical symptoms and mechanisms. These reactions can be analysed in the acute phase by assessing different inflammatory markers on peripheral blood using flow cytometry or at the resolution phase of the reaction mainly aimed at identifying the responsible drug. For this last purpose lymphocyte transformation test (LTT) has been the most frequently used and is based on the capacity of lymphocytes to proliferate after stimulation with a specific drug generally evaluated by the incorporation of either 3H-thymidine. In the last years, the possibility of evaluating the proliferative response using carboxyfluorescein diacetate succinimidyl ester and flow cytometry has improve the LTT capacity since enables one to phenotype the proliferating cells in terms of population structure and production of inflammatory markers. This technique will be key to fully characterising the effector mechanism triggered by the suspected drug.
Taken all together we can observe that flow cytometry helps the evaluation of DHRs giving the opportunity to perform more functional in vitro tests that mimic the effector mechanism involved in the DHRs. Multidisciplinary collaborations are needed to identify biomarkers and increasing our knowledge of the drug metabolites involved in the immunological mechanisms of DHRs that will improve the sensitivity and specificity of in vitro tests.

SELECTED ORAL COMMUNICATIONS PRESENTATIONS

CO_2454 INTRAEPITHELIAL LYMPHOCYTES SUBSETS IN CELIAC DISEASE DIAGNOSED PATIENTS

CO_2457 CHILDREN WITH COW´S MILK ALLERGY: THE IMPACT OF ORAL IMMUNOTHERAPY (OIT) IN THEIR IMMUNE PROFILE

18H30 - 19H00 COMMERCIAL SESSION by ALEXION | Room Sophia de Mello Breyner

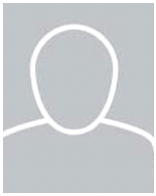
Avances en el diagnóstico de la HPN
Chair: Amparo Sempere - Hospital La Fe, Valencia
Speakers:
Alberto Orfão,
Centro de Investigación del Cáncer, Universidad de Salamanca (CSIC), Salamanca

Teresa Caballero,
Hospital Universitario Virgen del Rocío, Sevilla

Antonio Almeida,
Instituto Portugués de Oncología, Lisboa

18H30 - 19H00 COMMERCIAL SESSION by MERCK | Room Fernando Pessoa

STUDY OF IMMUNOLOGICAL SYNAPSE FORMATION USING FLOWSIGHT® AND IMAGESTREAM® IMAGING FLOW CYTOMETERS.



Clara Cigni,
Merck S.p.A., Vimodrone (MI), Italy

Abstract: During adaptive immune response, the activation of T lymphocytes by antigen-presenting cells (APC) results in the formation of an immunological synapse. Following contact, the formation of immune synapses requires multiple rearrangements in the actin cytoskeleton and the recruitment of adhesive and signaling molecules to the T cell-APC interface. During the past decades, the immunological synapse was studied using fluorescence microscopy. However, the interpretation of microscopic imagery could be subjective and laborious to analyze statistically, especially for the study of rare events like immunological synapse. In order to overcome these problems, Amnis® imaging flow cytometers were used. ImageStream®X and FlowSight® are multispectral imaging flow cytometers able to generate high resolution microscope images of cells in suspension. In particular, the ImageStream®X and FlowSight® systems allow to register multiple parameters for each cells, including brightfield, darkfield (SSC) and up to 10 fluorescent markers at high speed, thanks to the CCD camera and the time-delay integration (TDI) technologies. Due to these characteristics, ImageStream®X and FlowSight® combine the speed and sensitivity of flow cytometry with the resolution and detailed imagery of microscopy, overcoming the limitations of both techniques. Using the IDEAS® analysis software and its optimized wizards, we were able to demonstrate that both FlowSight® and ImageStream®X can distinguish the cells conjugates with an organized immunological synapse and assess their frequency. Moreover, the ImageStream®X can furtherly discriminate the specific location of adhesion and signaling molecules within the interface of the immunological synapse. In conclusion, the innovative design of Amnis® cytometers and the ease of use of IDEAS® software bring outstanding performances, opening the door to the study of an extensive range of applications, like the immunological synapse formation.

19H00 - 20H00 SIC GENERAL MEETING | Room Sophia de Mello Breyner

20H00 - 22H00 WELCOME COCKTAIL at Centro Cultural de Belém

26th May 2017

09H00 - 10H00 PLENARY SESSION | Room Sophia de Mello Breyner

Chair: Artur Paiva

ADVANCES IN THE FLOW CYTOMETRY DIAGNOSIS AND MONITORING OF HEMATOLOGICAL MALIGNANCIES

Alberto Orfão, MD, PhD
Departamento de Medicina and Servicio General de Citometria (NUCLEUS), Centro de Investigacion del Cancer (Instituto de Biologia Molecular y Celular del Cancer; CSIC-USAL), Universidad de Salamanca, and IBSAL, Salamanca, Spain

Abstract: Flow cytometry immunophenotyping has long proven to be of great clinical utility in the diagnosis classification and monitoring of haematological malignancies. This is mainly due to the fact that flow cytometry allows for objective and robust multiparametric analysis of thousands of cells per second. However, along the years, both the protocols used for instrument set up and sample preparation, and the specific panels of reagents applied have varied substantially among laboratories. This is mostly due to continuous introduction of new technological advances in the equipment and reagents (e.g. potential for the measurement of more colors and more cells, broad availability of compatible fluorochrome and fluorochrome-conjugated antibody reagents and an increased availability of antibodies clones per CD marker), together with co-existence of multiple, only partially overlapping, consensus-based antibody panels.Despite such consensus guidelines have been proposed to standardize the antibody panels, they have been only partially successful. Since 2012, the EuroFlow consortium has introduced a new concept a strategy about how to apply flow cytometry for the immnunophenotypic diagnosis of haematological malignancies based on the use of fully standardized procedures and panels of reagents in order to allow clinical comparisons of immunophenotypic data obtained for individual samples against data bases of normal and leukemic/lymphoma patient samples. This permits automated gating and alarming for any altered cell population in the sample, identification of relevant technical problems, and a software-guided diagnostic help. Together with the introduction of new informative markers and software tools immunophenotyping this has paved the way for more robust, sensitive and reproducible diagnosis of haematological malignancies. In addition, the new tools and strategies have also proven useful in other fields such as the diagnostic screening and classification of primary immunodeficiencies and immune monitoring.

10H00 - 11H45 PARALLEL SESSION III | Room Sophia de Mello Breyner

CYTOGENOMIC ANALYSES SHOW EXPANDED GENOMES IN THE PUCCINIALES (RUST FUNGI) AND SUGGEST PLOIDY VARIATION ALONG THEIR LIFE CYCLES

Pedro Talhinhas, PhD
LEAF - Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal.

Abstract: Rust fungi (Basidiomycota, Pucciniales) are biotrophic plant pathogens with complex life cycles (up to five spore types). The urediniosporic infection cycle is frequently the most important in disease dissemination as the only stage capable of repeating itself. The cell nuclear content of rust fungi is thought to follow that of other Basidiomycota, with haploid nuclei throughout the life cycle, only becoming diploid upon karyogamy in telia and immediately returning to the haploid state as meiosis takes place leading to the formation of basidiospores. Recently, using genome size quantification techniques, the presence of 1C, 2C and a low proportion of 4C nuclei was detected in different stages of the urediniosporic cycle of several rust fungi. These results suggest the presence of diploid nuclei that supposedly only occur in teliospores, and compatible with the occurrence of karyogamy and meiosis prior to urediniospore formation, although endopolyplody or other

parasexuality phenomena cannot be ruled out. This unexpected phenomenon seems to be transversal to the Pucciniales. Moreover, the estimation of genome size ca. 60 rust species sets the average Pucciniales genome at ca. 380 Mbp, ranging from 70 to 2489 Mbp (the average fungal genome is ca. 44 Mbp), with no apparent phylogenetic structuration. Such genome size variations may be due to polyploidy phenomena, and may be linked to the nuclear content variation along rust life cycles.

THE MAMMALIAN SPERMATOZOA: IS IT TIME FOR FLOW SPERMETRY?

Fernando Peña, DMV, PhD
Laboratory of Equine Reproduction, Department of Medicine, Faculty of Veterinary Medicine, University of Extremadura, Cáceres, Spain

Abstract: As blood, the mammalian ejaculate is a cellular suspension in liquid plasma (seminal plasma). Moreover, has a heterogeneous nature, with different phenotypes present in the spermatozoa. As an example only 5-10% of the spermatozoa in the ejaculate will finally be mature enough to fertilize the egg, after completion of the maturational process called capacitation. While flow cytometry has been used since the late 80s, initially for evaluation of sperm DNA, in the last decade an extraordinary development of flow cytometry applied to sperm has occurred, and multicolor panels are now used in the laboratory of the authors. Flow cytometry has multiple applications in clinical andrology and animal science, where market of semen is a major component of the animal breeding industry. Flow cytometry is routinely used for multiple andrological applications including assessment of the quality of the sperm DNA, integrity and functionality of the membrane, determination of spermptosis, and oxidative stress, and mitochondrial activity. Also phosphoflow applications have been developed for sperm assessment. This lecture will be an update of current applications of flow cytometry in sperm analysis, discussing the interest of the technique for the study of sperm biology as well as clinical and biotechnological applications.

PHENOTYPE AND FUNCTION OF RARE IMMUNE CELLS

Andrea Cossarizza, MD, PhD
University of Modena and Reggio Emilia School of Medicine, Modena, Italy

Abstract: The possibility to detect, count and perform functional analysis of rare cells is opening a new field in the world of cell analysis, and it is giving relevant information either to the basic scientist or to the clinician. The term “rare” is typically referred to those events that have a frequency of 0.01% or less. In the last year, my group has focussed on the development of new methodologies and techniques for studying rare events, and studies were performed on several different types of cells and different pathologies.
Applications of rare cell analysis include, among others, the detection in blood of tumours such as metastatic breast cancer cells or neuroblastoma cells infiltrating the bone marrow, monitoring of minimal residual disease, detection of stem cells and rare HIV-infected cells in peripheral blood, identification of antigen (Ag)-specific T cells, invariant natural killer T cells, and analysis of mutation frequencies in genetic toxicology.
In my talk, I will present and discuss the methods for the detection of some rare cell populations of interest for immunologists, along with some crucial technical issues, and with the requirements to isolate and enumerate such cells in peripheral blood.

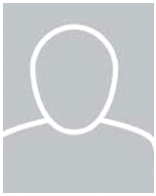
SELECTED ORAL COMMUNICATIONS PRESENTATIONS

CO_2429 EVALUATION OF THE ETHANOL TOLERANCE BY FLOW CYTOMETRY FOR WILD AND MUTANT SYNECHOCYSTIS STRAINS

CO_2431 DESIGN OF MASS CYTOMETRY PANELS FOR CLINICAL STUDIES OF PATIENTS WITH SYSTEMIC AUTOIMMUNE DISEASES

Stem Cells and Transplantation
Chairs: Artur Paiva & Alexandre Salvador

MINIMAL RESIDUAL DISEASE AND IMMUNE RECONSTITUTION AFTER STEM CELL TRANSPLANTATION



Noemi Puig,
Salamanca, Spain

(RE) PROGRAMING HAEMATOPOIESIS AND IMMUNITY

Filipe Pereira, PhD
Center for Neuroscience and Cell Biology (CNC),
Universidade de Coimbra, Coimbra, Portugal

Abstract: Once committed, the differentiated state of a cell is normally stable and can be inherited through cell division. Under certain conditions cell fate can, however, be modified or reversed. Mouse and human differentiated cells can be reprogrammed to pluripotency or directly converted to unrelated somatic cell fates by combinations of transcription factors (TFs). Here I will discuss the requirements to reprogram fibroblasts to Hematopoietic Stem Cells (HSCs) and Dendritic Cells (DCs) using combinatorial TF approaches. I hypothesize that direct cell fate reprogramming can be employed for hematopoietic regeneration as well as to induce immunity.
We have recently demonstrated the direct reprogramming of mouse fibroblasts into clonogenic hematopoietic progenitors with the combination of TFs, Gata2, Gfi1b, cFos and Etv6 (Pereira et al, Cell Stem Cell 2013). These four TFs induce a dynamic, multi-stage hemogenic process that progresses through an endothelial-like intermediate. As such, it recapitulates developmental hematopoiesis in vitro (Pereira et al, Dev Cell 2016). We now show that the same blood forming process can be induced in human skin-derived fibroblasts with a similar combination of TFs. Reprogrammed fibroblasts undergo morphological and transcriptional changes, display cell surface phenotypes of human HSCs, and repopulate immunodeficient mice. Interestingly, we have mapped the genomic targets of hemogenic TFs uncovering unexpected mechanisms underlying hematopoietic reprogramming. In addition to a regenerative strategy we are applying cellular reprogramming to induce immunity. We are employing direct reprogramming to induce DCs from mouse fibroblasts. Induced DCs express the main components of the antigen presenting machinery at the cell surface, are able to engulf particles, secrete inflammatory cytokines and present antigens to T-cells.
Collectively this work has provided insight into HSC and DC transcriptional networks as well as remarkable information on the specification on both cell-types. This knowledge may be applied for patient-specific reprogrammed cell generation without transiting through pluripotency. Our work may have important implications for hematopoietic development, disease modeling and generation of cells for replacement therapies as well as the development of more efficient cancer immunotherapies.

IMMUNE RECONSTITUTION FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

Maria Soares, PhD
Instituto de Medicina Molecular, Lisbon, Portugal

Abstract: Chronic Graft versus Host disease (cGVHD) is a major limiting factor for the success of allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT). We evaluated the association between the kinetics of Regulatory T cells (TREG) and Conventional CD4+Foxp3-T cells (TCON) reconstitution and the development of cGVHD. We performed a 2 year phenotypic analysis by multiparametric flow cytometry using fresh blood from 39 patients undergoing allo-HSCT after a reduced intensity conditioning regimen. The data obtained is suggestive of decreased thymic output and increased differentiation into terminally differentiated effector cells in patients developing cGVHD. This likely impacts on the reconstitution of naïve TCON and TREG compartments in these patients. We speculate that the inability to generate and maintain the more immature TREG subsets may lead to decreased TREG numbers potentially resulting in decreased control of effector T cells contributing to the development of cGVHD.

SELECTED ORAL COMMUNICATIONS PRESENTATIONS

CO_2443 PRESENCE OF EOSINOPHILIA AFTER PEDIATRIC LIVER TRANSPLANTATION AND ITS RELEVANCE TO GRAFT DYSFUNCTION PROCESS
CO_2445 CYTOMEGALOVIRUS SPECIFIC CELLULAR IMMUNE RESPONSE AS A MARKER OF THE OUTCOME OF LIVER TRANSPLANTATION FOR CHRONIC HEPATITIS C VIRUS INFECTION

11H45 - 12H15 COFFEE BREAK and Poster Viewing

Immunophenotype of T and NK cells in acute and chronic lymphoproliferative Syndromes
Chairs: Teresa Molero & Maria Arroz

T-CELL DIFFERENTIATION AND ITS IMPLICATIONS FOR THE DIAGNOSIS, CLASSIFICATION AND MONITORING OF ALL T

Alberto Orfão, MD, PhD
Departamento de Medicina and Servicio General de Citometria (NUCLEUS), Centro de Investigacion del Cancer (Instituto de Biologia Molecular y Celular del Cancer; CSIC-USAL), Universidad de Salamanca, and IBSAL, Salamanca, Spain

Abstract: T-ALL accounts for around 10-15% of all ALL patients both in children and in adults. Despite T-ALL was initially associated with a worse clinical outcome -vs. B-cell precursor (BCP)-ALL-; later on diagnosis of T-ALL was associated with intensification of therapy, which has led to a significant improvement in the outcome of T-ALL patients. Therefore, immunophenotyping provides critical information for the assessment of the blast T- cell lineage in T-ALL and its differential diagnosis with other subtypes of acute leukemia and lymphoblastic lymphomas such as BCP-ALL, acute myeloblastic leukemia (AML) and mixed lineage acute leukemia (MPAL). In addition, immunophenotyping by flow cytometry allows for subclassification of the disease and minimal residual disease (MRD) monitoring. Currently, the WHO defines T-cell lineage based on the presence of CyCD3, usually in association with a strong CD7 expression with or without other T-cell markers. However, current knowledge about the normal bone marrow (BM) and thymic T-cell maturation pathways suggests the need for both revisiting the definition of the very early T/NK/DC precursors, and the potential inaccurate diagnosis of early T-cell leukemias with the need for more objective criteria for a robust diagnosis of the disease, as proposed recently by the EuroFlow group based on the use of automatic comparison of individual patient immunophenotypic profiles vs. well-defined data bases of normal and acute leukemia samples stained with the ALOT tube.
In addition, extended immunophenotyping allows sub-classification of T-ALL into different subgroups based on their maturation features (early-pre-T, pro-T, pre-T, common and mature T-ALL). However, such classification typically shows marginal cytogenetical/molecular correlations, as well as clinical impact. This is probably due to the fact that it focuses more on the molecules that are similarly expressed in T-ALL blasts and their normal T-cell precursor counterparts, than in their phenotypic differences. Despite the above virtually all T-ALL cases show phenotypic aberrations that can be used to monitor MRD after therapy and MRD monitoring in T-ALL has proven to be of great clinical utility for patient risk stratification and MRD-adapted treatment intensification with improved survival. Despite, multiple aberrant phenotypes are usually detected in most T-ALL patients, a common antibody MRD panel that could be routinely applied to every T-ALL case, independently of the phenotype of blast cells at diagnosis, is still missing and under construction, to be combined with high-sensitive next generation flow MRD detection, as recently propose for BCP-ALL.

IMMUNOPHENOTYPE OF T LYMPHOCYTES IN CHRONIC LYMPHOPROLIFERATIVE SYNDROMES

Julia Almeida, MD, PhD
Departamento de Medicina and Servicio General de Citometria (NUCLEUS), Centro de Investigacion del Cancer (Instituto de Biologia Molecular y Celular del Cancer and IBSAL; CSIC-USAL), Universidad de Salamanca, Salamanca, Spain

Authors: Julia ALMEIDA[1], Juan FLORES-MONTERO[1], Antonio LÓPEZ[1], Susana BARRENA[1], Paloma BÁRCENA[1], María JARA-ACEVEDO[1], Noemí MUÑOZ[1], Noemí PUIG[2], Ton LANGERAK[3], Margarida LIMA[4], Jacques J. VAN DONGEN[5], Alberto ORFAO[1], on behalf of EuroFlow Consortium.

Institutions: [1] Departamento de Medicina and Servicio General de Citometria (NUCLEUS), Centro de Investigacion del Cancer (Instituto de Biologia Molecular y Celular del Cancer and IBSAL; CSIC-USAL), Universidad de Salamanca, Salamanca, Spain, and IBSAL; [2]Department of Hematology, University Hospital, Salamanca, Spain; [3]Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherland; [4]Department of Hematology, Santo António Hospital, Porto, Portugal; Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands

Abstract: Mature T-cell malignancies are a biologically and clinically heterogeneous group of disorders that result from clonal proliferation of mature post-thymic lymphocytes. This wide group of chronic lymphoid malignancies is still much less understood than their B-cell counterpart, owing to their rarity and biologic heterogeneity, and to the fact that most categories lack distinctive genetic markers or recurrent biological abnormalities. Nonetheless, major advances on molecular characterization have been reached in some categories (WHO2016) in the last years; also, great efforts have been recently performed, as regards the characterization of tumor cells and the identification of their normal counterpart (i.e. naïve CD4+ T cells, central memory CD4+ T cells, follicular helper T cells or regulatory T cells have been claimed to be the normal counterpart of T-cell prolymphocytic leukemia, Sezary syndrome, angioimmunoblastic T-cell lymphoma of adult T-cell leukemia/lymphoma). Despite this, challenges remain in the subclassification of major disease groups within peripheral T-cell neoplasms; actually, the most common WHO "category" of mature T-cell neoplasms is peripheral T-cell lymphoma not otherwise specified –composed of a kind of "wastebasket" subtype defined by exclusion criteria–, which accounts for approximately one third of all these disorders. Based on this background, the EuroFlow Consortium (to which our group belongs) aimed to design a diagnostic strategy by 8-color flow cytometry to better classify T-CLPD into specific WHO categories. Our results clearly showed further contribution of the marker combinations used to distinguish between normal/reactive and aberrant/malignant T-cells -except for a few CD8+ T-LGL cases- and at the same time it allowed a more precise classification of T-CLPD into most WHO entities, particularly within the CD4+ T-CLPD.

IMMUNOPHENOTYPE OF NK LYMPHOCYTES IN ACUTE AND CHRONIC LYMPHOPROLIFERATIVE SYNDROMES

Margarida Lima, MD, PhD
Head of the Laboratory of Cytometry, Hematology Department, Hospital de Santo António, Centro Hospitalar do Porto (HSA/CHP); Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto (ICBAS/UP); Oporto, Portugal

SELECTED ORAL COMMUNICATIONS PRESENTATIONS

CO_2452 FLOW CYTOMETRY: MEASUREMENT OF PERIPHERAL BLOOD EXPRESSION/ LYMPHOCYTES OF CUTANEOUS T LYMPHOMAS
CO_2453 PERIPHERAL BLOOD VΔ1 T CELLS IN B-CLL EXHIBIT A CYTOTOXIC PROFILE, LIKE THE OTHER T CELL SUBPOPULATIONS

Vesicles, exosomes, microparticules

Chairs: Lola Martinez & Bruno Costa Silva

EDUCATION OF BONE MARROW-DERIVED CELLS DURING METASTASIS BY TUMOR-SECRETED EXOSOMES

Hector Peinado, PhD
Laboratory of Microenvironment and Metastasis at CNIO, CNIO Universidad Autonoma de Madrid, Madrid, Spain

Abstract: Cancer is a systemic disease, while most of the research effort has been focused on analyzing the primary tumor, there is a lack of information on how the tumor microenvironment influences metastasis. The importance of the microenvironment in metastasis is now fully acknowledged. Exosomes are secreted vesicles carrying lipids, proteins, RNA and DNA molecules. By carrying these molecules, and facilitating their cell-to-cell transfer, exosomes can modulate the behavior of resident cells that can impact disease progression. Our studies demonstrated that tumor exosomes are a major tumor-derived factor that acts systemically to promote bone marrow-derived cells (BMDCs) recruitment to the tumor and metastatic microenvironments. We showed that exosome secretion by melanoma cells influences BMDC mobilization and recruitment to pre-metastatic and metastatic niches, thus promoting metastasis in a process that we have termed “education”. We have recently analyzed the role of tumor-secreted exosomes in lymph node pre-metastatic niche formation. We found that melanoma-derived exosomes promote lympho(angio)genesis and enrichment of molecular adhesion-, autoimmune- and cytokine-related expression signatures in lymphatic endothelial cells (LECs). Our results support a role of tumor-secreted exosomes in promoting cellular and molecular alterations in the lymph node microenvironment fostering metastasis. In addition, we started the characterization of secreted exosomes by flow cytometry and particle fractionation to determine the characteristics of secreted vesicles. Analysis of exosome content in human lymphatic fluid suggest that proteomic cargo and particles are increased in melanoma patients opening the possibility of the use of circulating vesicles in lymphatic fluid as biomarkers and as a source of novel biomarkers to predict metastatic potential.

PAST, PRESENT AND FUTURE OF EXTRACELLULAR VESICLES ANALYSIS BY FLOW CYTOMETRY

Òscar Fornas, PhD
Flow Cytometry Unit. Pompeu Fabra University (UPF) and Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology (BIST). Barcelona, Spain.

Abstract: Extracellular vesicles (EVs) are circular fragments of membrane released by the cells with specific lipid, protein, and RNA contents reflective of parent cell status. This characteristic makes them to be increasingly recognized for their role in intercellular communication and as biomarkers of diseases. The size-range of the EVs secreted by cells is between 70-1000 nm, being the EVs with a size below 200 nm the most abundant. The challenge in the EVs field for diagnostic purposes in a clinical setting include the time required for EV isolation and analysis, as well as the lack of suitable methodology that has been the main challenge of EVs analysis during last decade. We developed a new methodology to identify and isolate EVs by Flow Cytometry with a resolution of 30-40 nm. This novel

methodology provides an easy, reproducible and reliable analysis of EVs improving all existing ones. With this novel approach is not necessary to concentrate the EVs, avoiding inconsistent results that are obtained by using different isolation protocols and complex settings for the cytometer. We dramatically reduced the time consuming from 2 days to 1 hour. This is the first time where sorting of exosomes is successfully performed because has been confirmed and validated by complementary technologies such as confocal and electron microscopy. Our new method resolves the lack of robustness and reliability in exosomes analysis in the current flow cytometry approaches.

IMMUNOSUPPRESSIVE EXOSOMES PRESENT IN HUMAN OVARIAN TUMOR MICROENVIRONMENTS RAPIDLY AND REVERSIBLY ARREST T CELL ACTIVATION

Paul Wallace
Department of Flow & Image Cytometry,
Roswell Park Cancer Institute, Buffalo, NY

Abstract: Virus size membrane bound extracellular vesicles isolated from ascites fluid from ovarian cancer patients have biophysical and compositional characteristics similar to vesicles called exosomes. The tumor-associated exosomes inhibit an early activation endpoint (translocation of NFAT from the cytosol into the nucleus) of a significant portion of virus (EBV and CMV) specific CD8+ T-cells that are stimulated with viral peptides presented in the context of Class I MHC. Early and late activation endpoints of peripheral blood CD4+ and CD8+ T-cells (of unknown specificity) stimulated with immobilized antibodies to CD3 and CD28 are also significantly inhibited by the exosomes. The inhibition of the T-cells is induced directly and rapidly (after just a 2h pulse with the exosomes), and occurs coincidentally with the exosomes binding to and internalization by the T-cells. The early arrest in the activation occurs without a loss of viability in the T-cells. The immune suppressive exosomes in the tumor microenvironment and the ability to block their T-cell inhibitory activity represent a potential therapeutic target to enhance the anti-tumor immunity of quiescent tumor-associated T cells, and to prevent the functional arrest of endogenous or adoptively transferred T-cells upon their entry into the tumor.

SELECTED ORAL COMMUNICATIONS PRESENTATIONS

CO_2432 MULTICENTER HARMONIZATION OF FLOW CYTOMETERS IN THE CONTEXT OF THE MULTICENTRIC PRECISESADS PROJECT

CO_2439 NEXT GENERATION FLOW CYTOMETRY IN THE MONITORING OF MONOCYTIC-LINEAGE ALTERATIONS DURING FOLLOW-UP OF MYELODYSPLASTIC SYNDROMES AND ACUTE MONOBLASTIC/MONOCYTIC LEUKEMIA

14H00 - 15H15 LUNCH

Advances in primmary immunodeficiencies screening and diagnosis by flow Cytometry

Chairs: Martin Perez-Andrés & João Farela Neves

NEW STANDARDIZED STRATEGIES IN THE ORIENTATION OF LYMPHOID DEFECTS BY FLOW CYTOMETRY: COMBINED IMMUNODEFICIENCIES AND PRIMARY ANTIBODY DEFICIENCIES ANALYSIS BY FCM

Martín Perez Andrés, PhD
Dep. Medicine-Serv. Cytometry, Cancer Research Center (IBMCC-CSIC/USAL) and University of Salamanca, Salamanca, Spain

Authors: Martin Perez-Andres,[1] Mirjam van der Burg,[2] Tomas Kalina,[3] Elena Blanco,[1] Sonia de Arriba,[4] Juan Torres-Cañizales,[5] Eduardo Lopez-Granados,[5] Jan Philippé,[6] Carolien Bonroy,[6] Cristina Serrano,[7] Marcela Vlkova,[8] Anne-Kathrin Kienzler,[9] Marjolein Wentink,[2] Ester Mejstříková,[3] Menno van Zelm,[10] Alberto Orfao,[1] Jacques J.M. van Dongen[11] on behalf of the EuroFlow PID consortium

Institutions: Dep. Medicine-Serv. Cytometry, Cancer Research Center (IBMCC-CSIC/USAL) and Univ. of Salamanca, Salamanca, Spain; [2]Dept. of Immunology, Erasmus MC, Rotterdam, The Netherlands; [3]Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic; [4]Department Pediatrics and Allergology, University Hospital of Salamanca, Salamanca, Spain; [5]Immunology, Universitario La Paz, Madrid, Spain; [6]Department of Laboratory Medicine, University Hospital Ghent, Ghent, Belgium; [7] Servicio Hematologia. Fundacion Jimenez Diaz. Madrid, Spain; [8]Institute of Clinical Immunology and Allergology, St Anne`s University, Brno, Czech Republic; [9]BRC-Translational Immunology Lab, University, Oxford, UK; [10]Dept. of Immunology and Pathology, Central Clinical School, Monash University, Melbourne, Australia, [11]Dept. of Immunohematology and Blood Transfusion (IHB), Leiden University Medical Center (LUMC), Leiden, The Netherlands

Abstract: Primary immunodeficiencies (PID) are inherited disorders of the immune system, generally presenting with recurrent, severe and sometimes life-threatening infections. To date, more than 300 genes have been identified that can be mutated in PID patients, that affect the functional abilities and/or numbers of one part of the immune system or one cell type. Since most of PID patients (60-65%) have a defect in the lymphoid system, involving B- and/or T-cells alone or in combination with other cells, flow cytometric immunophenotyping of lymphoid cells plays a central role in the stepwise diagnostic workup of patients suspected of PID. An accurate immunophenotypic diagnosis is of utmost importance for guiding genetic testing, whether targeted to specific genes or whole genome sequencing. It also contributes to understand the clinical heterogeneity in disease presentation and outcome, even within genetically homogeneous disease entities. Finally, immunophenotyping supports treatment decisions and patient monitoring such in case of immunoglobulin replacement therapy and in hematopoietic stem cell transplantation or gene therapy.

Besides “genetics-first” approach using whole exome sequencing (WES) or whole genome sequencing (WGS) has been postulated for PID diagnosis by some investigators , flow cytometry offer considerable advantages for the clinical routine: 1) WES or WGS approaches are too slow and too demanding for routine first-choice diagnostics, 2) different genetic defects might show a different clinical course as compared to those previously reported for the affected gene (e.g. hypomorphic defects), 3) even when the genetic alteration has bene fully defined, immunological studies are required to identify the consequences of the defect for the immune system.

Because of the central role of immunophenotyping, many PID centers have developed multi-color flow

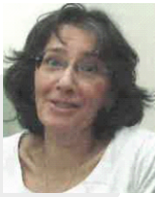
cytometric protocols and antibody panels for diagnosis and classification of PID. However, variability among these single center initiatives (sample processing, antibody panels, immunostaining procedures, instrument setup, sample measurement and data analysis) in combination with the the low incidence and the clinical-immunological heterogeneity of PID, hamper prospective validation in large patient series and age-matched healthy controls, and limit the exchange of data between PID centers. Accordingly, we aimed to develop a tool that would be universal for all lymphoid PIDs and offer high sensitivity to disclose a lymphoid defect. This approach takes advantage of the recently designed and validated EuroFlow procedures and tools resulting in standardized pre-analytical and analytical EuroFlow procedures, PID-specific ≥8-color antibody panels, and software tools for multidimensional data analysis. This strategy has been validated in >400 PID patients, and immunological alterations have been defined using a group of >200 healthy controls of different age-groups (from cord-bood to 80 years-old) as a reference. Together with this, new lymphoid subpopulations has been identified that might be of interest for diagnosis and monitoring particularly for primary antibody deficiencies (like dissection of memory B-cells and plasma cells compartments according to IgH subclasses). Together with strategy, an algorithm was designed for guiding immunophenotypic PID studies, based on available clinical and laboratory information. In summary, this strategy enables a sensitive screening, followed by in-depth analysis as cost-effective step-wise strategy that has been validated for a broad applicability for many different PID of the lymphoid system (not targeted on a few types of PID), based on the broad overview of all relevant subsets in a single analysis.

FLOW CYTOMETRY IN THE IDENTIFICATION OF INNATE CYTOTOXIC DYSFUNCTION

Yenan Bryceson, PhD
Karolinska Institutet, Stockholm, Sweden

Abstract: Cytotoxic lymphocytes, encompassing T cell and NK cell subsets, can kill target cells through directed release of specialized lysosomes, so called cytotoxic granules. They can identify and eradicate infected and malignant cells. Lymphocyte cytotoxicity also contributes to immune homeostasis, through killing of activated immune cells. Congenital defects in lymphocyte cytotoxicity are associated with a life-threatening hyperinflammatory syndrome, hemophagocytic lymphohistiocytosis (HLH), typically presenting in infancy or childhood. Individuals with more subtle impairments in lymphocyte cytotoxicity may also present with or related disorders later in life. In this talk I will provide a background to the mechanisms of lymphocyte cytotoxicity and discuss the clinical manifestations of impaired lymphocyte cytotoxicity. Contemporary diagnostic strategies for the identification of primary defects in lymphocyte cytotoxicity, largely based on flow cytometry, will be presented.

MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASE - STRATEGIES FOR DIAGNOSIS BY FLOW CYTOMETRY



Júlia Vasconcelos
Centro Hospitalar do Porto, Oporto,
Portugal

Abstract: Mendelian susceptibility to mycobacteria disease (MSMD) is a group of rather rare primary immunodeficiencies, etiologically and clinically heterogenous regarding severity. Patients are prone to develop disseminated infections by many environmental nontuberculosis mycobacteria, several

Mycobacterium bovis (BacilleCalmete-Guérin,BCG) vaccine substrains, *Mycobacterium tuberculosis* and other intramacrophagic pathogens, mostly *Salmonella*. Patients with MSMD may have mutations in 7 genes that encode for the following proteins involved in the IL-12 dependent, IFN-γ mediated immunity: IFN-γ Receptor 1, IFN-γ Receptor 2, IL-12 Receptor β1, IL-12p40, signal transducer and activator of transcription 1 (STAT1), IFN-γ regulation factor 8 (IRF8), for IL-12 production by myeloid dentritic cells CD1c+ and interferon-stimulated gene 15 (ISG15). Deficiency in other proteins that protect from Mycobacteria infections may also cause MSMD: gp91PHOX, involved in the oxidative burst; GATA2, implicated on early hematopoiesis; nuclear factor kB essential modulator (NEMO) causing transcription of several cytokines; TyK2, associated with hiperIgE. Some of these proteins can be evaluated by FCM (flow cytometry), allowing a rapid and skilful diagnosis that may guide the molecular diagnosis and also the imperative treatment. We show our experience on evaluation, in whole blood, of some proteins of the IL-12/IFN-γ axis: surface expression of IFNγR1 on monocytes, surface expression of IL-12Rβ1 on CD25+ blasts T cells, intracellular expression of IL-12p40 in monocytes and intracellular expression of IFN-γ in T cells. If a GATA2 deficiency is suspect, the characteristic severe monocytopenia and B and NK lymphopenia is easily recognized by FCM. An algorithm for diagnosis of MSMD, guided by the frequency of the defect and the clinical features, is showed, and the results of some cases reports are presented. About 20-50% of patients with MSMD still don't have an etiological cause, so new genes or gene products are yet to be discovered.

References:

1. Steven M. Holland and Jean-Laurent Casanova: Inherited Disorders of the Interleukin-12-Interleukin-23/Interferon-g Circuit- Primary Immunodeficiency Diseases- A Molecular and Genetic Approach, Chapter 35, 2014: 450-466,
2. Waleed Al-Hertz, Aziz Bousfiha, and al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Imnunodeficiency –Frontiers in Immunology, 2014
3. Isabel Caragol, Miguel Raspall et al: Clinical Tuberculosis in 2 of 3 siblings with Interleukin-12 Receptor b1. Clinical Infeccious Diseases, 2003: 302-306

SELECTED ORAL COMMUNICATIONS PRESENTATIONS

CO_2441 NATURAL KILLER CELLS IN ACUTE MYELOID LEUKAEMIA PATIENTS: PHENOTYPIC AND FUNCTIONAL ANALYSIS

CO_2442 IDENTIFICATION AND ENUMERATION OF DISTINCT SUBSETS OF MONOCYTE-MACROPHAGES IN DIFFERENT HUMAN TISSUES THROUGHOUT LIFE USING 9-COLOR FLOW CYTOMETRY

Monitoring Immunotherapy for Cancer
- Session with the collaboration of SPO & SEOM
Chairs: Gabriela Sousa, Luis De la Cruz, Paulo Rodrigues-Santos

IINTRODUCTION TO CANCER IMMUNOTHERAPY

Bruno Silva-Santos, PhD
Instituto de Medicina Molecular, Faculdade de Medicina,
Universidade de Lisboa, Lisbon, Portugal

Abstract: Immunotherapy is changing the paradigm of cancer treatment. It has recently become an accomplished and groundbreaking strategy to deal with resistant advanced tumors. Much of the success comes from the clinical application of checkpoint inhibitors, antibodies that block the molecular breaks, the surface receptors CTLA-4 and PD-1, on T cell activation. Unleashed T cells are highly efficient at eliminating malignant cells and providing durable responses that prevent disease recurrence. The immunomodulatory antibodies approved for clinical use, anti-CTLA-4, anti-PD-1 and anti-PD-L1, have obtained unanticipated results in metastatic melanoma, lung, bladder and kidney carcinomas, and are delivering promising results in various other solid tumor types. At the same time, autologous cellular therapies, particularly those where modified T lymphocytes are infused back into patients, are producing the goods in hematological B-cell malignancies, with response rates above 90% in B-cell acute lymphoblastic leukemia. These were obtained with total T cells transduced with chimeric antigen receptors (CARs) that target the B-cell marker, CD19, which are being approval by regulatory authorities this year.
Our own studies with hematological tumors have provided seminal data on the potential of using a specific T cell subset,γδ T lymphocytes, to target aggressive cancers. We characterized the differentiation of a novel subset of Vδ1+ PBLs expressing natural cytotoxicity receptors (NCRs) that directly mediate killing of leukemia cell lines and chronic lymphocytic leukemia patient neoplastic cells. The stable expression of NCRs is associated with high levels of Granzyme B and enhanced cytotoxicity against lymphoid leukemia cells. Thus, NCR+ Vδ1+ T-cells, which we termed Delta One T (DOT) cells, constitute a novel, inducible and specialized killer lymphocyte population with high potential for immunotherapy of human cancer.

IMMUNODYNAMICS ASSAYS FOR CANCER IMMUNOTHERAPY

Paulo Rodrigues-Santos, PhD
FMUC - Immunology Institute, Faculty of Medicine, University of Coimbra
CNC - Immunology and Oncology Laboratory, Center for Neurosciences and Cell Biology, University of Coimbra, Coimbra
CIMAGO - Center of Investigation in Environment, Genetics and Oncobiology, Faculty of Medicine University of Coimbra, Coimbra, Portugal.

Abstract: Blockade of immune checkpoints is transforming human cancer therapeutics. Soluble and membrane-bound receptor–ligand immune checkpoints are the most druggable forms using agonist or antagonist antibodies. Although their implication in immune response during cancer therapy is theoretical obvious, it has been difficult to establish the best biomarkers for monitoring these new therapies. This lack of information extends to almost all immunologically targeted agents, raising new

challenges for developing immune endpoints that correlate systemic and intratumoral immune effects with systemic dose, toxicity, and efficacy for cytotoxic therapies. Refining immune endpoints to better inform clinical trial design represents a high priority challenge.
Immunodynamics was defined as a way to evaluate the impact of a drug or a therapy on the immune system. Assessment of the presence, functionality, and phenotype of infused and persisting adoptive cells, together with the impact of the infused cells on patient immunobiology and tumor microenvironment is one of the goals of immunodynamics assays. Complementary approaches including molecular, cellular and tissular assays are being used to accomplish this task. The quest for biomarker discovery results from the combination of pre-clinical studies using animal models and human in vitro results from patients included in clinical trials.
In our lab we use a multi-parametric flow cytometry strategy for the analysis of the expression of several immune checkpoint inhibitors by different T, B, NK, monocyte and dendritic cell subsets. We recently added the analysis of xMAP (Luminex®) in a 59-plex panel of soluble molecules (14 immune checkpoints and 45 cytokines, chemokines and growth factors). Gene expression profiles of cell sorted populations and miRNA-mediated immune regulation is also under investigation. Functional studies complete our portfolio of laboratory investigations aiming at the immune monitoring in cancer therapy.

MONITORING NK CELL-BASED IMMUNOTHERAPY IN THE ELDERLY

Rafael Solana, MD, PhD
University of Extremadura and IMIBIC - Reina Sofia University Hospital - University of Cordoba, Spain.

Abstract: Natural killer (NK) cells are innate lymphocytes with a central role in the defence against tumour and virus-infected cells. NK cell activity is finely tuned by the balance between the signals from activating and inhibitory receptors. Activating receptors, like NCRs (NKp30, NKp46, NKp44) and NKG2D, sense the altered expression of their ligands on the cell surface of cancer cells. On the contrary Killer-cell immunoglobulin-like receptor (KIR) and CD94/ NKG2A are inhibitory receptors that recognize HLA class I molecules whose expression is often altered on tumour cells. Different therapies have been designed to enhance the antitumor response of NK cells including stimulation with cytokines adoptive therapy with autologous or allogeneic expanded NK cells. Checkpoint blockade of inhibitory receptors and the use of agonist antibodies to stimulate activating receptors are emerging areas of research. Altered expression of NK cells activating receptors has also been described in cancer patients, probably as a consequence of chronic exposure to ligands on tumour cells. The effect of NK cell-based cancer immunotherapy aiming to reverse the tumour-induced NK cell dysfunction observed in cancer patients should be evaluated by monitoring their phenotype and function. Cancer is primarily considered a disease of old age, and it is well known that ageing is associated to alterations in NK cells including a diminished expression of several activating receptors. Thus age and cancer may synergistically contribute to alterations in NK cell-mediated tumour immunosurveillance supporting that special caution should be taken into account in the monitoring of NK cell reconstitution after immunotherapy when elderly patient are considered.

SELECTED ORAL COMMUNICATIONS PRESENTATIONS

CO_2427 DNA FLOW CYTOMETRY AS A CLINICALLY USEFUL PROGNOSTIC TOOL IN FEMALE BREAST CANCER. A LONG-STANDING 25-YEAR EXPERIENCE
CO_2455 EVALUATION OF T CELL-MEDIATED ANTITUMOR IMMUNE RESPONSE IN HEPATOCELLULAR AND CHOLANGIOCARCINOMA AT TUMOR AND PERIPHERAL LEVEL

17H00 - 17H30 COFFEE BREAK and Poster Viewing

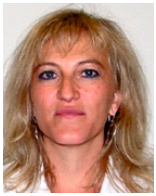
17H30 - 18H00 COMMERCIAL SESSION by Beckman Coulter | Room Sophia de Mello Breyner

ClearLLabLS: Is it the definitive solution for the lymphoid neoplasm screening?
Speaker Dr. Jose María Raya Sánchez
Head of Section of the Hematology Laboratory, Hospital Universitario de Canarias-Universidad de La Laguna

18H30 - 19H30 – GECLID and SIC Groups Presentations | Room Sophia de Mello Breyner

20H30 - 24H00 – CONGRESS DINNER at Navio Opera

MINIMAL RESIDUAL DISEASE DETECTION IN HEMATOLOGICAL MALIGNANCIES: RECENT ADVANCES AND FUTURE PERSPECTIVES



Julia Almeida, MD, PhD
Departamento de Medicina and Servicio General de Citometria (NUCLEUS), Centro de Investigacion del Cancer (Instituto de Biologia Molecular y Celular del Cancer and IBSAL; CSIC-USAL), Universidad de Salamanca, Salamanca, Spain

Authors: ALMEIDA J[1], FLORES-MONTERO J[1], SANOJA-FLORES L[1], LECREVISSE Q[1], THEUNISSEN PMJ[2],MEJSTRIKOVA E[3], VAN DER VELDEN VHJ[2], VAN DONGEN JJ[4], ORFAO A[1], on behalf of EuroFlow Consortium.

Institutions: [1]Departamento de Medicina and Servicio General de Citometria (NUCLEUS), Centro de Investigacion del Cancer (Instituto de Biologia Molecular y Celular del Cancer; CSIC-USAL), Universidad de Salamanca, Salamanca, Spain, and IBSAL; [2]Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherland; [3]Childhood Leukaemia Investigation Prague, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University, University Hospital Motol, Prague, Czech Republic; [4]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands

Abstract: Monitoring of minimal residual disease (MRD) in patients suffering from haematological malignancies is now routinely used in clinical settings. Some decades ago, it became an attractive approach for MRD investigation, because of the demonstration of the expression of aberrant phenotypes in leukemic cells in >85-90% of leukemia/lymphoma cases and the higher sensitivity (<10-4) vs. conventional methods, for the detection of low numbers of aberrant cells among a great majority of normal/regenerative hematopoietic cells. Currently, MRD monitoring either by flow or molecular techniques has proven to be one of the most relevant prognostic factors in these neoplasms, particularly in acute leukemias, chronic-B-cell lymphoproliferative disorders and multiple myeloma. To obtain MRD data with good sensitivity, acquisition of large numbers of cells is a prerequisite; however, the sensitivity of conventional flow cytometry is usually not high enough to detect aberrant cells at levels <10-5, required for some applications, which is at disadvantage vs. PCR. Another disadvantage of classical flow MRD is that the applied immunostaining protocols, antibody panels, and gating strategies differ significantly between centers and between treatment protocols, and are in fact highly subjective expert procedures. Consequently, results of flow-based MRD methods usually have much less interlaboratory comparability than PCR-based methods.
To try to solve the limitations of classical flow cytometry in detecting MRD, the EuroFlow Consortium aimed to design standardized 8-color immunophenotyping protocols for specific and reliable MRD measurement in different hematological malignancies, to identify aberrant cells at a sensitivity levels of tiv–p (i.e. at least comparable to PCR), through two steps: (1) application of novel software tools and principal component-based and canonical analyses, to select the most informative markers in distinguishing tumor cells from their normal counterpart; and (2) to increase the sensitivity of the approach, through the development of a protocol to stain and acquire large numbers of cells (>4 million), which allows theoretical sensitivities of ≤10–p. pplication of novel software tools and principal component-based and canonical analyses, to select the most informative markers in distinguishing tkemia (BCP-ALL) and multiple myeloma (MM). For both diseases, after several cycles of design-test-evaluate-redesign, the antibody tubes were tested on pathological samples and normal/regenerating bone marrow samples, followed by the assessment of the contribution of each antibody, until satisfactory results were obtained. Although the specific optimized combination(s) of markers used is a key factor for optimal identification of aberrant cells and discrimination from their normal

counterparts, another critical factor is the number of cells analyzed. Therefore, a new protocol was developed (The Euroflow bulk lysis protocol), allowing a 10-fold increase in the number of cells evaluated, in the context of fully standardized laboratory protocols. The novel protocol ("New Generation Flow", NGF) is faster, standardized and reproducible, vs. classical cytometry, and even in MM it does not require a diagnostic sample or patient-specific probes. Then, the EuroFlow-NGF approach developed (with a sensitivity close to 10–N) was validated in a multicenter manner, and it showed similar (MM) or higher (BCP-ALL) applicability vs. conventional flow-MRD, together with a significantly increased sensitivity for MRD detection.

10H00 - 11h00 PLENARY SESSION III | Sophia de Mello Breyner Room
Accreditation of Flow Cytometry Laboratories
Chairs: Catarina Martins & Juana Ciudad

ISO 15189 AND ISO/IEC 17025, STANDARDS FOR (CLINICAL) LABORATORIES

Catarina Martins, PhD
NOVA Medical School | Faculdade de Ciências Médicas,
Universidade NOVA de Lisboa, Lisbon, Portugal
Quality Auditor, IPAC – Portuguese Institute for Accreditation, Caparica, Portugal

Abstract: All over the world, accreditation by the standard ISO/IEC 17025 - General requirements for the competence of testing and calibration laboratories has been a reality for a long time in several fields of laboratory science, being even obligatory in several ones. The major goal of such a standard is to achieve harmonization of good laboratory practices. Biomedical diagnostic laboratories have resisted the implementation of ISO/IEC 17025, eventually due to the aseptic language of the standard, which must respond to a diversity of applications. Though ISO/IEC 17025 can also be applied to clinical fields, different countries developed individual codes and guidelines aiming to respond to the needs of medical laboratories, in a way better than ISO/IEC 17025.
More recently, ISO (International Organization for Standardization) developed ISO 15189 - Medical laboratories - Particular requirements for quality and competence. A panel of international experts was called upon, in order to answer the requests for a more clinically driven standard, adapting the regular accreditation nomenclature and processes to the medical laboratories. Recognizing the importance of such a document, countries like Australia, Latvia, or more recently France, The Netherlands, and The United Kingdom are bringing this standard mandatory to all medical laboratories, which now have to adapt and respond adequately to its requirements. Although the expansion of the standard has been rather slow, efforts are being made, concretely in Europe, to reinforce its application, for instance in countries such as Portugal and Spain.
With disadvantages, but mainly several advantages, ISO 15189 is undoubtedly the most common global reference for quality in medical laboratories, including clinical flow cytometry facilities. Auditors and accreditation bodies believe it is crucial to increase the knowledge on this standard amongst medical laboratories personnel, and clinical staff. Not only to achieve a solid implementation of ISO 15189, which can respond globally to the harmonization needs in the medical laboratory, but also in order to continue improving the standard and its applicability to the daily routine.

UNDERGOING ACCREDITATION IN FLOW CYTOMETRY LABS: STEPS TO FOLLOW AND CRITICAL POINTS

Ulrich Sack, MD, PhD
Institute of Clinical Immunology, Medical Faculty
at the University of Leipzig, Leipzig, Germany

Abstract: Flow cytometric methods are well established in patients' diagnostics and preparation of cellular therapeutics. These topics are subject to regulatory systems that have been as far as possible harmonized within the European community. By growing implementation of ISO 15189 based standardization in European laboratory diagnostics, flow cytometry laboratories are more and more challenged to introduce compliant quality management systems. Although in most countries accreditation of such laboratories is not yet compulsory, proof of following these rules is widely

requested. In general, adherence to quality management systems is considered to be very hard for cytometry. Therefore, we analyzed consequences of accreditation process for cytometry laboratories and investigated flow cytometrists'attitudes and misgivings according these requirements. As major challenges, staff qualification, adaptation of multicolor antibody panels, and quality assessment has been identified.

References
ISO 15189:2012. Medical Laboratories — particular requirements for quality and competence, ISO, Geneva.
Sack U, Barnett D, Demirel GY, Fossat C, Fricke S, Kafassi N, et al. Accreditation of flow cytometry in Europe. Cytometry B Clin Cytom 2013 May;84(3):135-42.
Dorn-Beineke A, Sack U: Quality control and validation in flow cytometry. Lab. Med. 2016;40(2):65-79.

FLEXIBLE SCOPE IN FLOW CYTOMETRY: EXPERIENCE OF A LABORATORY ACCREDITED BY UNE-EN-ISO 15189

Juana Gil - Herrera, MD, PhD
Hospital General Universitario Gregorio Maragor,
Madrid, Spain

Abstract: The Cellular Immunology Laboratory at the Hospital General Universitario "Gregorio Marañon" in Madrid achieved the Spanish national accreditation agency (Entidad Nacional de Acreditación, ENAC) first accreditation for flow cytometry in Immunology according to ISO 15189 standard in 2008. We got a fixed accreditation scope for lymphocyte counting including T (CD3+) cells along with T helper (CD3+CD4+) and T cytotoxic/suppressor (CD3+CD8+) subsets, B (CD19+) and NK (CD3-CD16/CD56+) cells. From that time on, we have developed several improvements and modifications that allowed us to progress and switch into a flexible scope during the last renewal audit in October 2016. Our current scope will let us include the quantitation of additional lymphocyte memory subsets, which are very useful in the clinical setting of immunodeficiency.

11H00 - 11H30 COFFEE BREAK

Immunology (SPI)
Chairs: Margarida Saraiva & Manuel Vilanova

ANALYSING THE IMPACT OF T CELL SUBSETS ON BRAIN COGNITIVE FUNCTION

Julie C. Ribot, PhD
Instituto de Medicina Molecular, Faculdade de Medicina,
Universidade de Lisboa, Lisbon, Portugal

Abstract: γδ T cells are known to populate multiple tissues, such as the skin, gut or lung, where they make major contributions to local physiology. Here we investigated whether γδ T cells could play a role in brain cognitive function, given that ab T cells were recently shown to be involved in learning behavior of mice: whereas IFN-γ producing subsets were detrimental, their IL-4-producing counterparts played a pro-cognitive role. We found that γδ T cells infiltrate the meningeal spaces from the brain of naive C57/BL6 mice already at birth and persisted throughout life. Strikingly, at 1 week of age, meningeal γδ T cells differentiated into IL-17 (but not IFN-γ) producers, which seemingly depended on IL-1β/IL-23 and retinoic acid. In fact, γδ T cells were the major source of IL-17, whereas ab T cells mostly provided IFN-γ in situ. To test whether IL-17-producing γδ T cells influenced the cognitive performance of mice, we scored the behavior of WT, TCRδ-/- and IL-17-/- mice in classical neuroscience paradigms assessing learning capacities. We observed that, contrary to WT controls, mice deficient for γδ T cells or IL-17 displayed impaired short-term/working memory in the Y maze paradigm, but a normal long-term spatial memory in the Morris water maze. We are currently identifying the underlying molecular mediators; interestingly, we found reduced levels of Brain Derived Neurotropic Factor (BDNF), a major player in memory and dementia, in the hippocampus of TCRδ-/- and IL-17-/- mice compared to WT controls. Altogether, our data suggest that γδ T cells regulate brain cognitive functions through a novel IL-17-dependent mechanism.

CHOREOGRAPHING IMMUNITY AND TOLERANCE INDUCTION IN THE THYMUS

Nuno Alves, PhD
Thymus Development and Function Laboratory
Instituto de Investigação e Inovação em Saúde (I3S)
Instituto de Biologia Molecular e Celular (IBMC), Oporto, Portugal

Abstract: Our laboratory is focused on studying the development and role of the thymus, the organ responsible for the generation of T cells that are simultaneously responsive against pathogens and self-tolerant. Thymic activity is not regular throughout life and deficits in T-cell production arise in several pathophysiological states, including with age, infection and chemotherapy. Besides, the failure in the deletion of autoreactive T cells in the thymus can lead to autoimmunity. Hence, the regular function of the thymus must be tightly controlled in vivo. Within the thymus, thymic epithelial cells (TECs) provide key inductive microenvironments for the development of T cells that arise from hematopoietic precursors. As a result, genetic defects that impair normal TEC differentiation are linked to immunodeficiency and autoimmunity, which makes the study of TECs of fundamental, and clinical, importance to understand the normal development and response of the immune system. TECs are divided into two specialized functionally distinct cortical (cTECs) and medullary (mTECs) subtypes, which derive from common bipotent TEC progenitors. Still, we know very little about the genetic basis that controls cTEC/mTEC lineage divergence from TEC progenitors. Interleukin-7 (IL-7) is a chief cytokine for T-cell development and a particular subtype of TECs produces it. We previously provided the first temporal-spatial analysis of thymic IL-7 niche in vivo. Over the last years, we have studied the

development of IL-7-expressing TECs relatively to the establishment of mature thymic epithelial microenvironments. We take a global approach to investigate TEC differentiation, which integrates the study of molecular processes taking place at the single-cell level to the analysis of in vivo mouse models.Ultimately, as T-cell immune reconstitution relies on the bi-directional interplay between T-cell precursors and TECs, understanding the development and function of TECs is crucial to comprehend how the immune system achieves the equilibrium between immunity and tolerance.

METABOLIC CUES IMPLICATED ON MONOCYTE BIOLOGY

Ricardo Silvestre, PhD, Braga, Portugal
Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal
ICVS/3Bs-PT Government Associate Laboratory, Guimarães, Braga, Portugal.

Abstract: Emerging evidence indicates that innate immune cells tightly coordinate their metabolic programs with the carbon and bioenergetic needs to support the immunological functions in response to pathogens, tumors or inflammatory diseases. This metabolic adaptation requires energy and precise control of cellular metabolic pathways consuming glucose, fatty acids or amino acids. Perturbed metabolic fluxes thus imply decisive effects on immune cells activation eventuating in their ability to control a certain pathological condition. The extent to which metabolic changes coordinate immune cell function or occur consequently remains to be established. Since development to adult life, monocytes home from the blood stream to tissues where they become resident macrophages. These cells display different phenotypes that may be due to the microenvironment found on the tissue during differentiation, or may already be imprinted during their passage on the bloodstream. In any case, the blood and tissue metabolic and inflammatory microenvironment occurring during chronic inflammations will impact decisively the biology of monocytes conditioning their phenotype. We are currently pursuing how the modification of systemic and cellular metabolism arising during inflammatory conditions impact the immune functions and by consequence the dynamic of immune response. Therefore, innovative therapeutic approaches or metabolic adjuncts are envisaged as a new mean to reorient host metabolic alterations towards immune protection.

CELL COMPETITION IN THE THYMUS

Vera Martins, PhD
Instituto Gulbenkian de Ciência, Lisbon, Portugal

Abstract: Most T lymphocyte development occurs in the thymus from progenitors of bone marrow origin in a process characterized by high cellular turnover. We found that thymus turnover is regulated by cell competition. During T lymphocyte development, cell competition occurs at a specific stage of differentiation. At that stage, precursors differ slightly in their time of thymus residency. Specifically, 'young' hematopoietic precursors (that recently seeded the thymus) coexist with 'old' precursors (residing for longer in the thymus). These cells share the same surface markers that identifies them as sharing the same differentiation stage, but young precursors induce the clearance of the old, thereby promoting thymus turnover. This is a particularly dynamic process, as winner cells progressively differentiate into more mature stages. Impairment or disruption of cell competition is permissive to self-renewal and persistence of the old precursors. These maintain thymus function autonomously for some weeks with de novo production and export of T lymphocytes. However, prolongation of this property invariably leads to development of T cell acute lymphoblastic leukemia (T-ALL) with strong similarities to the human disease.

13h00-13h15 CLOSING CEREMONY | Sophia de Mello Breyner Room

CO2427 DNA Flow Cytometry as a clinically useful prognostic tool in female breast cancer. A long-standing 25-year experience

António E. Pinto Teresa Pereira, Giovani L. Silva, Saudade André
Serviço de Anatomia Patológica do Instituto Português de Oncologia de Lisboa Francisco Gentil, E.P.E., and Centro de Estatística e Aplicações e Departamento de Matemática do Instituto Superior Técnico, Universidade de Lisboa

Introduction: This work reviews our 25-year experience using DNA flow cytometry parameters, ploidy and S-phase fraction (SPF), as prognostic/predictive biomarkers in female breast cancer.

Methods: Since 1990, DNA flow cytometry has been routinely used in our institution to analyse, in a prospective manner, surgical specimens and fine-needle cytological aspirates of breast cancer patients. The series herein reviewed (eight studies published between 1997 and 2015) encompassed > 1100 patients, who were diagnosed and treated according to standardized therapeutic protocols. Pathological staging (pTNM) and tumour differentiation were evaluated according to WHO classification and Nottingham grading criteria, respectively. Hormone receptors, Ki-67, and ERBB2 protein expression were determined by immunohistochemistry. ERBB2 gene amplification was evaluated by SISH. Fresh/frozen samples were analysed by DNA flow cytometry, following methodological and quality control recommendations widely accepted. Patients' end point outcomes were the disease-free (DFS) and overall survival (OS), and the prognostic impact of variables was statistically assessed using multivariate Cox hazard regression models.

Results: a) Aneuploidy and high SPF are significantly associated with histopathological and molecular features related to unfavourable prognosis (high tumour grade, lack of hormone receptors, ERBB2 overexpression), b) DNA ploidy and SPF (for DFS, in a short-term study) are independent prognostic factors, c) SPF appears to be a better prognostic marker of cell proliferation than Ki-67 expression, d) DNA aneuploidy is a significant indicator of worse prognosis in node-negative patients (for DFS) and, like high SPF, in node-positive disease, as well as in advanced breast cancer, and e) patients with multiploid/hypertetraploid tumours have the worst prognosis. Of note, the novel/interesting finding that DNA aneuploidy identifies subsets of early breast cancer patients with well differentiated Grade 1 and moderately differentiated Grade 2 tumours that exhibited poor clinical outcome, as these patients might benefit from adjuvant chemotherapy, particularly in Luminal A and Luminal B/ERBB2 negative endocrine-responsive disease.

Conclusion: Overall, our data indicate that DNA flow cytometry provides significant prognostic/predictive information that is biologically relevant and clinically useful in breast cancer management.

CO2428 Blastic plasmacytoid dendritic cell neoplasm. A case with different inmunophenotype and chromosomal aberrations

González-Martínez G, Campillo JA, Blasco A, Alfaro R, Galian JA, Moya-Quiles MR, Mrowiec A, Gimeno L, García-Alonso A, Alamo JM, Muro M, Minguela A.
Immunology Service. Clinical University Hospital Virgen Arrixaca. Instituto Murciano de investigación biosanitaria (IMIB). Murcia, Spain.

Introduccion: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare and aggressive myeloid neoplasm generally occurring with skin lesions and leukemic diffusion, with or without involvement of the bone marrow. The aim of this work is describe new phenotypic and genetic aberrations in a BPDCN case from a 75 year old man with several lymphadenopathies, initially diagnosed as lymphoma in the histology.

Methods: Bone marrow and peripheral blood immunophenotypic analysis was performed in a 8-color FACSCanto-II flow cytometer (Becton Dickinson) and analyzed in Diva Software (BD) by using lymphoma and acute myeloid leukemia antibody panels. Fluorescence in situ hybridization (FISH) was

used to analyze deletions of P53, 5q31m 7q31, 7q22 and 20q, as well as translocations of RARA, AML1, MLL and inversion of Cr-16. Other chromosome translocations were discarded by Hemavision Screen kit (DNA diagnostic). CGH-Array was performed to extend the genetic characterization (CIALAB).

Results: Peripheral blood immunophenotype study detected 70.0% of medium size (FSC) and low granulation (SSC) blastic immature cells with the following phenotype CD45low CD34- CD1a- CD117lowDRhi CD4+ CD7low CD10hi CD11b- CD11c- CD13- CD14- CD15- CD16- CD33low CD36+ CD43+ CD56hi CD64- CD66- CD83- CD123hi ILT3+ MPO- Tdt-, NG2hi, without T or B lymphoid, platelet or red line markers, compatible with the diagnosis of BPDCN.Complete absence of leukemic cells was evidenced in the bone marrow. CD10 and NG2 are not common markers in this pathology.As NG-2 is associated with MLL translocation, PCR screening was performed to rule out the possibility of acute monoblastic leukemia with aberrant markers.Duplication of RUNX1T1 (ETO) and deletion of 20q were observed by FISH. CGH-Array detected several deletions common in this pathology (9p, 12p, 13q), but 10p deletion and 20qwas additionally found.

Conclusion: The interest of this case is the importance of considering the diagnosis of BPDCN not only in patients with cutaneous involvement (though it is the most frequent compromised tissue for this disease), but also in cases with lymphadenopathy with or without bone marrow involvement. CD4 and CD56 markers were subsequently confirmed in the lymphoid tissue supporting the initial immunophenotype diagnosis of BPDCN. Our case is one more example to improveour knowledge of this rare neoplasia. We describe new phenotypic (very high expression of CD10 and NG2) and genetic aberrations (10p and 20q deletions) not previously reported that could have been involved on its atypical presentation.

CO2433 The proliferation index of bone marrow cells may assist the differential diagnosis of chronic myelomonocytic leukemia vs. monoblastic/monocytic leukemias

Pilar Leoz*, Alejandro Berkovits, Valentina Guerri, Carlos Fernández, Susana Barrena, Antonio López, Juana Ciudad, Miriam Fierro, Rosana Rivas, Miryam Santos, Alberto Orfao*, Sergio Matarraz*.

Author Affiliations: Servicio de Citometría (NUCLEUS) and Department of Medicine, Centro de Investigación del Cáncer (IBMCC, University of Salamanca-CSIC) and IBSAL, Salamanca, Spain.

Introduction: Overlapping features often hamper the differential diagnosis of chronic myelomonocytic leukemia (CMML) vs. acute monoblastic/monocytic leukemias (AMML) in clinical routine. Herein the proliferation index (PI) of different bone morrow (BM) cell subsets was investigated by multiparameter flow cytometry as a potentially useful tool for discrimination of both disease entities.

Methods: Among 151 BM samples corresponding to healthy subjects (NBM; n=74), CMML (n=33; CMML-1, n=24 and CMML-2, n=9) and AMML (n=44; monoblastic leukemia, n=33 and monocytic leukemia, n=11), the PI (percentage of S+G2/M cells) was studied within the more immature BM cell component (i.e. CD34+ cells and/or leukemic cells) and residual monocytic, neutrophil and erythroid lineage cells by flow cytometry, including the DRAQ5 stain.

Results: As expected, the highest proliferation level was depicted by NBM erythroid cells, followed by CD34+ hematopoietic precursors, neutrophil and monocytic cells (PI of 28%, 15%, 5% and 4%, respectively).
The analysis of proliferation of the more immature BM cell component from all the monocytic leukemias investigated revealed that CD34+ cells from CMML patients had normal proliferation levels (PI of 15%), while significantly decreasing in AMML (CD34+) leukemic cells vs. NBM (7% vs. 15%; $p<0.001$). When comparing CMML vs. AMML patients, the proliferation of CD34+ cells from CMML-1 was significantly higher than (CD34+) leukemic cells from monoblastic and monocytic leukemias (PI of 16% vs. 8% and 4%; $p<0.001$), while no differences were observed among CD34+ cells from CMML-2 vs. leukemic cells from both groups of AMML patients (PI of 3%). As noted above, a tendency to a lower PI of the immature (CD34+ cells) compartment is observed from monoblastic to monocytic leukemia (8% vs. 4%, respectively; $p>0.05$).
Conversely, AMML monocytic-lineage leukemic cells depicted a significantly increased proliferation vs. monocytic-lineage cells from both NBM and CMML (PI of 7% vs. 4% and 4%, respectively; $p\leq0.01$). Such

overall increased PI was mostly related to the enhanced proliferation of monoblastic leukemia cells, which was around twice the PI of monocytic cells from all other patients (LMMC1, LMMC2 and monocytic leukemia; 8% vs. 3%, 4% and 4%, respectively; p=0.002). However, no differences were found in the PI of the neutrophil-lineage among the groups here investigated.
Finally, the proliferation of the erythroid-lineage cells was found significantly decreased in AMML, as compared to NBM (23% vs. 28%: p=0.009). Interestingly, this was at the expense of erythroid cells from monocytic leukemia, which significantly decreased vs. all the other groups of patients (monoblastic leukemia, CMML-1 and CMML2; 14% vs. 26%, 30% and 24%, respectively; p≤ 0.003).

Conclusion: The detection of decreased levels of proliferation, particularly among erythroid cells might help the differential diagnosis of CMML-2 vs. monocytic leukemia, whereas an enhanced proliferation of leukemic cells is particularly recurrent in monoblastic leukemia vs. all the other monocytic leukemias.

CO2452 Flow cytometry: measurement of peripheral blood expression/lymphocytes of cutaneous T lymphomas

T. Castaño; R. Mata; A. Yeguas; R. Córdoba; P. Llamas; C. Serrano
Department of Hematology; Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain.

Introduction: Cutaneous lymphomas are a heterogeneous group of entities, mostly derive from T lymphocytes, within extranodal involvement are largely confined to the skin at the time of diagnosis. Sezary Syndrome (SS) is the peripheral expresion of clonal T cells from mycosis fungoides, and are generally CD3+ CD4+ with loss of the pan-T antigen.
The Aim of the study is to analyze peripheral expression from mycosis fungoides diagnosed in our center

Methods: We retrospectively analyzed peripheral blood subpopulations by Flow Cytometry from 57 patients in the last eight years (2008-2016). 72% were middle-aged men (50-60 years old) and the skin was the only organ affected in 95% of the patients analyzed.

Results: 50/59 Patients were an I-A stage and had a normal total white blood cell count, with lymphopenia <1000 cells/microl only in 31%. If we analyzed lymphocyte subpopulations, 19% presented CD3+CD4+ <500 cells / microl and 19% presented <100 NK cells/microl and the remaining cell lines analyzed (B lymphocytes, monocytes, neutrophils and eosinophils) were not altered.
Only 3/59 were diagnosed of SS with a mean total leukocyte count at diagnosis of 12,000 cells/microl and with lymphopenia of B, NK and polyclonal T lymphocytes. They were all CD4+, CD45Ro and CD5+ with loss of intensity of CD3+/TCRab in two patientes and bimodal distribution of CD7 in the three cases. All patiens were CD26 negative and CD2dim. Other antigens tested were negative (CD10, CD30, CD56, CD8, TCRgd, CD1a, CD34).

Conclusion: Cutaneous T-cell lymphomas are increasingly common in our country, however, taking into account the results of our study and the low incidence of expression in peripheral blood (<5% of cases) with exclusive cutaneous involvement in most of them perhaps we could limit the use of flow cytometry as a diagnostic and follow-up technique to assess response to the treatments used, to a limited group of patients.
As findings to take into account:
- Regardless of the stage of the disease, a significant proportion of patients will present lymphopenia at diagnosis.
- Eosinophilia has not been objectified in any of the cases studied, a finding that is often the reason for consultation in many cases to initiate the search for a possible cutaneous T-cell lymphoma.
- Other pan-T antigens others than CD7 can be change although in the literature CD7+ is more frequent.

CO2453 Peripheral blood Vδ1 T cells in B-CLL exhibit a cytotoxic profile, like the other T cell subpopulations

Catarina Simões[1,2]; Isabel Silva[1]; Sandra Silva[1]; Joana Lima[3]; Gilberto Marques[3]; Adriana Roque[4]; André Ribeiro[4]; Letícia Ribeiro[4];Artur Paiva[1]

[1]Unidade de Gestão Operacional de Citometria, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

[2]Universidade de Aveiro, Aveiro, Portugal
[3]Serviço de Patologia Clínica, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[4]Serviço de Hematologia Clínica, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Introduction: Vδ1 T cells, a minor subpopulation of T cells (1 to 10% of circulating T lymphocytes), are mainly found in peripheral tissues and are responsible for rapid innate-like immune responses against infectious agents. However, they have also been reported to have an antitumor capacity mediated by the ability of these cells to recognize stress molecules expressed by tumour cells and trigger cytotoxic responses.
Considering this, this study aims to quantify and phenotypically characterize peripheral blood Vδ1 T cells, as well as CD4+, CD8+, CD4+CD8+, and γδ (Vδ1 negative) T cells in B-cell chronic lymphocytic leukaemia (B-CLL), low-count monoclonal B-cell lymphocytosis (LC-MBL), high count (HC) MBL, and healthy individuals (control group), inferring the contribution of these cells to the overall immune response against these entities.

Methods: Were enrolled in this study 27 B-CLL (12 females, 15 males, mean age 72±10; 3 patients with del13q14 and 1 patient with del17p), 8 HC-MBL (1 female, 7 males, mean age 71±14, 1 patient with del17p), 7 LC-MBL (4 females, 3 males, mean age 70±11), and 6 controls (2 females, 4 males, mean age 73±7). The phenotypic characterization of the different T cell subpopulations was performed by flow cytometry using the following combination of monoclonal antibodies: CD27/ CD3/ CD4/ CD8/ CD45/ Vδ1/ CD69/ granzyme B. The samples were processed in BD FACS Canto II and analysed by Infinicyt® analysis software. The results were statistically evaluated with IBM SPSS Statistics 23.

Results: We observed an increasing percentage of granzyme B-producing Vδ1 T cells from control (16,5%) to LC-MBL (27,4%), HC-MBL (31,7%), and B-CLL (36,3%), although not reaching statistical significance. The same profile was observed for γδ (Vδ1-) T cells: controls (7,2%); LC-MBL (22,4%), HC-MBL (20,3%), and B-CLL (29,2%). These findings were also observed for all the other T cell subsets under study ($p<0.05$ for CD4+ T, CD8+ T, and CD4+CD8+ T cells, comparing B-CLL with controls). This increasing cytotoxic activity was accompanied by a decreasing percentage of CD27+ T cells in all T cell populations under study. No differences were observed for the percentage of Vδ1 T cells within peripheral blood T cells.

Conclusions: Vδ1 T cells like other T cell subsets from B-CLL and HC-MBL patients display higher frequencies of cells with cytotoxic potential.

CO2455 Evaluation of T cell-mediated antitumor immune response in hepatocellular and cholangiocarcinoma at tumor and peripheral level

Carmen Martín-Sierra[1]; Ricardo Martins[2,3]; Paula Laranjeira[1], Ana Margarida Abrantes[4,5]; J. Guilherme Tralhão[2,3]; Maria Filomena Botelho[4,5]; Dulce Cortes[3]; Emanuel Furtado[3]; Francisco Castro Sousa[2]; Artur Paiva[1]
[1]Unidade de Gestão Operacional em Citometria, Centro Hospitalar e Universitário de Coimbra, Portugal.
[2]Department of Surgery, Surgery A, Faculty of Medicine, Centro Hospitalar e Universitário de Coimbra, Portugal.
[3]Unidade de Transplantaçao Pediátrica e de Adultos, Centro Hospitalar e Universitário de Coimbra, Portugal.
[4]Departamento de Biofísica, IBILI, Faculdade de Medicina, Universidade de Coimbra, Portugal.
[5]Centro de Investigação em Meio Ambiente, Genética e Oncobiologia (CIMAGO), Faculdade de Medicina, Universidade de Coimbra, Portugal.

Introduction: Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are primary malignancies of the liver. In order to reveal the alterations of immune response during carcinogenesis, the interactions between cancer cells and immune cells have been broadly studied. Moreover, the identification of highly diverse tumor-infiltrating leukocyte (TIL) subsets, and their distinct functions in the tumor niche, has been an important development in onco-immunology. In fact, for HCC, Th17 cell infiltration has been correlated with poor prognosis.
The aim of this study is to identify potential correlations between tumor type and stage and T cell-mediated antitumor immune responses in patients with these malignancies. For that, we studied

peripheral blood (PB) and tumor infiltrating NK and T cells subsets in these two types of cancer at the time of surgery.

Methods: A group of 14 patients with HCC and CCA in different stages with a mean age of 64.6±11.2 and 2 control individuals with a mean age of 62.0±14.0 were analyzed. PB and tumor biopsy samples were immediately processed for the analysis of cytokine production (IL-17 and IFN-γ) by T lymphocytes using flow cytometry. All participants signed written informed consent before entering in the study.
Results: Tumor infiltrating CD4+ T cells presented a higher percentage of IFN-γ and IL-17, in comparison with their PB counterparts ($p<0.05$). The same tendency was found for CD8+ T cells ($p>0.05$). Interestingly, the percentage of CD8+ T cells infiltrating the tumor was higher in CCA than HCC. We found no significant differences between PB and tumor infiltrating NK cells.

Conclusions: By studying the profile of cytokine production in HCC/CCA patients, we can help to elucidate the role of the inflammatory process in these malignancies. Moreover, this knowledge can allow a better understanding of the interactions between immune components and tumor cells, helping to identify new clues for the mechanisms by which these tumors initiate, grow and survive under immune attack.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklowdowska-Curie grant agreement number 675132".

IMMUNOLOGY

CO2441 NATURAL KILLER CELLS IN ACUTE MYELOID LEUKAEMIA PATIENTS: PHENOTYPIC AND FUNCTIONAL ANALYSIS

Raquel Tarazona Beatriz Sanchez-Correa, Ignacio Casas, Juan M. Bergua, Alejandra Pera, Carmen Campos, Nelson López-Sejas, Fakhri Hassouneh, Mª José Arcos, Helena Bañas, Esther Duran, Rafael Solana, Raquel Tarazona
Immunology Unit, University of Extremadura, Caceres, Spain

Introduction: Natural Killer (NK) cells were originally defined as lymphocytes characterized by their natural capacity to kill tumour cells and virus infected cells without the requirement of prior sensitization. NK cells have demonstrated a role in leukaemia control. Acute myeloid leukaemia (AML) is a hematologic disease that generally affects older adults. It is well known that age is associated with changes in the frequency, phenotype and function of different subpopulations of immune cells, including NK cells, a phenomenon called immunosenescence. The aim of this work was to analyse NK cell phenotype and function in AML patients stratified by age and to analyse the possibility of modifying their function by cytokines.

Methods: Peripheral blood mononuclear cells were obtained from AML patients (21-86 years) from the Hospital San Pedro de Alcantara (Caceres, Spain) before specific anti-leukaemia therapy. Age-matched healthy donors (18-87 years) were also included in the study. The ethics committee of the participating institution approved the study, and informed consent was obtained in accordance with the Declaration of Helsinki. Diagnosis was established by cytological criteria based on the French-American-British classification. Cell surface analysis was carried out by flow cytometry using MACSQuant cytometer. NK cells were defined as CD3−CD56+ cells within the lymphocyte gate and the expression of NK cell receptors was referred to this population. NK cells were cultured in the presence or absence of IL-15. For co-culture experiments NK cells were cultured with AML blasts or with the human cell lines K562 and 721.221. NK cell degranulation assay were performed to determine NK cell functional capacity. NK cell cross-talk with dendritic cells was also analysed. Statistical analyses were done using SPSS-15.

Results: NK cells in AML patients show diminished expression of NKp46, NKp30, DNAM-1 and CD94/NKG2C activating receptors that contribute to impaired NK cell function and in consequence, to AML blast escape from NK cell immunosurveillance. We have analysed the hypothesis that the expression of CD112 and CD155 (DNAM-1 ligands) on leukemic blasts induces a decreased expression of the activating receptor DNAM-1 on NK cells. Receptor-ligand crosslinking is involved in downregulation of activating receptors such as DNAM-1, NKp30 and NKp46. An inverse correlation between CD112 expression on leukemic blasts and DNAM-1 expression on NK cells was found. In vitro culture with IL-15 upregulates the expression of NKp30 activating receptor on NK cells from AML

patients that improve NK cell activation against leukaemia cells and increase NK cell-mediated maturation of dendritic cells.

Conclusions: NK cell phenotype in AML patients resembles that found in healthy elderly individuals supporting that NK cells from young AML patients are senescent cells, probably as consequence of chronic stimulation with activating ligands on leukaemic blasts. In elderly AML patients both age- and AML-associated alterations can contribute to the defective NK cell function observed. Some phenotypic changes in NK cells have been correlated with disease progression and survival. NK cell-based immunotherapy has emerged as a possibility for the treatment of AML patients. Further understanding of age-associated defects of NK cell function is necessary to define adequate therapeutic strategies in older AML patients.

CO2442 Identification and enumeration of distinct subsets of monocyte-macrophages in different human tissues throughout life using 9-color flow cytometry

Daniela Damasceno[1,2]; Julia Almeida[1,2], Cristina Teodosio[3]; Martín Pérez Andrés[1,2]; Wouter BL van den Bossche[4]; Juan Flores-Montero[1,2]; Sandra de Bruin[3]; Sonia de Arriba[5]; Alfonso Romero[6]; Noemi Puig[7];Luís Muñoz-Bellvis[8]; Jacques van Dongen[3]; Alberto Orfao[1,2]

[1]Cancer Research Centre (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS) and Department of Medicine, University of Salamanca, Salamanca, Spain; [2]Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; [3]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands; [4]Department of Immunology, Erasmus University Medical Center, Rotterdam, The Netherlands; [5]Servicio de Pediatria, Hospital Universitario de Salamanca, Salamanca, Spain; [6]Centro de Salud Miguel Armijo, Sanidad de Castilla y León, Salamanca, Spain; [7]Service of Haematology, University Hospital of Salamanca, Salamanca, Spain; [8]Unidad de Cirugía Hepatobiliopancreática, Departamento de Cirugía, Hospital Universitario de Salamanca-IBSAL, Salamanca, Spain *Equal contribution

Introduction; Mononuclear phagocytes include circulating monocytes (e.g. classical (CD14+/CD16-) monocytes (cMo), intermediate (CD14+/CD16+) monocytes (iMo), and non-classical (CD14het/CD16+) monocytes (ncMo), and tissue macrophages (TiMas). Despite extensive research on the monocyte-macrophage system, the precise nature of maturational links between populations, functional differences, and age-related distribution are not well-known.
Here we have analysed 5 different monocytic subpopulations -from cord blood (CB) to peripheral blood (PB) specimens throughout life-, to determine age-reference intervals for those subsets; in addition, we evaluated the distribution of monocytic subpopulations in spleen, lymph node (LN) and PB paired samples.

Methods: A total of 11 CB and 171 PB, 4 spleen and 4 LN from healthy individuals, were investigated for the distribution of CD62L+ cMo, CD62L- cMo, iMo, and SLAN- ncMo and SLAN+ ncMo. PB samples were stratified according to the following age groups: newborns=4; 1 to 6 months=14; ≥6m<1y=10; ≥1<2y=13; ≥2<5y =18; ≥5<8y=18; ≥8<13y=17; ≥13<16y=6; ≥16<20y=5; ≥20<30y=13; ≥30<50y=14; ≥50<60y=12; ≥60<70y=9; ≥70<80y=9; ≥80y=9. Tissues other than CB and PB were collected from 4 adult donors (mean age of 69y), together with their paired PB samples.
For the multiparametric flow-cytometry analysis, more than 5x106 leukocytes were stained with a single nine-color combination of fluorochrome conjugated monoclonal antibodies against: CD14, CD16, CD36, CD45, CD62L, CD64, CD300e (IREM2), HLADR and Slan. Data acquisition was performed in a LSR FORTESSA X-20 cytometer (BD), and analysed using the Infinicyt software (Cytognos).

Results: The absolute number of PB cMo reached a peak in CB specimens (932 cells/μL), decreasing hereafter (273 cells/μL) until poverty (>16≤20y), when another peak of cMo was identified (though not so high as in CB: 365 cells/μL), mainly at the expenses of CD62L- cMo. Then, the absolute number of PB cMo decreased and remained stable until 50y-old subjects (197 cells/μL), increasing again in the elderly (329 cells/μ). No significant gender-related changes were found. In turn, PB iMo, and ncMo showed an age-related kinetics similar to cMo, except for CB and newborn groups: the higher number of iMo (53 cells/μL) and ncMo (68 cells/μL) was registered days after the delivery, remaining constant until 50

years old and coming up again in the second half of life, mainly due to an increase in the Slan- compartment. Of note, the distribution of the different monocytic subsets were different in the three tissues analysed in paired subjects: accordingly, in PB the great majority of cMo expressed CD62L, while the CD62L+/CD62L- ratio was inverted both in LN and spleen. Moreover, iMo were increased in LN, and nearly no ncMo were detected, while spleen showed higher frequency of ncMo (both Slan- and Slan+) vs both PB and LN.

Conclusions: Our study shows that the circulating monocyte pool dynamically changes during ageing and among different tissues in humans, which can be useful to establish a frame of their distribution, to be used as reference for pathological conditions. Further, the finding that the different subsets here studied are differentially distributed in tissues may give light on the dynamic trafficking of these cells and therefore in their specific functional roles.

CO2443 Presence of eosinophilia after pediatric liver transplantation and its relevance to graft dysfunction process

Catarina Maia[1]; Isabel Silva[1]; Susana Pedreiro[1]; Sandra Ferreira[2]; Cristina Gonçalves[2]; Susana Nobre[2]; Isabel Gonçalves[2]; Artur Paiva[1]

[1]Operational Management Unit of Cytometry/Clinical Pathology Service, Coimbra Hospital and University Centre
[2]Pediatric and Adult Liver Transplantation Unit, Coimbra Hospital and University Centre, Coimbra, Portugal

Abstract:

Introduction: In recent decades, several studies have reported that a higher eosinophil count (>500 cells/μL or 10% of total of leucocytes) can be a sign of acute cellular rejection, particularly in the first month after liver transplantation, and has also been associated with chronic liver graft dysfunction.
Therefore, in this work we evaluated if the presence of acute graft dysfunction (AGD) or recurrent graft dysfunction (RGD) are associated with phenotypic and functional alterations on several immune blood peripheral cells in eosinophilic and non-eosinophilic patients.

Methods: This study was developed in Coimbra Hospital and University Centre. We studied 59 children submitted to pediatric liver transplantation (28 females and 31 males, whose average age were 9,4 ± 5,3. These children were divided in 4 groups according the presence of eosinophilia (EOS) and AGD or RGD: 41 patients without peripheral eosinophilia (with AGD/RGD (n=8 and n=5, respectively) or without AGD/RGD (n=33 and n=36, respectively)) and 18 children with eosinophilia (with AGD/RGD (n=4 and n=6, respectively) or without AGD/RGD (n=14 and n=12, respectively)). The expression of CD123, CD33, CD15, CD16, CD14, HLA-DR and IgE bounded to its high-affinity receptor on peripheral blood monocytes, basophils, neutrophils, eosinophils and dendritic cells, as well as, the characterization of the different B cell compartments (transitional, naïve, memory and plasmablasts), was performed by flow cytometry. The quantification of T cells producing TNF-α, IFNg, IL-2, IL-6, IL-9 and IL-17, γδ T cells producing TNF-α, IFNg and NK cells producing TNF-α, IFNg was also done by flow cytometry, after in vitro activation with PMA/ionomycin.

Results: While in eosinophilic patients with AGD, we only observed a high tendency to an increase of plasmablasts IgM+ and a statistical significant increase in percentage of CD8+ T cells, the eosinophilic patients with RGD presented a significant increase in HLA-DR expression on intermediate and non-classical monocytes and of CD15 expression on classical monocytes.
Regarding the non-eosinophilic patients, in -EOS/+AGD group there weren't significant alterations. In -EOS/+RGD group, we observed an increase of CD15 expression on classical monocytes, of B cells frequency and of CD27+ memory B cells, accompanied by a decrease of these cells expressing IgG and an increase of those expressing IgM. No statistical significant differences were observed in T cell functional compartments.

Conclusions: Considering our preliminary results, either AGD or RGD seemed to induce few alterations in innate immune cells. Nonetheless, we observed some significant changes in IgM+ memory B cells and IgM+ plasmablasts, the first one associated with RGD in non-eosinophilic patients and the latter with AGD in eosinophilic patients, which could constitute important peripheral markers to monitoring liver

pediatric transplantation.

CO2445 Cytomegalovirus specific cellular immune response as a marker of the outcome of liver transplantation for chronic Hepatitis C virus infection.

Carmina Pallarés[1,2]; Ângela Carvalho-Gomes[1,2]; Victoria Aguilera[1,2]; Marina Berenguer[1,2,3]; F. Xavier López-Labrador[4,5]

[1]Hepatología y trasplante hepático, Instituto Investigación Sanitaria La Fe, Hospital U. y P. La Fe, Valencia, Spain
[2]CIBER-EHD, Instituto de Salud Carlos III, Madrid, Spain
[3]Department of Medicine, Universitat de Valencia, Spain
[4]Virology Laboratory, Genomics and Health Area, FISABIO-Salud Pública, Conselleria de Sanitat, Valencia, Spain
[5]CIBER-ESP, Instituto de Salud Carlos III, Madrid, Spain.

Introduction: Hepatitis C virus (HCV)-related cirrhosis is the main indication for liver transplantation (LT) in the western world. There are several factors associated with the severity of recurrent hepatitis C, including CMV reactivation. It is unclear whether changes in the cytomegalovirus (CMV)-specific cell-mediated immune response (CMI) might result in an inadequate control of HCV replication post-transplantation. CMV-specific CMI is known to induce a modification in the pool of T lymphocytes and may indirectly result in an inadequate control of other infections. We hypothesized that an altered CMV-specific CMI could be immunopathogenic resulting in an inadequate control of viral replication, and subsequent development of graft fibrosis. Our aims were: (i) to evaluate if the CMV-specific CMI allows for stratification of slow versus fast fibrosers, and (ii) in patients with CMV replication, to evaluate if the CMV-specific CMI can predict which patients will control CMV replication spontaneously.

Methods: A total of 300 whole-blood samples were obtained prospectively from 25 patients who underwent liver transplantation to evaluate T-cell specific responses. Patients included 16 HCV-positive at LT, nine of them with treatment-induced resolved HCV infection before LT. Samples were collected just before LT and at several time points after transplantation (week 1, 2, 4, 6, 8, 12, 16, 20, 24, 36 and 48). PBMCs were isolated and cryopreserved. CMV-specific CMI (T-cells) was assessed by intracellular cytokine staining (ICS). Cells were thawed and stimulated with CMV peptide pools: pp65, IE-1 and IE-2 and a positive control (SEB) for 6hr. An 8-color flow panel was developed for the BD FacsCanto-II including the intracellular staining with CD107a-APC, TNFa-PE and IFNg-FITC, a live/dead stain and the cell surface antibody mix (CD3-BV510, CD4-PE-Cy7, CD8-PerCPCy5.5, CD69-BV421). All possible combinations of functional T-cell subpopulations were analyzed using boolean gates with the FlowJo (V10.2) software.

Results: CMV reactivation after LT occurred in 12/25 patients (10 D+/R+ and 2 D+/R-). Of these, 9/16 patients with active HCV infection at LT reactivated CMV and 8 also showed severe HCV disease recurrence. In baseline pre-LT samples, polyfunctional pp65 and IE-1, but not IE-2, specific CD8+ T-cell responses were higher in patients who did not reactivate CMV after transplantation. Finally, when comparing either CMV-specific or polyclonal T-cell responses between slow versus fast fibrosers, polyfunctional CD8+ T-cells were increased in slow fibrosers compared to fast fibrosers, although differences were not statistically significant, possibly due to the small sample size. Further studies are ongoing evaluating the association between CMV-specific CMI and CMV infection as well as infections by other pathogens.

Conclusion: CMI ICS assays warrant further evaluation of the role of CMV-specific T-cell responses in (i) the control of CMV reactivation, and (ii) in the outcome of the graft after liver transplantation.

This work is Supported by Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Spanish Ministry of Economy Directorate of Science (Project PI13/01770), CIBER-EHD and CIBER-ESP and co-financed with Fondos Feder - Una manera de hacer Europa.

CO2454 INTRAEPITHELIAL LYMPHOCYTES SUBSETS IN CELIAC DISEASE DIAGNOSED PATIENTS

Blas-Espada, Javier[1]; Gonzalo-Hernández, Raquel[1]; Castañon-Morales, Susana[1]; Farrais-Villalba, Sergio[2]; Serrano-del Castillo, Cristina[1]

[1] Department of Immunology, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain.
[2] Department of Gastroenterology. Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain.

Introduction: The enumeration of intraepithelial lymphocytes (IELs) subsets (total Lymphocytes, CD3+γδ+, and innate lymphoid cells) by flow cytometry (FC), named as "IEL lymphogram", constitutes a useful tool for celiac disease (CD) diagnosis but is poorly understand its useful in gluten-free diet monitoring and its correlation with serological markers. The aim of this study was to quantify IELs by FC in CD patients, analyzed their value as a marker of different clinical profiles as well as evaluated their correlation with histological data, antibodies levels and gluten-free diet follow up.

Methods: We retrospectively study 59 consecutive IEL lymphogram from biopsies of CD patients during diagnosis or follow-up at ''Hospital Universitario Fundación Jiménez Díaz" between February 2016 and March 2017.
The lymphogram, was performed by FC in all patients in a single biopsy at the same time of diagnostic endoscopy for histological analysis.
TCRγδ-FITC, CD103-PE, CD3-PerCP, CD45-Pc7 and CD7-APC fluorochrome-conjugated monoclonal antibodies (mAbs) were used to evaluate IELs. IEL cells were selected gating on the basis of their side scattering and CD45 expression.
Clinical and histological data were collected from medical history. Duodenal biopsies were categorized according to Marsh- Oberhuber classification.

Results: 59 patients with CD, 56/59 older than 18 years with a female/male ratio=3.54).3 were selective IgA deficiency (5%) and only 5 were children (8%). Overall, 78% were female (ratio female/male= 3.54). Median age was 37 years (±15.80) [7-76].
28 of 59 patients were previously CD diagnosis and were on a gluten-free diet (GFD group) and 31 of 59 patients were diagnosed at the time of the study so they realized free diet (FD group).
The mean percentage of total IELs was 24.75 (±8.94), 21.63 (±9.44) and 27.56 (±7.56), CD3+γδ+ IELs was 25.66 (±15.85), 20.73 (±11.39) and 30.11 (±18.05) and Innate IELs was 1.95 [0.01-47], 2.05 [0.05-30] and 1.38 [0.01-47] to overall population, GFD Group and FD Group respectively.
No linear tendency between percentage CD3+γδ+ IELs and Anti-tTG levels
(Spearman r = 0.1293, P = 0.3424) and Low negative tendency between percentage Innate IELs and Anti-tTG levels (Spearman r = -0.3585, P = 0.0072) were observed.
No linear tendency between percentage CD3+γδ+ IELs and percentage of Innate IELs with GFD time (Spearman r = 0.1475, P = 0.4538 and Spearman r = 0.0673, P = 0.7335 respectively) were observed.
No significant differences were observed in percentage of IELs subsets between different histopathology lesions in both group of study. No differences were found in patients with concomitant H.pylori infection, patients with other immune conditions or between different age group patients.

Conclusions: Total lymphocytes and CD3+γδ+ IELs subsets in our cohort of study were elevated according to data reported in the literature.
CD3+γδ+ IELs were higher in FD than in GFD while innate lymphocytes were similar.
Innate IELs subset and not CD3+γδ+ had a low correlation with anti-tTG levels so it seems that this population would be more sensitive to change in anti-tTG levels.
IELs subsets does not give additional clinical information about histological lesion, concomitant H.pylori infection or immune condition to the diagnosis.

CO2457 - Abstract - Children with cow´s milk allergy: the impact of oral immunotherapy (OIT) in their immune profile

Miguel Dias[1]; Catarina Martins[2]; Magna Correia[3]; Susana Piedade[3]; Ângela Gaspar[3]; Inês Mota[3]; Filipe Benito-Garcia[3]; Glória Nunes[2]; Teresa Lopes[2]; Ana Cruz[4]; Luís-Miguel Borrego[2,3]

[1]Faculty of Sciences and Technology, Universidade de Coimbra, Coimbra, Portugal
[2]CEDOC, Chronic Diseases Research Center, NOVA Medical School|Faculdade de Ciências Médicas;

Universidade Nova de Lisboa, Lisbon, Portugal
[3]Hospital CUF Descobertas, Serviço de Imunoalergologia, Lisbon, Portugal
[4]Faculty of Sciences and Technology (FCT NOVA), Universidade Nova de Lisboa, Lisbon, Portugal

Introduction: Allergy to cow's milk proteins (CMPA) is the most frequent allergy in children. Up to 85% of CMPA children overcome this allergy till reaching the age of 3 years old. If tolerance is not achieved, it is designated as persistent CMPA, being frequently a severe and potentially life threatening condition, with a mandatory strict eviction of CMP ingestion. In these cases, oral immunotherapy (OIT) has proven to be an effective strategy for achieving tolerance, though not fully understood. With this study, we aimed to assess the immune profile of CMPA children after OIT (CMPA-OIT), in order to understand OIT's underlying immune mechanisms.

Methods: CMPA-OIT children and matched healthy controls were recruited. Circulating B and T-cells compartments were evaluated by flow cytometry, in a 4-color BD FACS Calibur, with a pre-validated panel of monoclonal antibodies (CD3, CD4, CD8, CD16+CD56, CD19, CD24, CD25, CD27, CD38, CD45, CD127). Specific IgE (sIgE), IgA (sIgA) and IgG4 (sIgG4) to milk, alpha-lactalbumin, beta-lactoglobulin and casein allergens were determined by fluoroenzyme immunoassay, in a UniCAP 100 system.

Results: Compared to controls, CMPA-OIT children presented increased eosinophils ($p=0.0481$) and NK-cells ($p\leq0.0076$; absolute counts and percentages), and decreased absolute counts and percentages of B-cells ($p\leq0.0193$), CD4+CD25+ ($p\leq0.0001$) and CD8+CD25+ ($p\leq0.0001$) T-cells. No differences were observed between both groups CD4+CD25+CD127- regulatory T-cells.
Within CMPA-OIT children, we have studied the correlation of the immune parameters with eviction period duration, OIT duration, and elapsed time after OIT conclusion (post-CMTP). While CMP avoidance was positively correlated with CD4+CD25+ T-cells percentages ($r=0.56$; $p=0.0016$), OIT duration was negatively correlated with CD4+CD25+ T-cells absolute counts ($r=-0.41$; $p=0.031$),
A negative correlation ($r=-0.45$; $p=0.014$) was found between sIgE for milk and post-CMTP time. Interestingly, we have also observed a strong positive correlation between CMP avoidance and sIgAs to alpha-lactalbumin, beta-lactoglobulin and casein ($r>0.46$; $p\leq0.0121$). B-cells counts were also negatively correlated ($r<-0.466$; $p<0.022$) with the levels of sIgEs and sIgAs to alpha-lactalbumin and beta-lactoglobulin.
Finally, we have divided CMPA-OIT patients according to the class of sIgE after OIT and we have observed a decrease in CD4 T-cells counts ($p=0.0121$) in those with at least one sIgE class 3, compared to patients with all sIgEs below class 3.

Conclusions: CMPA patients after OIT present decreased circulating B-cells and activated T-cells. Moreover, OIT duration affects the presence immune activation, which is supported by the negative correlation observed with activated T-cells. Patients with longer periods after OIT showed lower levels of sIgE for milk, possibly traducing the efficacy of treatment throughout time, as suggested in literature1. Avoidance of CMP intake promotes the generation of sIgA, though its role is still unclear. CMPA-OIT children with decreased B-cells counts also have higher levels of sIgE and sIgA (proven in our study by the first time, as far as we know). An increased B-cell differentiation towards antibody-secreting cells, and their subsequent migration into the tissues may explain this scenario, but further studies are needed to complete and confirm these findings.

1 Salguero, C., Chacón, Á. (2016). Immunological Changes in Specific Oral Tolerance Induction for Cow's Milk Allergy. International Journal of Allergy Medications, 2(1), pp.2–5.

ADVANCES AND NOVEL APPLICATIONS OF FLOW CYTOMETRY

2429 Evaluation of the ethanol tolerance by flow cytometry for wild and mutant Synechocystis strains

Teresa Lopes da Silva1; Teresa Lopes da Silva1, Paula C. Passarinho1, Ricardo Galriça1, Afonso Zenóglio1, Patrícia Armshaw2, J Tony Pembroke2, Con Sheahan3, Alberto Reis1, Francisco Gírio1
Laboratório Nacional de Energia e Geologia, Unidade de Bioenergia, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal

Introduction: The global energy crisis pressures to reduce greenhouse gases have driven the search for

renewable energy alternatives to replace traditional energy sources. The production of bioethanol has received increasing attention recently, being one major type of biofuel that can be blended with gasoline for use in current internal combustion engines powertrains.
Synechocystis sp. strain PCC 6803 is a model cyanobacterium for genetic manipulation and has been widely used to produce bioethanol. Within the ongoing FP7 DEMA project (Direct Ethanol from MicroAlgae) the utility of this model cyanobacterium to produce ethanol at a low cost is being investigated (http://www.dema-etoh.eu/en/).

Methods: To implement a bioethanol production process using cyanobacteria, the ethanol produced will be required to be continuously extracted from the culture media via a membrane-based technological process, to prevent detrimental effects on the biomass. Therefore it is essential to define the bulk ethanol concentration maximum threshold, in the cultivation media, that the cyanobacteria cells can tolerate. For the first time flow cytometry was used to evaluate the effect of initial ethanol concentrations on cyanobacterial strains of Synechocystis PCC 8063 [wild-type (WT), and ethanol producing recombinants (UL 004 and UL 030)] in batch cultures. The work was carried out at LNEG, Lisbon, Portugal. Carbofluorescein diacetate and propidium iodide stains were used to evaluate the enzymatic activity and membrane integrity of the strains. Ethanol recombinants, containing one or two metabolically engineered casettes, were designed towards the development of an economically competitive process for the direct production of bioethanol from microalgae through an exclusive autotrophic route.

Results: The three Synechocystis strains behaved differently in the presence of ethanol. The biomass concentration was reduced by 25% relatively to the absence of toxic, at initial ethanol concentrations of 10 g.L-1, 15 g.L-1 and 20 g.L-1 for the WT, UL 004 and UL 030 strains, respectively. For the WT strain, the proportion of cells with enzymatic activity (PCEA) progressively decreased as the ethanol concentration increased and the culture aged, while UL 004 PCEA showed a pronounced reduction only for initial ethanol concentrations above 10 g.L-1, relatively to the control. UL 030 PCEA was always above 80 % for initial ethanol concentrations in the range of 0-20 g.L-1. For all the strains, the proportion of cells with intact membrane (PCIM) followed similar profiles to the PCEA profiles. The cyanobacteria morphology was also affected by the presence of ethanol. For WT and UL 004 strains, as the initial ethanol concentration increased and the cultures aged, the cells aggregated and formed filamentous structures. UL 030 cells also aggregated with the ethanol concentration increase and age, but in a lesser extent, and did not form filaments.

Conclusions: The recombinant Synechocystis strains tested (UL 004 and UL 030) were more tolerant to the presence of ethanol than the WT strain, and the most efficient ethanol producer (UL 030 containing two copies of the genes per genome) was also the most tolerant to ethanol.

Acknowledgments
The authors acknowledge the European Commission for supporting the EC DEMA project (FP7 – Grant agreement # 309086).

2431 Design of Mass Cytometry Panels for clinical studies of patients with Systemic Autoimmune Diseases

Concepción Marañón; Paulina Rybakowska, Marta Alarcón-Riquelme
Centro GENYO. PTS Granada (Spain)

Introduction: Mass cytometry (CyTOF/HELIOS) offers the possibility of studying immune networks at a single cell level with approximately 40 probes targeting different markers. Therefore, different population landscapes and their functional responses can be studied. For these reasons this technology is the best for identifying cells and pathways involved in the pathogenesis of heterogeneous and overlapping diseases like systemic autoimmune diseases (SADs). The PRECISESADS multicentric project aims at the molecular re-classification of the SADS combining molecular and cellular "-omics", considering all the diseases jointly and not as separate entities.
The first part of this investigation was to set up a methodology for the study of the functionality of different circulating leukocytes in blood samples of SADs patients using the mass cytometry HELIOS instrument.

Methods: A panel of surface markers and intracellular cytokines were designed using the MaxPar panel

designer. Validation of monoclonal antibodies was performed by conventional flow cytometry. In-house conjugation to the metal isotopes was carried out using the Maxpar antibody labeling kit. Whole blood was stimulated with various stimuli: LPS (TLR4), polyI:C (TLR3), R848 (TLR7/8) and R837 (TLR7). Part of the sample was frozen, using different stabilizing methods and stored at -80ºC until acquisition. The other part was stained for surface and cytokine markers and acquired on HELIOS. Conventional biaxial plot gating and the SPADE algorithm are used for data analysis.

Results: A panel for surface and intracellular cytokines was specially designed for the PRECISESADS study. Antibody clones were validated and conjugated to the metal isotopes. Surface and cytokine panels were combined and cytokine production was detected across different stimuli and different cell populations in HELIOS.

Conclusions: We build the basis for future studies using functional mass cytometry for cellular and molecular reclassification of SADs.

Funding: PRECISESADS project Grant No. 115565

2432 Multicenter harmonization of flow cytometers in the context of the multicentric PRECISESADS project

Concepción Marañón Christophe Jamin, Lucas Le Lann, Damiana Álvarez, Nuria Barbarroja, Tineke Cantaert, Julie Ducreux, Aleksandra Dufour, Velia Gerl, Natja Kniesch, Esmeralda Neves, Elena Trombetta, Marta Alarcón-Riquelme and Jacques-Olivier Pers
GENYO Center, PTS Granada

Introduction: The innovative medicine initiative (IMI) multicentric project called PRECISESADS will study 2600 patients of systemic autoimmune diseases (SADs) and 666 controls in order to identify clinically relevant biomarkers for precise SADs molecular reclassification. Among other -OMICs data, multi-parameter flow cytometry analysis will be carried out in 11 different centers using 4 different flow cytometer models. Therefore, the integration of data coming from the different centers and platforms requires a fine mirroring of all the instruments and a continuous quality control procedure.

Methods: Nine Duraclone 8-color tubes (Beckman Coulter) were designed for the flow cytometry analysis on whole blood samples. One flow cytometer chosen as a reference fixed the MFI of 8 fluorochome-conjugated antibodies using VersaComp capture beads. The 10 other centers adjusted their own PMT voltages to reach the same MFIs. Afterwards, every center acquired Rainbow 8-peaks beads data on a daily basis to follow up the stability of the instruments over time, and corrected the PMT voltages if required. Standard operation procedures for standardized blood staining and data acquisition were established.

Results: Control blood samples were dispatched to every center and stained using a Duraclone tube following the SOP. Comparison of leukocytes frequencies and cell surface marker MFI demonstrated the close sensitivity of all cytometers, allowing a multicenter analysis. Regular intercalibration using VersaComp beads evidenced the stability of the data over time.

Conclusions: The effective multi-center harmonization enables the constitution of a workable wide flow cytometry database that can be confidently exploited to search for clinically relevant signatures in SADs patients.

Funding: PRECISESADS project Grant No. 115565

2439 Next Generation Flow Cytometry in the monitoring of monocytic-lineage alterations during follow-up of myelodysplastic syndromes and acute monoblastic/monocytic leukemia

Sergio MATARRAZ[1]; Pilar LEOZ[1]; Carlos FERNANDEZ[1]; Quentin LECREVISSE[1]; Ana YEGUAS BERMEJO[2]; Enrique COLADO[3]; Juan FLORES-MONTERO[1]; Alejandro BERKOVITS[1]; Vincent van der VELDEN[4]; Susana BARRENA[1]; Antonio LÓPEZ[1]; Jacques van DONGEN[4]; Alberto ORFAO[1]

Author Affiliations: [1]Cytometry Service (NUCLEUS), Department of Medicine and IBSAL, Cancer Research Center (IBMCC, University of Salamanca-CSIC), Salamanca, Spain; [2]Hematology Service,

University Hospital of Getafe, Spain; [3]Central University Hospital of Asturias, A.G.C. Hematology Laboratory; [4]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands.

CORRESPONDENCE TO: Prof. Alberto Orfao, MD, PhD
Servicio General de Citometría.
Centro de Investigación del Cáncer
Campus Miguel de Unamuno
37007-Salamanca. Spain
Telephone number: +34-923 29 48 11
Fax number: +34-923 29 47 95
E-mail: orfao@usal.es

Introduction: Clonal involvement and subsequent impaired production of monocytic-lineage cells is a recurrent finding in myelodysplastic syndromes (MDS). Further stepwise accumulation of oncogenic events may translate into maturation blockades and altered phenotypic patterns among the monocytic (and/or any other) bone marrow (BM) cell subset. The eventual arrest of prior dysplastic monocytic maturation may occur at distinct levels of monocytic ontogeny, ensuing transformation into acute monoblastic or monocytic leukemia (AMML). Although monocytic-lineage alterations are often evidenced in MDS and AMML diagnosis, conventional flow cytometry and cytological procedures are frequently inconclusive, due to technical- and/or expert-related limitations.
Next-generation flow cytometry (NGF) provides a multidimensional dissection of hematopoietic cell development. In monocytic-lineage malignancies, this translates into a highly sensitive and automated method for detection of baseline and incoming (at diagnosis and follow-up, respectively) maturation blockades and phenotypic alterations, as well as an objective expert-independent evaluation of their degree of deviation from normal patterns.

Methods: We analyzed by 10-dimensional NGF the monocytic maturation of 37 MDS patients and 15 subjects with AMML at diagnosis and distinct follow-up BM (up to seven follow-up BM), which were compared to a database of normal monocytic differentiation based on (n=94) age-matched normal BM samples.

Results: As expected, maturation blockades, together with phenotypic alterations of monocytic cells (≥3 SD from normal reference values) were automatically detected in virtually all MDS patients at diagnosis. These mainly consisted of defective intensity of CD11b, CD13, CD36, CD33, CD64, CD300e and CD35, altered HLA-DR expression, asynchronous CD14 vs. CD35 vs. CD300e patterns, aberrant CD34 and cross-lineage CD56 expression. At follow-up BM, aberrant phenotypes and maturation blockades persisted in a significant fraction of MDS cases.
In all AMML cases (100%), phenotypic alterations were detected at variable levels along with monocytic differentiation, even among cases without evidence of relapsing blasts at follow-up; all these later cases ended up showing relapsing disease at further follow-up BM. The relapsing leukemic phenotypes observed at follow-up BM closely resembled those detected at diagnosis in only 2 AMML patients (13%), while the remaining 13 cases showed dynamically changing relapsing phenotypes and maturation blockades at the different time points evaluated.

Conclusion: Next Generation Flow Cytometry provides a high-sensitivity method for detection of persistent and changing altered phenotypes and maturation blockades within monocytic differentiation from MDS and AMML at diagnostic vs. follow-up, even in the apparent absence of relapsing/resistant clonal cells by conventional approaches. Hence, it is confirmed that searching for (leukemic) cells resembling baseline phenotypic characteristics appears insufficient for an appropriated assessment of residual disease.

POSTERS
HEMATOLOGY

P2437 Frequency and number of clonal plasma cells in peripheral blood (PB) in plasma cell neoplasm (PCN) by Next Generation Flow

Sanoja-Flores Luzalba1 Flores-Montero Juan1, Paiva Bruno2, Puig Noemí3, García-Mateo Arazanzu4, Garcés Juan Jose2, García Omar1, Corral-Mateos Alba1, Burgos Leire2, Pontes Roberia5,

Hernández-Martín José4, Prosper Felipe2, Merino Juana2, Vidriales María Belén3, García-Sanz Ramón3, Palomera Luis6, Ríos Rafael7, Del Cañizo María Consuelo3, Durie Brian8, Mateos María Victoria3, van Dongen Jacques JM9, San Miguel Jesús F.2 and Alberto Orfao1 on behalf of the EuroFlow Consortium and the International Myeloma Foundation
1Cancer Research Center (IBMCC-CSIC/USAL-IBSAL); Cytometry Service (NUCLEUS) and Department of Medicine, University of Salamanca, Salamanca (USAL), Spain; 2Clinica Universidad de Navarra; Applied Medical Research Center (CIMA), IDISNA, Pamplona, Spain (UNAV); 3Department of Hematology, Hospital Universitario de Salamanca, IBSAL; IBMCC (USAL-CSIC), Salamanca, Spain; 4Department of Hematology, Complejo Asistencial de Segovia, Segovia, Spain; 5Faculty of Medicine, Federal University of Rio de Janeiro and Institute of Pediatrics and Childhood Care, Rio de Janeiro, Brazil; 6Hospital Universitario Lozano Blesa, Zaragoza, España; 7Hospital Virgen de las Nieves, Granada, España; 8Cedars-Sinai Samuel Oschin Cancer Center, Los Angeles, CA, USA; 9Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.

Introduction: Detection of clonal plasma cells (CPC) in PB from PCN patients has been frequently reported as an adverse prognostic factor. However, the percentage of PCN patients showing CPC in PB depends on the sensitivity of the techniques used. Here, we investigated for the first time the frequency and number of CPC in PB of PCN patients by NGF, and correlated them with subtype of PCN disease and their risk category.

Methods: A total of 414 samples from 226 PCN patients -188 paired PB/bone marrow (BM) and 38 additional PB samples, from 139-monoclonal gammopathy of undetermined significance (MGUS), 25 smoldering multiple myeloma (SMM), 49 MM (4 diagnosed by extramedullary disease with low BM involvement) and 13 solitary plasmacytomas (SP)- and, 10 PB/BM from healthy donors (HD), were studied. For sample preparation, the EuroFlow Bulk Lysis Standard Operating Procedure and the EuroFlow-IMF MM MRD antibody panel combination (Tube 1: CD138BV421/ CD27BV510/ CD38FITC/ CD56PE/ CD45PerCP-Cy5.5/ CD19PE-Cy7/ CD117APC/ CD81APC-C750, and; Tube 2: identical to Tube 1 except for cyKappaAPC/cyLambdaAPC-C750); were used. Measurement employed FACSCanto II instruments (BD Biosciences) and the Infinicyt software (version 1.8.0 Cytognos) for data acquisition (median of 11.1x106 cells/sample) and analysis, respectively. Risk stratification of MGUS and SMM was established by the Mayo Clinic index (for both patients groups) and, the proportion of CPC from all BM-PC and the Salamanca score, respectively. MM patients were grouped by the R-ISS score. ROC analysis was used to define the most accurate cut-off to discriminate between MM and MGUS cases based on the number of PB CPC.

Results: Overall, CPC were detected in all MM and SMM patients (100%) and 60% of MGUS cases (p 0.0001). In contrast, the proportion of MM patients with extramedullary disease showing circulating CPC was significantly lower (25%), and SP-patients with (41%) or without (0%) progression to MM vs MM (p 0.0001). Despite the majority of MGUS showed circulating CPC their number was significantly lower than in SMM and in MM (p 0.0001 and p 0.03, respectively). A cut-off value of 0.0011% of CPC and 54 CPC/mL, or a PB clonal/normal PC ratio of 0.075, all allowed discrimination between MGUS and MM cases in 83%, 84% and 81% of cases (p 0.0001). Of note, circulating CPC showed a significant correlation (ρ=0.75; p 0.0001) with the percentage of CPC in BM being typically detected once the later represented ≥60% of all BM-PC. Within MGUS the proportion of cases with CPC in PB and their number increased in Mayo Clinic score 0 to 1 and 2 (p 0.001); similarly, they were also found higher in MGUS cases with ≥95% of CPC from all BM-PC (p 0.01). In contrast, no significant differences were found in SMM patients with different normal/clonal PC ratios. Finally, R-ISS stage II and III MM patients displayed higher levels of circulating CPC in PB than stage I patients (p 0.03).

Conclusions: NGF emerges as the most sensitive method to detect circulating CPC in PB of patients with PCN; their number being associated with more aggressive and malignant subtypes of the disease and risk groups.

P2440 KIT D816V positive mast cell leukemia associated with acute myeloid leukemia: a case report

Marta Sofia Lopes Maria dos Anjos Teixeira, Catarina Lau, Patrícia Seabra, Vanessa Mesquita, Cláudia Casais, Jorge Coutinho, Margarida Lima.
Serviço de Hematologia Clínica, Centro Hospitalar do Porto, Porto, Portugal

Introduction: Mastocytosis is rare neoplasm defined by abnormal expansion/accumulation of clonal mast cells (MC). Mast cell leukemia (MCL) accounts for <1% of all mastocytosis, it may appear de novo or secondary to a previous MC disorder, and it may be associated with other clonal hematologic non–MC disease (1,2). Diagnosis is based on the presence of ≥20% atypical MC in the bone marrow (BM) or ≥10% in the peripheral blood (PB). The neoplastic MC have an abnormal immature and/or activated phenotype; the KIT D816V mutation is detected in <50% of cases and a normal karyotype is found in most patients. Symptoms of MC activation and involvement of the liver, spleen, peritoneum, gastrointestinal tract and bones are relatively frequent, and skin lesions occur in 1/3 of the patients. No effective treatment exists and the median survival time is <6 months.

Case report: A 41 years old woman presented with one-month history of asthenia, anorexia, fever, epigastralgias and diarrhea. She had hepatosplenomegaly, without skin lesions or palpable lymphadenopathies, and she mentioned a past history of an intermittent maculopapular rash, episodes of flushing, primary hypothyroidism and an anxiety disorder. Peripheral blood showed pancytopenia, 6% blast cells (BC) and 20% MC. Serum biochemistry revealed elevated lactate dehydrogenase, cholestasis, hypoalbuminemia, hypogammaglobinemia and increased tryptase levels (184 g/L). The BM smears showed 23.7% myeloblasts and 16.0% morphologically abnormal toluidine blue positive MC. Flow cytometry of the BM aspirate revealed 12% myeloid BC (CD34+CD117+lowCD13+CD33+); 34% of CD34-CD117+CD13+CD33+ promyelocytes with aberrant phenotype; a maturation blockage at the myeloblast/promyelocyte level (abnormal CD11b/CD13/CD16 pattern, with virtually no CD16+ neutrophils); and a heterogeneous population of CD117+CD203c+ MC (16% BM cells) most of which had an immature (CD123+, cytoplasmic carboxypeptidase +) and aberrant (CD25+CD30+) immunophenotype. The activating KIT D816V mutation was found in MC, BC and other BM cells. The BM karyotype was normal, as did genetic testing for other specific alterations, including t(15;17), t(8;21), inv(16), t(9;22), FLT3 internal tandem duplication and NPM-1 mutations. Abdominopelvic TC scan showed hepatomegaly, mild splenomegaly, and retroperitoneal adenopathies. Digestive endoscopy exhibited a congestive erythematous gastric and duodenum mucosa, and skeleton radiography did not show osteolytic lesions. The diagnosis of MCL associated with acute myeloid leukemia was assumed. The patient started corticosteroids and oral cromoglycate, and received two cycles of cladribine, followed by two courses of idarubicin + cytosine arabinoside. One month later, the serum tryptase was of 8.03 g/L, and flow cytometry revealed 1.5% of myeloid BC, 54% maturing granulocytic cells, and 0.03% MC in the BM. The patient received HLA-haploidentical allogeneic hematopoietic stem cell transplantation (HSCT). The outcome was favorable, with complete hematological remission 7 months after HSCT (16 months after the diagnosis).

Discussion and conclusions: We present this case because of the rarity of MCL, given emphasis on the role of flow cytometry to establish the diagnosis and to the immunophenotypic characteristics of the neoplastic MC.

References: (1) Stone RM and Bernstein SH. Mast Cell Leukemia and Other Mast Cell Neoplasms. In: Holland-Frei Cancer Medicine. 6th edition. Kufe DW et al., editors. Hamilton: BC Decker; 2003. (2) Georgin-Lavialle S et al. Mast cell leukemia. Blood 121:1285-95, 2013.

P2446 PROSPECTIVE MULTICENTRIC EVALUATION OF PREDICTOR PARAMETERS FOR PAROXYSMAL NOCTURAL HEMOGLOBINURIA DIAGNOSTIC SCREENING.

Martin Perez-Andres1, Marta Morado2, Alex Freire Sandes3, Enrique Colado4 Dolores Subirá5, Paloma Isusi6, María Soledad Noya7, María Belén Vidriales8, Amparo Sempere9, José Ángel Díaz10 Alfredo Minguela11, Beatriz Álvarez12, Cristina Serrano13, Teresa Caballero14, Maria de las Mercedes Rey15, Ana Pérez Corral16, María Cristina Fernández Jiménez17, Elena Magro18, Angelina Lemes19, Celina Benavente20, Helena Bañas21, Celine Castejon22, Matheus Vescovi Gonçalves3,Alberto Orfao1 on behalf of the PNH working group of the Iberian Society of Cytometry (SIC)

1Cancer Research Centre (IBMCC, CSIC-USAL), Institute of Biomedical Research of Salamanca (IBSAL), and Cytometry Service (NUCLEUS) and Department of Medicine, University of Salamanca, Salamanca, Spain, 2Serv. Hematologia. Hosp Univ. La Paz. Madrid, Spain, 3División de Hematología y Citometría de Flujo, Fleury Group, São Paulo, Brazil, 4Serv. Hematologia. Hosp Central Asturias. Oviedo, Spain, 5Serv. Hematologia. Hosp. Univ. Guadalajara. Guadalajara, Spain, 6Serv. Hematologia. Hosp Basurto. Bilbao, Spain, 7Serv Hematologia. Complejo Hosp. Univ. A Coruña. A Coruña, Spain, 8Serv. Hematologia. Hosp. Univ de Salamanca. Salamanca, Spain, 9Serv. Hematologia. Hosp. Univ. La Fe. Valencia, Spain, 10Serv.

Hematologia. Hosp Univ. Santiago de Compostela. Santiago de Compostela, Spain, 11Serv. Inmunologia. Hosp. Virgen de la Arrixaca. Murcia, Spain, 12Lab. Central Comunidad de Madrid. Madrid, Spain, 13Serv Hematologia. Fundacion Jimenez Diaz. Madrid, Spain, 14Serv Hematologia. Hosp. Univ. Virgen del Rocio. Sevilla, Spain, 15Lab Unificado Donosti. Hosp de Donosti. San Sebastián, Spain, 16Hosp. Gregorio Marañon, Madrid, Spain, 17Serv. Hematologia. Compl. Hosp de Toledo. Toledo, Spain, 18Serv. Hematologia. Hosp. Univ. Principe de Asturias, Alcalá de Henares, Spain, 19Serv. Hematologia. Hosp. Univ. Dr. Negrin. Las Palmas de Gran Canaria, Spain, 20Serv Hematologia. Hosp. Clínico San Carlos, Madrid, Spain, 21Serv. Hematologia. Hosp. San Pedro Alcántara, Caceres, Spain, 22Balague Center, Hospitalet de Llobregat, Spain.

Introduction: Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal and non-malignant hematopoietic stem cell disorder characterized by partial or absolute deficiency of glycophasphatidilinositol (GPI)-anchored cell membrane proteins. Although recommended medical indications for PNH testing by flow cytometry has been firmly stablished, different international clinical studies have shown heterogeneous prevalence of PNH. Routine analytical parameters related with disease activity may potentially contribute to a more accurate selection of samples for PNH screening by flow cytometry.

Methods: Information about 3,938 samples tested by flow cytometry for PNH diagnostic screening was prospectively collected from 2010 to 2014. Data collected included medical indications leading to PNH screening and laboratory parameters analyzed at diagnosis: blood cell counts (red blood cells, reticulocytes, platelets, leukocytes, monocytes and neutrophils) and related hematological variables (hemoglobin and mean corpuscular value -MCV-), serum levels of bilirubin, lactate dehydrogenase LDH, and creatinine. Information about recent transfusion was also recorder. In order to obtain comparable results, LDH and haptoglobin (hapto) values were normalized with the upper and lower limit of normal values (ULN and LLN respectively) from the corresponding center. Conventional flow cytometry methods were used for the analysis of the expression on mature neutrophils and monocytes. A PNH positive result was defined when GPI-deficient cells were in two or more different cell lineages in a frequency higher than 0.01% of total leukocytes.

Results: Patients with GPI-deficient cells (vs. negative cases) had higher serum normalized LDH/ULN values (2.2±2.6 vs. 1.2±1.7, p<0.001) and MCV (99±9 vs 92±11 fL, p<0.001), and decreased normalized hapto/LLN values (1.1±1.6 vs 2.5±3.7, p <0.001), hemoglobin levels (9.6±2.5 vs 11.1±2.8 g/dL, p <0.001), WBC (3,809±2,164 vs 5,593±4,380 x 109cells/L, p< 0.001), RBC (2.9±0.8 vs 3.8±1.0 x 109cells/mcL, p< 0.001), and platelet counts (94±90 vs 166±131 × 109/L, p<0.001). Both normalized serum LDH/ULN and haptoglobin/LLN levels retained their association with the presence of GPI-deficient cells when patients were classified based on their medical indication leading to PNH screening, including Aplastic Anemia (1.4±1.6 vs. 1.0±1.7 and 1.3±1.6 vs. 2.9±3.4, LDH/ULN and hapto/LLN- p<0.05), myelodysplastic syndromes (1.4±1.1 vs. 1.0±0.7 and 0.8±1.3 vs. 3.0±4.4, p<0.05), non hemolytic anemia/cytopenia (1.9±2.4 vs. 1.1±1.5 and 1.2±1.7 vs. 3.0±4.4, p<0.001) and hemolysis (4.4±3.7 vs. 2.3±3.0 and 0.2±0.2 vs. 0.8±1.2, p<0.001).
Multivariate analysis showed that normalized LDH and haptoglobin are the only biochemical parameters that can predict PNH clone detection in all clinical groups. Cases with increased LDH (LDH/ULN>0.8) and/or decreased (hapto/LLN<0.6) values (vs. normal LDH and/or hapto values) were associated with a significantly higher proportion of PNH+ cases, in all medical indications: Aplastic anemia (54% vs. 18%, p=0.01), myelodysplastic syndrome (33% vs. 2%, p=0.001), anemia/cytopenia (24% vs. 6%, p<0.001), and hemolysis (30% vs. 8%, p<0.001)

Conclusions: Presence of GPI deficient cells is associated with biochemical parameters reflecting hemolysis and/or cytopenia. Among them, higher LDH levels and lower haptoglobin levels are significantly associated to the presence of a PNH clone, regardless of the medical indication for PNH screening.

P2448 CLL-like B-cell clones from MBLlo individuals persist at increased counts after seven years of follow-up

Authors: Ignacio Criado1, Arancha Rodríguez-Caballero1, Laura Gutiérrez1, Carlos E. Pedreira1, Wendy G. Nieto1, Cristina Teodosio1, Virginia Herráez1, Alfonso Romero-Furones2, Paulino Fernández-Navarro3, Julia Almeida1*, Alberto Orfao1* and the Primary Health Care Group of Salamanca for the Study of MBL. *Equal contribution

Institutions: 1Cancer Research Centre (IBMCC, CSIC-USAL), Cytometry Service (NUCLEUS) and Department of Medicine, University of Salamanca, Salamanca. Institute of Biomedical Research of Salamanca (IBSAL), Salamanca. Spain. 2Centro de Atención Primaria de Salud Miguel Armijo, Salamanca, Sanidad de Castilla y León (SACYL), Castilla y León, Spain. 3Centro de Atención Primaria de Salud de Ledesma, Salamanca, Sanidad de Castilla y León, Spain.
Keywords: low-count Monoclonal B lymphocytosis (MBLlo), follow-up, multiparametric flow cytometry, cytogenetics.

ABSTRACT:

Background: The presence of very low numbers of clonal B cells in peripheral blood (PB) of otherwise healthy individuals (low-count monoclonal B lymphocytosis -MBLlo-) is a common finding in the general population. Since the vast majority of clonal B cells from MBLlo subjects show a phenotype overlapping with CLL (chronic lymphocytic leukemia) cells, the former might represent either the normal counterpart of CLL or the earliest stages of the disease. Little information exists about both the clinical outcome of MBLlo subjects and the biological features of their B-cell clones over time.

Methods: The baseline study was constructed in 2008, when 80 out of 639 (12.5%) healthy individuals (>40y) were found to carry at least one PB CLL-like clonal B-cell population, using high-sensitive flow cytometry. A subset of them (n=49) has been followed for a median period of 84 months (range: 67-95 months). Besides physical examination and flow cytometry analyses, the most frequent CLL-related cytogenetic alterations [del13q14.3(D13S25), trisomy 12, de11q(ATM) and del17q(TP53)] were studied at baseline and at follow-up.

Results: A total of 64 CLL-like MBLlo clones (median size: 0.44 cells/ul, range: 0.027-66 cells/ul) were detected in PB of the 49 subjects at recruitment (in 15 cases ≥2 B-cell clones were detected in the same subject). In all subjects, B-cell clones persisted at reevaluation, phenotypically identical vs. baseline. Interestingly, we found a near-3-fold overall increase in the size of CLL-like B-cell clones after a 7y follow-up vs. baseline (median size: 1.22 cells/ul, range: 0.046-789 cells/ul; p:<0.001); in line with this, most clones (45/64; 70%) increased their size, while the remaining 30% maintained stable or slightly decreased numbers compared to time 0. From the genetic point of view, only 8/32 (25%) clones tested carried one cytogenetic alteration at baseline, del13q(D13S25) being present in 7/8 cases and trisomy 12 in the remaining one. Strikingly, re-evaluation after 7 years showed 36/56 clones (64%; p<0.01 vs. baseline) with cytogenetic alterations; again, the most common abnormality was del13q(D13S25) (34/36) followed by trisomy 12 (1/36) and del 17p(TP53) (1/36). No statistical association (p>0.05) was found between the change over time in the size of these clones and the presence of cytogenetic lesions. Three subjects developed lymphocytosis (median: 5.3x109 lymphocytes/l; range: 4.1x109-5.9x109/l) after 7 years; in these cases the clone size increased substantially over time and showed (2 of them) del13q14.3. Despite this, only 1/3 subjects evolved to more advanced stage of disease, who showed 5.9x109 lymphocytes/l with >500 clonal B cells/ul (thus reaching MBLhi) and del13q14.3.

Conclusion: Our results suggest that CLL-like MBLlo is not a transient condition, as all clones detected at recruitment were systematically present also at follow-up with an increase frequency of cytogenetic lesions. Despite, B-cell clones slightly increased in size and even acquired cytogenetic abnormalities, the likely to progress into an over CLL seems to be extremely rare. Further research is needed to better understand the significance of CLL-like B-cell clones in healthy individuals and to clearly identify those MBLlo subjects at risk to evolve to MBLhi/CLL.

P2450 INCREASE IN CLASSICAL SUBSET MONOCYTES IN PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA: A NEW CLUE FOR DIAGNOSIS?

Alicia Saez Salinas Cristina Lemes Quintana*, Angelina Lemes Castellano, Dolly Fiallo Suarez, Leslie González Pinedo, Maria Teresa Gómez Casares, Teresa Molero Labarta
Hospital Universitario de Gran Canaria Dr Negrín *Complejo Hospitalario Universitario Insular Materno-Infantil

Introduction
Chronic myelomonocytic leukemia (CMML) is a myelodysplastic / myeloproliferative neoplasm whose diagnosis is based on the presence of persistent monocytosis > 1x109 /L in peripheral blood and monocytes accounting for ≥10% of the white blood cell (WBC) differential count, measured for at least

3 months. Diagnosis could be difficult in cases such as reactive monocytosis or prefibrotic myelofibrosis.
Some authors have demonstrated an increase in the fraction of classical CD14+/CD16- monocytes with respect to non-classical fractions (CD14+/CD16+ and CD14 low/CD16+) in patients with CMML, and its posterior decrease with treatment. Furthermore, the non-classical subsets have been related to inflammatory diseases.
The goal of this study was the comparison of the distribution of different fractions of monocytes in peripheral blood in patients with criteria of CMML according to the WHO 2016 classification and in patients with reactive monocytosis.

Methods:18 patients with CMML from two hospitals were compailed. Out of those patients, 12 were male and 6 female. They were compared to 20 reactive monocytosis out of which 11 were male and 9 were female.

	Age (Mean)	WBC x10^9/L	Monocyte x10^9/L	Monocyte %	Hemoglobin gr/dL	Platelet x10^9/L
Controls Mean (range)	60,45 (40-77)	15,6 (6,2-26)	1,8 (1,5-2,7)	13,28 (6-29,4)	12,02 (8,8-15)	326 (72-682)
Cases Mean (range)	74,94 (57-90)	12,5 (3,3-32)	3,1 (1,0-10,3)	24,8 (11,8-55)	12,3 (7,4-16,2)	238 (214-661)

Table1. Features of patients

The distribution of the different monocyte subsets (CD14+/CD16-, CD14+/CD16+ and CD 14 low/CD16+) was analyzed by flow cytometry. They were labeled with these monoclonal antibodies: CD15FITC/CD64PE/CD33PerCP/CD16PECy7/CD14APC/CD38APCH7/HLA-DRV450/CD45V500.
The samples were labeled in accordance with the standard procedures of Euroflow. At least 50000 events were acquired in a FACSCANTO (BD) at the CD64 gate.

Results: Statistically significant differences were observed in the distribution of the three monocyte subsets of patients and controls ($p < 0.001$).
After the ROC Curve analysis, we were able to establish a cut-off point for the classical monocytic subsets of 97.5% with 77.8% sensitivity and 85% specificity for the diagnosis of CMML (Fig. 1).
It is noteworthy to mention the presence of 4 patients with CMML who did not exceed the 97.5% cut-off of classical monocytes (CD14 + / CD16-). Three of them were on treatment at the time of the flow cytometry analysis. And one was not on treatment but he had a personal history of psoriasis.

Discussion: The study of monocytic subsets by flow cytometry may be a useful tool in the differential diagnosis of reactive monocytosis vs CMML.
We were able to establish a cut-off point in the percentage of classical CD14+ / CD16- subset that discriminates patients with CMML. Expanding the number of cases and controls would further increase the sensitivity and specificity shown in our study.
Finally, it is important to highlight the possible use of flow cytometry as a tool to assess treatment efficacy by decreasing the percentage of classical monocyte subsets as it has been shown in our treated patients.

P2451 LST tube's utility in diagnosing classical Chronic Lymphocytic Leukemia (CLL).

Angelina Lemes Castellano Dolly Fiallo Suarez, Naylen Cruz Cruz, Cynthia Acosta Fleitas, Leslie González Pinedo, Alicia Saez Salinas, Teresa Molero Labarta
Hospital Universitario de Gran Canaria Dr Negrín *Complejo Hospitalario Universitario Insular Materno-Infantil

Introduction: the LST tube was designed by Euroflow with the objective of screening patients for chronic lymphoproliferative disorders (CLPD). Furthermore, it has contributed notably to the diagnosis of clinical entities such as CLL and mantle cell lymphoma (MCL) when combining with the second tube from the B-CLPD panel.

Objective: to demonstrate LST's utility in the diagnosis of classical CLL.

Methods: 18 patients were studied, with an average age of 65 years. All of them with newly diagnosed CLL after morphological, histochemical, cytometric, molecular and FISH studies. There were 28 control patients with different pathologies (9 were healthy, 4 had asma, 4 were obese, 4 with rheumatological syndromes, 3 were hypertensive, 2 with chronic renal failure, 1 with colon cancer, 1 dyslipidemia and 1 with Q fever). Their average age was of 38 years and none of them presented neither lymphocytosis nor alterations in hematological parameters.One-Flow's LST tubes, provided by Becton Dickinson, were used with the following desiccant antibodies' combination: CD20V450/CD45V500/CD8/antiLambdaFITC/CD56/antiKappaPE/CD5PercPCy5.5/CD19/TCRabPECy7/CD3APC/CD38APC-H7. In the patients' group, a second tube from Euroflow's B-CLPD panel was used as well.
Fluorescence targets as well as compensations were performed according to the instructions that were included in OneFlow "7C Setup beads" and "compensation kit". Samples were acquired with a "Facscanto II" Cytometer, with an average acquisition of 300,000 events.

Results: The "fluorescence" average intensity (MFI) resulted significantly reduced ($p<0,005$) in patients' lymphocytes for the following antigens: CD19/CD45/CD38/CD20/Lambda and kappa.MFI in light chains/CD38/CD20 were the variables that contributed the most to the diagnosis.

	Mean Fluorescence controls	Mean Fluorescence patients
Kappa	26201	3394
Lambda	7353	1094
CD38	1596	517
CD20	25653	4169

The decrease in MFI contributed significantly to the diagnosis in 99,8% in the case of light chains, 91% for CD38 and 100% for CD20. Other findings were: a) CD5's greater MFI in patients' clonal B-lymphocytes when compared to those in the control group (MFI 4686 vs 3091, respectively)($p<0,004$). b) There were no differences when studying the expression's intensity of the patients' non clonal residuals B-lymphocytes.

Conclusion: The implementation of One Flow LST's tube in Flow Cytometry is a very useful tool when screening and establishing diagnosis of classical B-CLL, without the need of including additional monoclonal antibodies in the study; thus, reducing costs without a loss in specificity.

P2459 Less represented bone marrow hematopoietic cell lines from Myelodysplastic Syndrome exhibit phenotypic and functional alterations

Cátia Simões1, Anabela Carvalho1, Isabel Silva1, Sandra Silva1, Susana Santos1, Susana Pedreiro1, Margarida Coucelo2, Paula Laranjeira1, Manuela Fortuna1, Emília Cortesão2, Helena Vitória3, Letícia Ribeiro2, Artur Paiva1

1- Operational Management Unit of Cytometry/Clinical Pathology Service, Coimbra Hospital and University Centre
2- Clinical Hematology Service, Coimbra Hospital and University Centre
3- Clinical Hematology Service, Tondela-Viseu Hospital Centre

Introduction: Myelodysplastic syndrome (MDS) is a heterogeneous group of myeloid neoplasms characterized by changes in the differentiation and maturation of myeloid cells. The contribution of immunophenotyping for the diagnosis of MDS is not well established, however, since 2014 the International Leukemia Net European Working Group recommends the integration of flow cytometry in the diagnosis of these entities.

In this context, the aim of this study was to evaluate if less-represented bone marrow (BM) cell lineages from MDS patients, such as basophil, eosinophil, plasmacytoid dendritic cell and mast cell lineages, were phenotypically and functionally different from normal/reactive BM.

Methods: This work was developed in Coimbra Hospital and University Centre. MDS patients (n=84) were distributed according to the World Health Organization classification as: 8 RCUD, 45 RCMD, 17 RAEB-1, and 14 RAEB-2. Based on the International Prognostic Scoring System, 18 cases were Low risk MDS, 19 were intermediate risk-1 and 16 were classified as intermediate risk-2 and high-risk. Additionally, we analysed 14 normal/reactive BM. In the different hematopoietic lineages, the percentage of cells in different maturation stages and expression of several receptors, like CD10, CD11b, CD13, CD33, CD34, CD35, CD44, CD45, CD64, CD117, CD123, CD133, and HLA-DR were studied. We also analysed the tryptase and eosinophil peroxidase gene expression in purified basophils and eosinophils, respectively, in 9 MDS samples and in 5 normal/reactive BM.

Results: We observed that MDS patients displayed a statistical significant increase in the expression of CD44 and CD45 in all hematopoietic lineages under study; of CD117 and HLA-DR in mast cell lineage, a decreased expression of CD13 in basophil lineage and of CD35 and CD64 in eosinophil lineage. Concerning gene expression analysis, we found, in MDS patients, an increased tryptase mRNA expression in basophils and eosinophil peroxidase mRNA in eosinophils.
Conclusions: Our results pointed to the occurrence of immunophenotypic and functional alterations in less-represented hematopoietic cell lineages from MDS patients, consistent with those published in granulocytic, monocytic and erythroid lineages, reinforcing the contribution of flow cytometry for the diagnosis of MDS, especially in the evaluation of dysplasia in less-represented BM cell lineages.

P2462 Functional alterations of T and NK cells from lymph node biopsies from B-cell chronic lymphoproliferative disorders

Ana Lopes1, Anabela Carvalho1, Isabel Silva1, Sandra Silva1, Susana Santos1, Susana Pedreiro1, Margarida Coucelo2, Marília Gomes2, Rui Bártolo1, Adriana Roque2, Letícia Ribeiro2, Artur Paiva1

1-Unidade de Gestão Operacional de Citometria do Serviço de Patologia Clínica, Centro Hospitalar e Universitário de Coimbra
2- Serviço de Hematologia Clínica, Centro Hospitalar e Universitário de Coimbra

Introduction: B cell chronic lymphoproliferative diseases (B-CLPD) are a heterogeneous group of entities representing about 80-90% of all chronic lymphoproliferative syndromes, in which can be observed a clonal expansion of B lymphocytes. Lymph node biopsy samples are currently used for the diagnosis of these entities and represent appropriate locations where interactions between B and T cells occur.

Objective: The main goal of this study was to quantify and evaluate the function of different subpopulations of T cells (CD8+ T cells, CD4+ regulatory T cells (Treg), Th1, and Th17 cells), NK cells, monocytes, and dendritic cells from normal/reactive and B-CLPD lymph node biopsies.
Material and Methods: 19 lymph node biopsies were evaluated, 7 normal/reactive, 5 with small lymphocytic lymphoma (SLL)/B-CLL, and 7 with B cell non-Hodgkin lymphomas (B-NHL). We quantified the distinct immune cell populations by flow cytometry, and evaluated the expression of IFNg in T and NK cells, and IL-17 in T cells, upon PMA plus ionomycin stimulation. IL-2 and IL-10 mRNA expression was further assessed in FACS-purified CD4+ and CD8+ T cells.

Results: We observed in SLL/B-CLL and NHL-B groups an increase in the frequency of activated T cells (expressing HLA-DR, $p<0.05$) and Treg cells, not reaching statistical significance in the latter cell population. We also found a tendency to a decrease of Th1/Tc1 cells, particularly in B-NHL group. Concerning the gene expression results, we found an increased IL-2 mRNA expression in both CD4+ ($p>0.05$) and CD8+ T cells ($p<0.05$) from SLL/B-CLL group, and an increased IL-10 mRNA expression in CD8+ T cells from both disease groups ($p<0.05$), which was more notorious in B-NHL. Moreover, a significant decrease in the frequency of CD56bright NK cells producing IFNg was observed in both disease groups ($p<0.05$).

Discussion/Conclusion: Our results, although preliminary, point to phenotypic and functional alterations on lymph node T and NK cells from B-CLPD, which may contribute to the prognosis of these entities.**P2468** BIOLOMINISCENCE IMAGING OF LEUKEMIC CELLS IN A MOUSE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA

Martínez, Andrea Ruth Losada Fernández Ignacio; Pérez-Chacón, Gema; Rosado, Silvia; Pérez-Aciego, Paloma; Vargas, Juan Antonio
Fundación LAIR

Introduction: Chronic Lymphocytic Leukemia (CLL) is the most prevalent leukemia in the adult population and remains an incurable disease. Mouse models of CLL are valuable tools for the elucidation of the underlying pathogenic mechanisms and for finding new therapies. The congenic New Zealand Black (NZB) strain is a model of CLL characterized by a spontaneous lymphoproliferation of B1 cells (B220low IgM+ CD5low). Unlike CLL, the number of circulating leukemic cells is low and do not correlate with tumor progression, hence making it difficult to follow-up disease progression and response to therapies.
To overcome these drawbacks, our goal was the generation of tumor cell lines (NZB-CLL) with a stable expression of a bioluminescent/fluorescent reporter by lentiviral transduction that permits detecting tumor cells in vivo with non-invasive imaging techniques.

Methods: Lentiviral transduced NZB-CLL cells (NZB-CLL-LV) were generated from diseased NZB spleen mononuclear cells grown in a co-culture system that selectively expands the tumoral cells, and transduced with a reporter construct encoding a fusion of eGFP and firefly luciferase. Phenotype was confirmed using flow cytometry and cell culture assays for luciferase activity. Transduced cells were transplanted into NOD.SCID mice and imaged using a high-resolution charge-coupled-device cooled camera. Fluorescence microscopy was used for the analysis of OCT-preserved tissue sections.

Results: Co-culture system lead to the long-term expansion of NZB-CLL lymphocytes, which could be maintained up to 5 months in vitro preserving phenotypic characteristics of the parental cells before and after lentiviral infection. The fusion construction was tested in a fibroblast cell line, showing a homogeneous and stable expression of eGFP and luciferase during 2 months in culture, both signals having a good correlation, thus allowing cells to be monitored by flow cytometry. Infection of NZB-CLL cells with this construction yielded high transduction rates (>85%). Cells transduced with the eGFP-Luc fusion or a control construction (NZB-CLL-LV) were transplanted into NOD.SCID mice with successful engraftment in both cases. Control mice (N=2) had leukemic NZB-CLL-LV cells infiltrating most lymphoid as well as several non-lymphoid tissues. Secondary lymphoid organs were enlarged with the majority of the tissue occupied by leukemic cells. Phenotype of control-transduced cells was maintained up to 9 months post-transplant, as assed by flow cytometry and fluorescence microscopy.
Cells transduced with the eGFP-Luc fusion were transplanted into one NOD.SCID mouse. This mouse is currently being followed for engraftment and disease progression using bioluminescence imaging (BLI). Luciferase signals compatible with spleen infiltration have been detected 30 and 60 days post-transplant, with enhanced signal at the latter time point, indicative of tumor growth.

Conclusions: Our co-culture system allows growing and maintenance of NZB-CLL cells during long periods of time before and after lentiviral infection with dual function reporter genes, and therefore the generation of genetically modified cell lines. These cells keep its original phenotype up to 5 months. Using BLI we were able to visualize the course of malignant disease including engraftment an expansion. This will allow us to track the neoplastic cells in vivo to assess in real time the effect of different pharmacological agents.

P2469 Lymphoid populations and regulatory T cells after allogenic stem cell transplantation and correlation with chronic cutaneous graft versus host disease

Paula Molés-Poveda (1),Lourdes Cordón (2), Amparo Sempere (2), Carlos Casterá (2), Felipe Casanova (2), Miguel Ángel Sanz(2), Rafael Botella-Estrada (1), Victoria Fornés (3), Jaime Sanz (2).
Hospital Universitario y Politécnico La Fe

Introduction: Nearly 15,000 allogenic hematopoyetic stem cell transplantation (allo-HSCT) are performed each year across Europe. Approximately 50% of these patients will develop a major late complication – chronic graft versus host disease (cGVHD) – a multi-organ allo- and auto-immune disorder affecting the skin, mucosae, lungs, liver, joints, and gastrointestinal genital tract which causes substantial morbidity and mortality.
Regulatory T cells (Tregs) promote immune tolerance to self- and alloantigens. In the context of allo-HSCT, Tregs inhibit GVHD by dampening the effects of donor immune cells in the grafted tissue.

Programmed death ligand 1 (PD-L1) is expressed on dendritic cells, tumour cells and normal tissue interact with PD receptors on T cells to modulate the balance of tolerance and immunity.
CTLA4 delivers an inhibitory signal to T cell activation and is required for T cell tolerance.
Our hypothesis was that Treg decreases in patients who developed cutaneous cGvHD and its absolute number correlates with disease severity.

Subjects and methods: We included 59 adult patients who underwent allo-HSCT for any medical condition from October 2015 to December 2016 in Hospital Universitario y Politécnico La Fe. Peripheral blood samples collected in EDTA tubes from April 2016 to March 2017 were obtained prospectively and were studied by flow cytometry at three different timepoints. In total 85 samples were processed (44, 29 and 12 at +100, +180 and +365 days, respectively). Cells were stained using combinations of monoclonal antibodies fluorochrome conjugated to study lymphoid populations (T, B and NK cells), PD-1, CTLA-4 and CD45RA expression on T cells and Tregs expressing nuclear transcription factor FoxP3.

Results: The median age of the patients was 42 years (18-70); 51% were female. Only 1 patient was transplanted for a severe aplastic anemia (1.2%) while the rest were transplanted for haematological malignancies (98.8%). Eleven out of 59 patients underwent a previous allo-HSCT. Thirty three out of 59 patients (56%) presented cutaneous cGVHD. Global cGVHD severity was grade 0, 1, 2 or 3 in 11%, 23%, 9% and 16%, respectively. Eight out of 59 patients (14%) relapsed.
T, B and NK lymphoid populations, as well as PD-1, CTLA-4 and CD45RA expression in T cells and Tregs were statistically analysed and neither the presence of Tregs nor the expression of CTLA4 or PD1 correlated with the presence of cutaneous cGVHD or with global its maximum severity.

Conclusion: Chronic GVHD remains the most common long-term complication of allo-HSCT. The discovery of valid biomarkers remains a significant challenge. Our results did not show correlation with the presence of cGVHD or its severity. However, some of our patients need further follow up until one year after transplantation. Additional studies in larger series should be performed to confirm these results.

P2501 DETECTION OF ENDOGENOUS ALKALINE PHOSPHATASE ACTIVITY IN INTACT LEUKEMIC CELLS

Laura G. Rico1, Jordi Juncà1,2, Mike D. Ward3, Jolene Bradford3 and Jordi Petriz1
1 Josep Carreras Leukaemia Research Institute, Badalona, Barcelona, Spain; 2 Institut Català d'Oncologia, Badalona, Barcelona, Spain, 3 ThermoFisher Scientific, Eugene, Oregon, USA.

Introduction: Alkaline phosphatase (ALP) is an enzyme highly expressed in pluripotent stem cells, embryonic stem cells, and embryonic germ cells. It has been classically used as a histochemical marker for the detection of these cells. Alkaline phosphatase activity is altered in some disease states, such as leukemia and lymphoma. With the aim to detect candidate malignant primitive progenitor populations in human leukemia and lymphoma cells, we modified the original ALP stem cell detection method based on the identification of alkaline phosphatase fluorescent cells in combination with flow cytometry immunophenotyping.

Methods: Peripheral blood and bone marrow samples from leukemia and lymphoma patients were studied at diagnosis, for minimal residual disease monitoring, and relapse. ALP staining was combined with leukemia immunophenotyping and no-lyse no-wash methods using the Attune™ NxT Flow Cytometer (Thermo Fisher Scientific). Blue Side scatter (B-SSC) and violet SSC (V-SSC) were detected to discriminate leukocytes. Vybrant™ DyeCycle™ Violet stain (DCV) was also used to discriminate nucleated cells from erythrocytes debris. Alkaline Phosphatase Live Stain was obtained from Thermo Fisher Scientific.

Results: Preliminary data obtained in our laboratory have shown that ALP can be expressed at high levels in leukemia. By using these newly developed panels, leukemic cells can be classified into different ALP functional states. Prospective comparison and classification of ALP+ cells also shows different subsets of primitive leukemic cells.

Conclusions: Our results suggest that the main differences in the activity of the enzyme, accordingly with previous observations showing that primitive stem cells express the highest phosphatase activity, could help to identify and differentiate new oligoclonal/pseudoclonal populations in patients with neoplastic malignancies. We have verified that this method gives accurate and reproducible

measurements and our preliminary results suggest that ALPhigh leukemic cells appear to sustain leukemogenesis over time.

IMMUNOLOGY

P2430 EOSINOPHILIA AFTER LIVER TRANSPLANTATION IS ASSOCIATED WITH IMMUNOPHENOTYPIC FEATURES ON THE OTHER PERIPHERAL BLOOD LEUKOCYTES

Catarina Maia1, Isabel Silva1, Susana Pedreiro1, Sandra Ferreira2, Cristina Gonçalves2, Susana Nobre2, Isabel Gonçalves2, Artur Paiva1
1Operational Management Unit of Cytometry/Clinical Pathology Service, Coimbra Hospital and University Center
2 Pediatric and Adult Liver Transplantation Unit, Coimbra Hospital and University Centre, Coimbra, Portugal

Abstract:

Introduction: In recent decades, several studies have reported that a higher eosinophil count (>500 cells/μL or 10% of total of leucocytes) can be a sign of acute cellular rejection, particularly in the first month after liver transplantation. Specifically, in children, the acquisition of persistent eosinophilia and different pathologies of allergic nature, might be associated with chronic liver graft dysfunction. However, the etiology of peripheral reactive eosinophilia, after pediatric liver transplantation, and their consequences on the liver graft dysfunction remain unclear. In this work we evaluated if the presence of eosinophilia induced phenotypic and functional alterations on peripheral blood basophils; monocyte subpopulations (classical, intermediate, non-classical and high affinity IgE receptor+ classical monocytes); plasmacytoid (pDC) and myeloid dendritic cells (mDC); B cell subpopulations (immature/transitional , naïve, IgA+, IgG+ or IgM+ memory (CD27+ and CD27-) or plasmoblasts, T cells (Th/c1; Th/c 17; Th9; Th producing IL-6 and γδ T cells) and NK cells (CD56bright and CD56dim).

Methods: This study was developed in Coimbra Hospital and University Centre. We studied 41 children submitted to pediatric liver transplantation (PLT) (20 females and 21 males) without peripheral eosinophilia (PLT - Eosinophilia) and 18 children (8 females and 10 males) with eosinophilia (PLT + Eosinophilia) at time of blood collection, whose average age were 9,7 ± 5,4 and 8,7 ± 5,0 respectively. The expression of CD123, CD33, CD15, CD16, CD14, HLA-DR and IgE bounded to its high-affinity receptor on peripheral blood monocytes, basophils, neutrophils, eosinophils and dendritic cells, as well as, the characterization of the different B cell compartments, was performed by flow cytometry. The quantification of the above described functional T cell subpopulations were also done by flow cytometry, after in vitro activation with PMA/ionomycin.

Results: We observed, in the eosinophilia group, a statistical significant increase in the expression of CD123 on eosinophils; of IgE bounded to its receptor on basophils, pDC, mDC and on a small subpopulation IgE+ classical monocytes and of HLA-DR on CD15+ classical monocyte subpopulation.
Concerning B cell populations, a significant increase in frequency and absolute counts of plasmablasts; of plasmablasts expressing IgA and of CD27+ memory cells expressing IgA was found in the eosinophilia group. Moreover, we also observed a higher frequency of Th17 cells, in the eosinophilia group. Interestingly a significant increase in the frequency of recurrent graft dysfunction was observed in PLT+Eosinophilia when compared with PLT-Eosinophilia (33,3% vs 12,2%).

Conclusions: Our results point to an IgE-mediating eosinophilia in PLT patients, since a significant increase of IgE bounded to its receptor was observed in all leucocytes that express FcεRI as well as higher levels of serum IgE, accompanied with significant alterations on B and T cell functional compartments and a higher degree of activation of eosinophils and classical monocytes, which, in some extension, could contribute on a higher incidence of recurrent graft dysfunction.

P2436 EFFECT OF CMV INFECTION AND AGEING ON THE EXPRESSION OF CD57, CD300A AND CD161 ON CD8+ T-CELLS

Rafael Solana Fakhri Hassouneh, Nelson Lopez-Sejas, Carmen Campos, Beatriz Sanchez-Correa, Raquel Tarazona, Alejandra Pera
University of Extremadura. Dept of Phisiology

Introduction: Immunosenescence is a progressive deterioration of the immune system with ageing. It affects both innate and adaptive immunity limiting the response to pathogens and to vaccines. As chronic cytomegalovirus (CMV) infection is probably one of the major driving forces of immunosenescence, and its persistent infection result in functional and phenotypic changes to the T-cell repertoire, the aim of this study was to analyze the effect of CMV-seropositivity and ageing on the expression of CD300a and CD161 inhibitory receptors, along with the expression of CD57 marker on CD8+ T-cells from healthy donors (n = 67) stratified by CMV-serostatus and age.

Methods: Peripheral blood from each subject was collected and followed by PBMCs isolation from each blood sample and CMV-specific IgG and IgM was determined by ELISA from plasma or sera of each sample. The percentage of cells expressing CD57, CD300a and CD161 was measured on CD8+ T-cells. Samples were acquired by multiparametric flow cytometry and analyzed with FlowJo v X 10.0.7 software. Boolean gating options were performed to analyze the co-expression of CD57, CD161, and CD300a markers and analysis of the phenotype profiles was performed by SPICE5.35 software. Ethical statement was approved by the Ethics Committee of the Reina Sofia University Hospital and all study participants provided informed written consent.

Results: Our results showed an increase of CD57 and CD300a expression on CD8+ T-cell in elderly CMV-seropositive donors when compared to young (CMV-seropositive and CMV-seronegative) and middle-age CMV-seropositive individuals. Whereas the expression of CD161 decreased progressively with age, being very low or null in the elderly compared to middle age and young individuals. CMV infection per se, increases the expression of CD57 on CD8+ T-cells, in young CMV-seropositive individuals compare to CMV-seronegative. While, CD300a and CD161 expression was only affected by age in CMV-seropositive individuals. Moreover, the co-expression analysis of CD57, CD161 and CD300a on CD8+T-cells showed that CD300a is expressed either alone or in combination with CD57 or CD161, while CD161+ T-cells were CD300a+ or CD300a-. Furthermore, the analysis of the phenotype profiles of CD57, CD161 and CD300a on CD8+ T-cells, showed that, in young and middle age individuals, the majority of CD8+ T-cells did not express any of the markers studied, while in old individuals the majority of cells are CD300+. On the other hand, the differences of the phenotype profiles of CD8+ T-cells were only due to age, and not to CMV latent infection.

Conclusions: Our results show that CMV latent infection and age contribute differentially to the phenotype of CD8+T-cells, highlighting the importance of including CMV serology in any study regarding immunosenescence. Additionally, our data support the potential use of CD57, CD300a and CD161 as biomarkers of immunosenescence.

P2447 CERVICAL LYMPHOCYTE POPULATIONS OF HIV POSITIVE WOMEN: ARE THEY RELATED TO HPV CO-INFECTION AND CERVICAL CANCER?

MJ Brito(1) A Quintas(1) F Ventura(3,4), C Martins(2), A Félix(2,5)
1 – Hospital Garcia de Orta, Almada, Portugal, 2 - CEDOC, Chronic Diseases Research Center, NOVA Medical School|FCM; Universidade Nova de Lisboa, Lisbon, Portugal,3 - NOVA Medical School|FCM; Universidade Nova de Lisboa, Lisbon, Portugal, 4 – Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal, 5 – Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG)

Introduction: Associations between HIV-related immunodeficiency, HPV infection and cervical carcinoma have been reported, even in controlled HIV infection, but there is scarce knowledge about the role of genital mucosa immunity.
Our study aims to characterize lymphocyte subsets in endocervical cytobrush (EC) samples of HIV+ women under cART therapy, with CD4>200 cells/uL and HIV viral load<40 and compare them according to the presence of HPV and cervical lesions.

Methods: 18 HIV+ and 13 HIV- women showing no colposcopic lesions were recruited. EC samples were collected to PBS, and incubated with monoclonal antibodies (CD3,CD4,CD8,CD16/CD56,CD19,CD45). Samples were acquired in a 4-color BD FACS Calibur and analyzed using Paint-a-Gate. For HIV+ women,

peripheral blood (PB) was also analyzed by flow cytometry, and viral load by real time PCR.
HPV typing was performed in Hologic Thin Prep® samples, using the DNA cobas® HPV test, which detects HPV16, HPV18, and a pool of High Risk (HR)-HPV types (31,33,35,39,45,51,52,56,58,59,66,68).
Liquid-based cytologies were reported according to the Bethesda system.
Statistical analysis was performed with GraphPadPrism 6 software. Significance was considered for $p<0.05$.

Results: 31 EC samples were collected and analyzed, 8 showed no lymphoid cells (5 HIV+; 3 HIV-). Percentages of T (CD4 and CD8), B and NK-cells were evaluated within total lymphocytes. Compared to HIV-, HIV+ women presented lower percentages of CD4 T-cells ($p=0.0147$), higher percentages of CD8 T-cells ($p=0.0025$) and lower CD4/CD8 ratios ($p=0.0041$). No significant differences were identified for total T, B and NK-cells between HIV+ and HIV- women.
In HIV+ women, different percentages of CD4 ($p=0.0479$) and CD8 T-cells ($p=0.0007$), and CD4/CD8 ratios ($p=0.0081$) were found between PB and EC samples. Nevertheless, parameters varied similarly and were significantly correlated in both sample types (CD4: $p=0.0004, r=0.7610$; CD8: $p=<0.0001, r=0.8710$; CD4/CD8 ratio: $p=0.0020, r=0.763$).
7/18 HIV+ women and 3/13 HIV- women presented HR-HPV (Table 1). No significant differences were identified in PB or EC lymphocyte subsets comparing HIV+ women with and without HR-HPV infection.
High-grade and low-grade squamous intraepithelial lesions (HSIL/LSIL) were identified in 5/18 HIV+ women, all with HR-HPV. A single LSIL was observed in HIV- women, whom also had HR-HPV. Higher percentages of circulating CD8 T-cells ($p=0.0460$) and a trend for lower CD4/CD8 ratios ($p=0.0568$) were observed in HIV+ women with HSIL/LSIL, compared to HIV+ women negative for intraepithelial lesion or malignancy (NILM).

Conclusions: According to our data it was possible to effectively identify cervical lymphoid populations and to understand that cervical CD4 and CD8 T-cells subsets are correlated with their circulating counterparts.
Moreover, as expected, we confirmed that cervical CD4 T-cells and CD4/CD8 ratios were decreased in HIV+ women compared to HIV- women.
Finally, the occurrence of higher percentages of circulating CD8 T-cells seems to be related with the presence of squamous intraepithelial lesions in HIV+ women, and eventually to HR-HPV, since it was found in all women with HSIL/LSIL.
Further conclusions are limited by the small number of samples analyzed so far, and so we aim to further evaluate the impact of the local and systemic immune imbalance in the development of cervical neoplasia.

Table 1 - HIV+ women: CD4/CD8 ratios (PB and EC), HR- HPV and Cervical Cytology.

HIV+ women	PB CD4/CD8	EC CD4/CD8	High Risk HPV	Cytology Report
1	0,43	*	Yes	**LSIL**
2	0,49	*	Yes	NILM
3	0,36	0,32	Yes	**HSIL**
4	0,94	*	Yes	NILM
5	1,79	1,29	Yes	**HSIL**
6	0,72	*	Yes	**HSIL**
7	0,43	0,25	Yes	**LSIL**
8	2,72	1,25	No	NILM

9	0,87	0,79	No	NILM
10	1,72	0,91	No	NILM
11	0,80	0,59	Yes	NILM
12	0,83	1,30	No	NILM
13	1,47	1,20	No	NILM
14	1,44	0,82	Yes	NILM
15	0,51	0,33	No	NILM
16	1,81	1,46	No	NILM
17	1,13	*	Yes	NILM
18	1,37	0,92	No	NILM

* Women without lymphoid cells in EC samples
PB – Peripheral blood samples
EC – Endocervical cytobrush samples

Bethesda cytology report system (2014)
HSIL – High-grade squamous intraepithelial lesions
LSIL – Low-grade squamous intraepithelial lesions
NILM – Negative for intraepithelial lesion or malignancy

P456 TEMPORAL EVOLUTION AND DIFFERENCES IN LYMPHOCYTE PROFILES IN SJÖGREN'S SYNDROME SSA+

Filipe Barcelos1, 2, 3, Catarina Martins3, José Vaz Patto1, Glória Nunes3, Teresa Lopes3, João Amaral4, Joana Cardigos5, Nuno Alves5, Jaime C. Branco3, 6, Luís Miguel Borrego2,3

1Instituto Português de Reumatologia, Lisboa, Portugal
2Hospital CUF Descobertas, Lisboa, Portugal
3CEDOC, Chronic Diseases Research Center, NOVA Medical School|Faculdade de Ciências Médicas; Universidade Nova de Lisboa, Lisboa, Portugal
4Faculdade de Medicina Dentária, Universidade de Lisboa, Lisboa, Portugal
5Hospital Sto. António dos Capuchos, Centro Hospitalar de Lisboa Central, Lisboa, Portugal
6Hospital Egas Moniz, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal

Introduction: Primary Sjögren's syndrome (SS) is a heterogeneous disease. While most patients have a mild clinical course, some have increased inflammatory activity and risk of potentially severe extra-glandular manifestations. Anti-SSA antibodies are the most typical humoral markers in SS, and are associated with increased clinical expression of the disease. We aimed to evaluate clinical and immune parameters of two populations of anti-SSA+ SS patients with different disease duration.

Methods: We have included 37 anti-SSA+ SS patients (classified according to the AECG criteria), 17 diagnosed <2 years (SS<2) and 20 with a diagnosis for >10 years (SS>10) and 22 healthy controls. Demographic, clinical, and laboratory data were collected, and sialometry, Schirmer I test, lacrimal break test (BUT), and keratitis evaluation were performed. Lymphocyte subsets were characterized by flow cytometry: follicular (Tfh) and regulatory (Treg) T cells, maturation stages of B cells, plasmablasts (PB) and regulatory B cells (Breg). Statistical analysis was performed with GraphPadPrism6, with significance for $p<0.05$.

Results: SS>10 patients were older ($p=0.0112$) compared to SS<2, but age at onset and time till diagnosis were similar. Lower salivary function was evident in SS>10 patients, in whom keratitis was also more frequent (55.0% vs 17.6%). Extraglandular manifestations (MEG) were more frequent in

SS>10 patients, who also had greater ESSDAI activity indexes compared to SS<2 (4.0 vs 1.8).
In the evaluation of lymphocyte subsets, SS patients presented less circulating lymphocytes (p≤0.0372), with lower counts of T cells (p=0.0008), B cells (p=0.0449), NK cells (p=0.0450) and CD4 T cells (p<0.0001). CD4 T cells percentages were also decreased in SS patients (p=0.0001), who had on the contrary higher CD8 T cells percentages (p=0.0006). Treg cells (p=0.0012), activated CD4+CD25+ T cells (p≤0.0004), and Th17 cells (p = 0.0003) were also diminished in absolute counts in SS patients. IL-21-producing TFh cells were increased in SS patients (p=0.0021) as well as TFh1 cells (0.0065). In the B cell compartment, percentages and absolute counts of memory subpopulations were decreased in SS patients, resulting in an increase of the Naïve/Memory B cells ratios (p=0.0248). SS patients had also lower CD24hiCD27+ B cells (p≤0.0006) and increased percentages of plasmablasts compared to controls (p=0.0347).
Within SS patients, lower percentages of Treg cells were observed in SS>10 (p=0.0241). Total B cell percentages were also decreased in SS>10 patients compared to SS<2 (p=0.0144). SS>10 patients exhibited a trend to decreased percentages of memory B cells, and on the contrary increased percentages of plasmablasts.

Conclusions: SS patients with anti-SSA antibodies and longer disease evolution have a greater impairment of salivary and lacrimal functions, and a greater occurrence of MEG and systemic activity. Although the small size of our cohort may limit the extend of our conclusions, our data suggest that SS evolution is accompanied by a reduction in Treg cells, along with alterations in the circulating B cell compartment, which may reflect the progression of the immune imbalance and its possible impact on the occurrence of more severe clinical manifestations.

P2463 EVALUATION OF CD4 T CELLS COUNTS AFTER CHANGES IN ANTIRETROVIRAL THERAPY WITH DOLUTEGRAVIR IN HIV PATIENTS

Manuela Rebordão Sandra Francisco, Diana Fernandes, Manuel Silva
Hospital Forças Armadas

Introduction: Antiretroviral therapy (ART) regimens used to treat individuals with HIV infection consists of a dual nucleoside combination plus a third agent from a different class. HIV-1 integrase is essential for viral replication; integrase inserts viral DNA into the cellular genome of the host CD4 cells. Loss of integrase activity disrupts the viral life cycle. Regimens using integrase strand transfer inhibitors (INSTIs) are considered to be the preferred third agent in combination with two nucleoside analogues. Dolutegravir (DTG), an INSTIs, has recently been approved for the treatment of HIV-1 disease in combination with other antiretroviral agents.
Aim: Our study wants to evaluate the therapeutic effect of switching the ART regimen, introducing DTG in the treatment of HIV patients, determined by CD4 T cells count and viral load changing.

Methods: We studied 28 Hiv-1 infected patients; all men with 56 average age (40-73) and treated with ART regimens for long time.We evaluated viral load and CD4+ T cells before and after changes in combination therapy. There were three different ART regimens. Drug classes combination were: Group 1) 14 patients with 2NRTIs (nucleoside and nucleotide reverse transcriptase inhibitors) plus 1NNRTIs (non nucleoside reverse transcriptase inhibitors) which switched the NNRTIs to DTG; Group 2) 10 patients with 2NRTIS plus 1 PI (Protease inhibitors) which switched the PI to DTG; Group 3) 4 patients with 2NRTIs plus 1 INSTI (Raltegravir) switched to DTG. Study´s median time after therapy changes was 13 months (10-21). Statistical analyses were done with parametric tests; SPSS-Wilcoxon test.

Results: Median CD4 T cells counts in our 28 patients, before and after therapy combination changes, were 29% (672.9 mm3) and 30.6% (754.7 mm3) respectively, showing a significant increase in CD4 T cells, p=0.009 (p=0.013). All patients had viral load undetectable before and after therapy changes. Evaluating separately the three groups, CD4 T cells had a significant increase only in group 1, p= 0.019. No statistically significant results were found with other changes in ART.

Conclusions: Changing ART regimen adding Dolutegravir may improve immunologic function, as we can see by CD4 T cells count increase and sustained virologic suppression. Results of this study, although a small population, indicate that Dolutegravir may have impact on patient's outcome leading to improvements in cellular immunity.

P2464 TREGS ABSOLUTE COUNT EVALUATION IN CGVHD TREATMENT WITH LOW-DOSE IL2: A CASE REPORT

Sousa ME Fragoso G, Godinho I, Palmeira C, Azevedo C, Pires A, Campilho F, Campos A, Martins G
Serviço de Imunologia do IPO-PortoFG, EPE e Serviço de Transplantação de Medula Óssea (STMO) do IPO-PortoFG, EPE

Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) can result in therapeutic graft versus tumor activity (GVTA) and toxic graft versus host disease (GVHD). Chronic GVHD (cGVHD) is a systemic inflammatory disorder with autoimmune manifestations and develops in more than half of patients who undergone HSCT. 1,2,3
Regulatory T cells (Tregs) suppress autoreactive lymphocytes and control innate and adaptative immune responses. Tregs are associated with tolerance and autoimmunity and prevent GVHD, after HSCT.1,2,3
Interleukin-2 is critical for Treg cell growth, survival and activity. Low-dose IL2 could enhance Tregs in vivo and suppress clinical manifestations of cGVHD. 1
In the present case, we evaluate the absolute count of peripheral blood (PB) lymphocytes subpopulations, including Tregs, by flow cytometry, during the administration of low-dose IL2 to a patient with cGVHD.

Methods: A 9 year male child, diagnosed with Schachman-Diamond Syndrom, with a marked neutropenia and thrombocytopenia, was submitted, on May 2016, to allotransplant with HCST of non relational donor. First, he developed acute GVHD (aGVHD), followed by cGVHD, refractory to several therapeutic lines, like high-dose of glucocorticoid.
Since January of 2017, he initiated treatment with low-dose of IL2, in association with Prednisolone (0.6mg/kg of body weight) and Imatinib (100mg/day) with good tolerance.
The PB samples were obtained at pre-IL2 administration (T0), and at 3 (T1), 9 (T2), 16 (T3), 23 (T4), 37 (T5) and 51 (T6) days after IL2 administration.
The absolute counts of lymphocytes subpopulations, like T cells subsets, were analyzed with 7-color immunofluorescence and spheres (TruCount, tubes; BD biosciences), using a CANTO II flow cytometer (BD biosciences) and the Infinicyt (Cytognos) software for the analyses of the files.
The panel for T cell subpopulations characterization was:
Tube 1: 4 /x /3/16+56/45/19/8/14 andTube 2: 4 /x /56/25/45/127/8/3, where the respective fluorocromes were: Pacific-Blue/X/FITC/PE/PerCP/PC7/APC/APC-H7
Treg cells were defined as: CD3+CD4+CD25++CD45+CD127-/+low and Tconventional (Tcon) were defined as: all the CD3+CD4+CD45+ not Tregs4

Results: During IL2 administration, the number of Tregs increased from 8 cell/µL at T0 (baseline), to 22 cell/µL at T1, 150 cell/µL at T2, 108 cell/µL at T3, 109 cell/µL at T4, decreased to 54 cell/µL at T5 and 23 cell/µL at T6.
The number of Tcon at T0 was 63 cell/µL and also increased during IL2 administration, to 93 cell/µL at T1, 740 cell/µL at T2, decreased to 410 cell/µL at T3, 269 cell/µL at T4, 90 cell/µL at T5 and 74 cell/µL at T6.
The Treg/Tcon ratio increased after IL2 treatment: being 2 times higher at T1 going up to 5 times at T5.

Conclusion: In this present case, the administration of low dose of IL2 enhanced Treg cells, with a markedly increase at T5 counts.
The Treg/Tcon ratio showed to be a good parameter to evaluate the expansion of Tregs. Although, the absolute number of Treg seemed to decrease, they were expanded in the CD4+ compartment.
Flow cytometry proved to be a useful technique to monitor the immune response to IL2 treatment.

References

1.Koreth Jj et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. N Egl J Med 2011; 365:2055-66
2.Bolton H A et al. Selective Treg reconstitution during lymphopenia normalizes DC costimulation and prevents graft-versus-host disease. J Clin Invest. 2015; 125(9):3627-3641.
3.Xhaard et al. Reconstitution of regulatory T-cel subsets after allogeneic hematopoietic SCT. Bone Marrow Transplantation (2014) 49, 1089-1092.
4.Lastovicka J. The phenotypic markers of CD4+CD25+ T regulatory lymphocytes. An International Journal, vol 2013 (2013), article ID119348.

P2466 A REAL-TIME FLOW CYTOMETRY ASSAY OF THE ACTIVITY OF NA+/H+ EXCHANGER IN WHOLE-BLOOD LEUCOCYTES AND HUMAN LEUKEMIC CELLS

Ana Giménez1, Guadalupe Herrera2, Beatriz Jávega1, Maria-Do-Ceu Monteiro3, Julián Ochando1, José-Enrique O'Connor1

1 The University of Valencia, VALENCIA, Spain
2 Incliva Foundation, VALENCIA, Spain
3 CESPU, Polytechnic Health Institute of the North, GANDRA, Portugal

Introduction: The Na+/H+ exchanger (NHE) is an integral membrane protein that extrudes H+ in exchange for extracellular Na+ and plays a crucial role in the regulation of intracellular pH (pHi). NHE is critical for the maintenance of pHi during ischemia, but there is growing evidence of a pivotal role for NHE in tissue injury during ischemia and reperfusion. We had previously set up (Dolz M et al. Cytometry A. 2004; 61:99) and validated (Lequerica JL et al. J Physiol Biochem. 2006; 62: 253) a flow cytometry kinetic method to measure the activity of NHE in different adherent cell lines. Here, we present an adaptation to determine NHE activity in leucocytes from human whole blood samples and to compare pHi regulation with human leukemic cells.

Methods: Samples (25 mL) of human whole blood anticoagulated with EDTA, citrate or heparin were incubated with 5 mL of CD45-PECy5 antibody for 15 min and diluted to 1 mL with bicarbonate-free RPMI 1640 medium containing 25 mM HEPES, to abolish the activity of other cellular pH-restoring systems. Then, 5 mL of the fluorescent pH-indicator BCECF-AM (1 mM) were added and the sample incubated for 15 min at 37° C in the dark with or without 4 mM ethyl-isopropyl-amyloride (EIPA). Samples were run on an Accuri C6 or a Gallios flow cytometers and the following parameters acquired: FS, SS, CD45-PECy5 red fluorescence, BCECF fluorescences at 525 nm (FL1) and 620 nm (FL2) and the Ratio FL2/FL1 (an estimation of pHi) and Time (up to 300 s). Sample was run for 10 s, then paused, and appropriate concentrations of sodium propionate (ProNa) or ammonium chloride were added to induce intracellular acidification or alkalynization, respectively. Data acquisition was re-started to show decreased (acidification) or increased (alkalinization) ratio FL2/FL1 and subsequent restoration oh pHi. Similar experiments were performed using Jurkat cells (T-cell leukemia) and U937 cells (monocytic leukemia).

Results: In whole-blood unlysed samples gating on CD45 showed clearly leukocyte subpopulations. Analysis of pHi showed that leukocyte subpopulations differed in resting pHi, granulocytes being more alkaline. Kinetic analysis of pHi following intracellular acidification or alkalinization was dependent on the type of anticoagulant used and showed heterogeneity in WBC responses. Selection of specific subpopulations on SS vs CD45 expression showed that lymphocytes do not recover pHi, whereas monocytes and granulocytes return rapidly to resting pHi. The participation of NHE is confirmed by the inhibitory effect of EIPA in all leucocytes. There were also differences between normal lymphocytes and Jurkat cells or normal monocytes and U937 cells.

Conclusions: This is a simple and convenient assay allowing to examine in real time the acidification and alkalinization responses in leucocyte subpopulations and in leukemic cells. The assay may be useful for basic and applied studies in the immuno-hematological and immunotoxicological areas. Supported by grant UV-INV-AE15-349700 (University of Valencia).

P2467 MULTIPARAMETRIC FLOW CYTOMETRIC ANALYSIS OF PERIPHERAL WHOLE BLOOD LYMPHOCYTE SUBPOPULATIONS AT ONSET OF TYPE 1 DIABETES

Aina Teniente-Serra1,2, Eduarda Pizarro3, Mª Teresa Julián3, Marco A Fernández4, Eva Mª Martínez-Cáceres1,2

1Immunology Department Hospital Universitari Germans Trias i Pujol, Badalona (Spain), 2Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona , Bellaterra (Spain), 3Department of Endocrinology, Hospital de Mataró (Spain), 4Flow Cytometry Facility, Germans Trias i Pujol Research Institute (IGTP), Campus Can Ruti , Badalona (Spain).

Introduction: Type 1 diabetes (T1D) is an autoimmune disorder characterized by destruction of pancreatic beta cells resulting in insulin dependency. Changes in T and B cell subpopulations in

peripheral blood of T1D patients have been described, but a comprehensive multiparametric flow cytometric analysis is still lacking.

Aim: To identify changes in the T and B peripheral blood cell compartments in patients at onset of T1D.

Material and methods: CD4+ and CD8+ T cells (including naïve, central memory, effector memory and terminally differentiated effector (TEMRA), Th17 and Tregs) and B cells subsets (naïve, unswitched memory, switched memory and transitional B cells) were analyzed in peripheral blood of T1D patients at onset (n=26) and healthy donors (HD; n=40) using 8-parameter flow cytometry.

Results: A decrease in the percentage of early and late effector memory CD4+ and CD8+ T cells (T CD4+: p=0.001 and p<0.001, T CD8+: p=0.046 and p<0.001), TEMRA CD4+ and CD8+ cells (p=0.003 and p=0.004, respectively) was found. In contrast, the percentage of naïve CD4+ T cells (p=0.010), and percentage and absolute counts of naïve CD8+ T cells (p<0.001 and p=0.001) were increased in peripheral blood of T1D patients compared with HD. Moreover, an increase in percentage of total B cells and transitional B cells was observed in patients compared with HD (p=0.015 and p=0.006, respectively). No changes were found either in Tregs or in Th17 subpopulations.

Conclusion: The observed changes in the percentage and/or absolute number of effector memory and naïve T cell subpopulations as well as transitional B cells at onset of T1D suggest that these cells can play a role in the pathogenesis of the disease.

P2471 PHAGOCYTOSIS IN DOLPHINS: A MODEL TO EVALUATE IMMUNOLOGICAL EFFECTS OF ENVIRONMENTAL TOXICS

Alicia Martínez-Romero1, Mar Felipo-Benavent2, Consuelo Rubio-Guerri2, José-Enrique O´Connor1,3

(1) Cytomics Core Facility, Príncipe Felipe Research Center (CIPF). Valencia, Spain
(2) Comunidad Valenciana Oceanogràfic Foundation. Valencia, Spain
(3) Cytomics Laboratory. Mixed Unit UVEG-CIPF. Valencia, Spain

INTRODUCTION: Dolphins are long-lived cetaceans, top predators in the marine environment. As they belong to the upper part of the aquatic food chain, cetaceans can bioaccumulate environmental contaminants. For this reason, dolphins and other cetaceans are considered sentinels of the marine environment. In addition to pollutants, different conditions leading to stress exert well-known effects on the immune system in laboratory animals and humans. However, knowledge of the immune system of cetaceans and its impairment by contaminants and by stress is still scarce. Immunomodulation induced by xenobiotics and stress may influence the responses of cetaceans against pathogens such as viruses, bacteria and parasites, thereby increasing susceptibility to infections. In this context, phagocytosis is one of the first defensive mechanism against external infections, and this process might be an indicator of immunological functionality. In fact, analysis of phagocytosis by flow cytometry (FCM) is now a frequent assay in human clinics and commercial products are available for routine studies. In this presentation we show the preliminary results of the application to cetaceans of a whole-blood commercial assay designed for the evaluation of phagocytosis in humans.

METHODS: Blood samples from healthy dolphins (Tursiops truncatus) were obtained from the Oceanogràfic of Valencia, Spain. Phagocytosis was analyzed using the IngoFlow® kit (EXBIO, Prague, Czech Republic). Briefly, heparinized peripheral blood was incubated with FITC-labelled Escherichia coli (E.coli FITC) at 37ºC for one hour and then washed to eliminate extracellular bacteria. For quenching the remaining extracellular and the surface bound bacteria trypan blue was included.. The percentage of FITC-positive of phagocytic cells was evaluated in a Cytomics FC500 flow cytometer (Beckman-Coulter, Brea, CA, USA). Forward scatter (FS) and side scatter (SS) signals were used to define the morphology of leukocytes. The propidium iodide staining included in the assay to label nucleated cells was detected at 620 nm fluorescence emission and phagocytosis was estimated from FITC fluorescence emission at 525 nm. Two negative controls were included in the same assay: incubation of whole blood with E.coli FITC on ice and pre-incubation of whole blood with 10 µM cytochalasin A for 30 minutes.

RESULTS: Application of the IngoFlow® kit to samples of healthy dolphins allowed to detect phagocytosis in whole blood samples in a comparable degree to human samples run in parallel

experiments with the same reagents and procedure. The percentage of phagocytic cells in healthy dolphins was typically between 60-80 % of monocytes and granulocytes.

CONCLUSIONS: The phagocytosis assay with the commercially available IngoFlow® kit can be easily and conveniently applied in dolphins in a similar way to its use for human samples. This assay can thus be applied to detect alterations in the immune functions in dolphins as a consequence of intoxication, sickness or stress, as for example in stranded individuals.

P2472 NK FREQUENCY AND CYTOTOXIC ACTIVITY IN RECURRENT MISCARRIAGE WITHOUT ANTIPHOSPHOLIPID SYNDROME

Irure J1, Asensio E1, Riesco L1, Arroyo JL2, López-Hoyos M1, San Segundo D1.

1Immunology Service. Hospital Universitario Marqués de Valdecilla-IFIMAV. Santander. Spain
2Regional

Introduction: Early stages of pregnancy involve complex processes including maternal hormonal changes as well as immune responses. Alterations in these aspects could lead to pregnancy loss. Specifically, natural killer (NK) cells may have an effect on reproductive performance, since elevated number of this type of cells have been found in women with recurrent miscarriage.The goal of this study was to determine the percentage and the cytotoxic activity of NK cells in women with recurrent miscarriage according to a previously stablished reference range.

Methods 9 samples from healthy women from 20 to 40 years and 40 samples from women with recurrent miscarriage (RM) without antiphospholipid syndrome and prior inmunomodulant treatment were analyzed in order to determine differences in the percentage of NK cells and the cytotoxic activity using flow cytometry. Cytotoxicity of NK cells was measure by the expression of CD107after co-culture with K562 cell line as previously described(1) and adquired on Navios 6C (Beckman Coulter Inc).

Results: The median frequency of NK cells was increased in healthy controls compared with RM group 15.0 [11.4-18.4] vs 11.13 [8.6-16.1] respectively, p=0.053. Moreover, the mean cytotoxic activity was significantly augmented in control group 34.6 (8.82) vs 22.7 (10.6), p<0.004. However, no correlation between % NK cells and cytotoxicity was observed in control group (r2 =0.13, p=0.34) whereas in RM group a negative correlation was observed (r2=0.13, p=0.02).

Conclusion: The patients with RM without treatment had lower frequency and cytotoxic activity of NK cells than controls but the clinical implication of these findings should be assessed in controlled prospective studies.

ADVANCES AND NOVEL APPLICATIONS OF FLOW CYTOMETRY

P2435 IMMUNOPHENOTYPIC CHARACTERIZATION OF THE ENDOTHELIAL CELLS ISOLATED FROM VARICOSE SAPHENOUS VEINS AND NORMAL SAPHENOUS VEINS USED IN BYPASS SURGERY

Autores: Cláudia Torres1,2, Rui Machado3, Margarida Lima2,4

1Doutoramento em Ciências Biomédicas, ICBAS/UP, Portugal (aluna);
2UMIB/ICBAS/UP;
3Serviço de Angiologia e Cirurgia Vascular, HSA/CHP, Porto, Portugal;
4Laboratório de Citometria, Serviço de Hematologia Clínica, HSA/CHP, Porto, Portugal.

Introduction: Varicose veins are a manifestation of chronic venous disease and affect about one third of the adult population. Although the predisposing factors for its development are known, its etiology and pathogenesis are not fully understood. In recent years, evidence has emerged that the formation of varicose veins may be secondary to cellular changes and / or extracellular matrix components of vessel walls, with the possible involvement of endothelial cells (EC).

Objectives: To characterize, by flow cytometry, to compare the immunophenotype of the EC from varicose saphenous veins (SV) with that of the EC from normal SV used in bypass surgery (controls).

Methods: EC were isolated by mechanical method from varicose SV segments of 15 patients undergoing varicose vein surgery and normal SV from 10 individuals undergoing bypass surgery (controls). Immunoblotting of EC was done with the following antibodies: anti-CD45 (pan-leukocyte); Anti-CD31 (PECAM, CE marking and intercellular junction molecule) and anti-CD146 (CE marking and intercellular junction molecule), anti-CD54 (ICAM-1 activation marker), anti-CD62E (E-selectin, activation marker), anti-CD106 (VCAM-1, activation marker) and anti-CD142 (tissue factor, procoagulant marker). The procedure used was that of lysis without washing. The samples were collected on a FACSCanto II cytometer (Becton Dickinson) and the data were analyzed with the INFINICYT program (Cytognos). EC were identified as CD45(-), CD31(+) and CD146(+) cells. The expression of the studied molecules was evaluated by the corresponding mean fluorescence intensity.

Results: We found that the EC of the varicose SV presented lower intensity of expression of all markers evaluated ($p < 0.05$), including intercellular junction molecules (CD31 and CD146), activation-related markers (CD54, CD62E, CD106) and pro-coagulant molecules (CD142), as compared to controls.

Conclusions: EC from varicose SV differ phenotypically from EC of normal SV and present lower expression of several molecules including, contrary to what was expected, of markers of EC activation and procoagulant activity. These alterations may result from the dysfunctionality and, possibly, from the lower viability of the EC from SV varicose veins, with a consequent decrease in the responsiveness to stimuli.

Correspondência para Cláudia Torres, Laboratório de Citometria, Serviço de Hematologia Clínica, Centro Hospitalar do Porto, Rua D. Manuel II, s/n, Porto; Telemóvel. +351 969015913; @-mail: torres.cb@gmail.com

P2438 INTRACELLULAR PH DETERMINATION BY FLOW CYTOMETRY TO EVALUATE THE EFFECT OF DIFFERENT PROTON PUMPS INHIBITORS IN TUMORAL CELLS.

Javier Godino Gómez1*, Eduardo Chueca Lapuente2,3, César Vallejo Ruiz1 and Elena Piazuelo Ortega2,3,4

1 Instituto Aragonés de Ciencias de la salud. Unidad de Separación Celular y Citometría
2 CIBERehd,Madrid,Spain
3 Instituto de Investigación Sanitaria Aragón,Zaragoza,Spain
4 Instituto Aragonés de Ciencias de la Salud, CIBA, Zaragoza, Spain.
* Correspondence: jgodino.iacs@aragon.es

Background: Disturbance of intra/extracellular pH gradient, as a consequence of overexpression of some membrane-bound proton transporters is a feature of neoplastic cells, which has a profound impact on cell metabolism and has been recurrently shown to be involved in neoplastic transformation as well as tumoral progression and metastasis. We aim to evaluate the impact of pharmacological inhibition of different H+ transporters on intracellular pH (pHi) of esophageal adenocarcinoma cells.

Methods: Two cell lines were used in this study, the OE33 cell line (ECACC), established from an adenocarcinoma of the lower esophagus arising in Barrett's esophagus and OACM5.1C cells (ECACC), established from a lymph node metastasis derived from a primary Barrett's esophageal adenocarcinoma. Cells were incubated for 2 hours in the presence of AZD3965 (100nM), a selective inhibitor of MCT1 lactate carrier and acetazolamide (100µM), a carbonic anhidrase inhibitor. The effect of glucose overload in culture medium (30mM final concentration) was also evaluated.
pHi was evaluated in cells by flow cytometry using the pH-sensitive fluorescent probe SNARF-1 AM. Briefly, 1 ml of cells (106 cells/mL) were resuspended and incubated with 2.5µl SNARF-1 AM (2mM) for 30 min at 37° C. pHi was determined by the 563/600 nm fluorescent ratio with a FACSAria cytometer following the nigericin calibration procedure. Calibration was achieved by resuspending dye-loaded cells in high-potassium buffers in the presence of the proton ionophore nigericin, which allows free H+ and K+ exchange across their concentration gradients, thus matching pHi to a set of known pH values. In our experiments the calibration curve had 5 pH points: 6.5, 7, 7.5, 8 and 8.5.
Experiments were performed at least in triplicate. Statistical analysis was performed by Student-t test. Results are expressed as mean ±SE of pHi values.

Results: Metastatic cells displayed a slightly more acidic pHi (7.29±0.06) than the non-metastatic

tumor cells (7.49±0.07). Neither acetazolamide treatment nor glucose overload altered pHi in both cell lines (7.55±0.08 in acetazolamide-treated OE33 cells vs 7.46±0.09 in control cells, p=0.08 and 7.29±0.05 vs 7.29±0.04, p=0.61 in OACM5.1C cells; 7.5±0.08 in glucose-treated OE33 cells vs 7.49±0.07 in control cells, p=0.21 and 7.32±0.05 vs 7.29±0.06, p=0.33 in the metastatic cell line). In contrast, treatment with AZD3965 significantly increased pHi values in the two cell lines (7.62±0.1 vs 7.49±0.07, p=0.03 for OE33 cells, and 7.52±0.03 vs 7.29±0.06, p=0.04 for OACM5.1C cells). These results indicate that in our cell model, increasing glucose concentration has no effect on pHi and that a blockade of MCT1 but not carbonic anhidrase transporters alters tumor pHi, suggesting a different role of the different proton transporters in the homeostasis of tumor pH in esophageal adenocarcinoma cells.

Conclusion: Inhibition of proton transport can affect pHi of cancer cells, and flow cytometry showed to be a reliable and useful approach to evaluate changes in pHi.

P2444 HOW TO RELIABLY CHOOSE SETTINGS FOR OPTIMAL RESOLUTION IN A MULTICOLOR FLOW EXPERIMENT

Tania López1, Ultan P.Cronin1, Miguel Ángel Sánchez-Luengo1, Lola Martínez1.
1.Flow Cytometry Core Unit. Spanish National Cancer Research Center (CNIO). Madrid-Spain.

Multicolor flow cytometry rapidly reveals a large amount of biological information about a single sample (1). Over the past few years, the number of parameters simultaneously analyzable in typical flow cytometry experiments has increased from the typical 2-4 color experiments to 8+ color experiments routinely run on the labs these days (2). Not only has this increased the power of flow cytometry, but it has also increased the importance of a proper experimental setup to ensure accurate and meaningful results (1). Certain factors such as fluorochromes spillover and the selection of the proper voltage for each detector have a direct influence on populations spread, which in turn, have a clear impact on population resolution, and a loss of accuracy and significance of the data.

The selection of the proper voltage for each detector, it's essential in order to obtain the minimum spread and the maximum resolution of our populations during acquisition (3). Instrument resolution is dependent on the electronic noise, which can be defined as the background signal caused by electronics. An increase on electronic noise will result in a decreased resolution sensitivity of our populations of interest (4). Currently, there are three different approaches that people use to select the optimal voltage for the experiment.

Conventionally, many users set up the voltage “by eye”, increasing it until they get the best separation between the positive and negative populations.
Manufacturers have developed over the years different approaches, such as the CST (Cytometer Setup Tracking) BDTM method, which looks at background and detector efficiency to determine a minimum PMT voltage setting for each detector, ensuring the populations will be set over the electronic noise. This method is based on the use of CST beads that contains dim, mid-range and bright emission intensities. These beads are only valid on BD DIVA instruments.
Another approach, described here, involves the assessment of the electronic noise of each PMT in your instrument and to employ the voltage required to obtain approximately 2.5 times the rSDEN (Robust Standard Deviation of the Electronic Noise) in each detector. This should ensure that we use a voltage where the electronic noise does not interfere with the measurements at the low end of the scale. This approach could be used for all cytometers and will employ your unstained beads or cells to perform such calculations.
Our goal here is to compare these methods and determine which is both convenient and best able to establish optimal, consistent and reproducible cytometer settings for multicolor immunophenotyping applications, while ensuring that the voltage settings will be sufficient to resolve various lymphocyte subpopulations present in mouse spleen using an 8-color inmunophenotyping panel and a FACS CANTO II flow cytometer equipped with 488nm, 640nm and 407nm lines.

References

1.BD Technical Bulletin. 2009. An Introduction to Compensation for Multicolor Assays on Digital Flow Cytometers. BD Biosciences.

2.Perfetto, S.P., Chattopadhyay, P.K. and M. Roederer. 2004. Seventeen-colour flow cytometry: unravelling the immune system. Nat Rev Immunol.4:648-655.

3.Maecker, H.T. and J. Trotter. 2006. Flow Cytometry Controls, Instrument Setup, and the Determination of Positivity. Cytometry Part A. 69A:1037-1042.

4.Kukuruga, M. Using the BDTM Cytometer Setup and Tracking (CS&T) System for Instrument Characterization and Performance Tracking. BD Biosciences.

P2449 BONE MARROW ASPIRATE'S SAMPLES' DESTINY: SYSTEMATIC OR ARBITRARY?

Leslie Gonzalez Pinedo Angelina Lemes Castellano, Cynthia Acosta Fleitas,Dolly Fiallo Suarez,Naylen Cruz Cruz, Teresa Molero Labarta
Las Palmas Gran Canaria

Introduction: The bone marrow (BM) aspirate has as one of its main objectives the performance of a profound morphological study of all the cellular elements that are present in the sample's smear. With a small amount of bone marrow material obtained by aspiration, a smear can be easily made just by an extension process of the substance that must be analyzed. The major characteristics of this material are of great relevance, as they are essential in order to determine whether the aspirate has been successfully made or not. In order for this to happen, no more than 0,1-0,5 ml of material is required and it is mandatory to observe the presence of "bone marrow lumps" in the sample. Objectives: To determine the concordance correlation between the cellular counts in BM smears obtained at different times throughout the same aspiration process in patients from the Department of Hematology between November 2016 – January 2017.

Methods: 30 patients' BM smears were studied at different moments throughout the aspiration process. The 1st smear was obtained by aspiring less than 0,5ml, whereas the 2nd one contained 2 ml of bone marrow material, mimicking in such way the practice used in Flow Cytometry for the obtention of material. May-Grunwald Giemsa staining technique was used in order to analyze the morphological features of the haematopoietic cells. A differential cellular count was made out of a total of 200 cells in each BM sample, all of which were analyzed by the same trained cytologist. Patients with haematological disorders were selected. Having received chemotherapy for the last 15 days prior to the study became an exclusion criterion for participation. With the aim of establishing the concordance correlation between both measurements, several analyzing methods were used, such as Bland-Altman graph, Passing-Bablok regression method and Lin's concordance correlation coefficient.

Results: Concordance between both measurements was extremely poor for all cellular elements, except for plasma cells, where a substantial correlation was observed. This finding could be explained by the clearly distinctive morphological features of plasma cells, which makes it almost impossible not to identify them correctly. The cellular populations in smear # 2 were systematically reduced, acting as main exceptions to this process both the neutrophils (probably related to a greater dilution of the sample) and monocytes (whose number resulted increased in half of the samples and decreased in the other half, no matter which smear was analyzed). The latter finding could be explained by an "irregular" distribution of the monocytic lineage throughout the BM probably related with its intrinsic motility.

Conclusions: Based on our study's results, that clearly showed that most of the cellular elements concentrate within the 1st milliliter of a BM aspiration, it can be reasonable to conclude that this 1st ml should be exclusively reserved for morphological studies, as these still constitute the basis for haematological diagnosis. The next 2ml should be saved then for further Flow Cytometry studies, as they also represent a very useful quantitative diagnostic tool.

P2458 THE USE OF FLOW CYTOMETRY TO STUDY SACCHAROMYCES CEREVISIAE VIABILITY AND CELL CYCLE PHASE IN BOTTLE-FERMENTED BEERS

Ultan P. Cronin1, Tania López1, Miguel Ángel Sánchez-Luengo1 & Lola Martínez1

Flow Cytometry Core Unit, Spanish National Cancer Research Center (CNIO), Madrid, Spain

Introduction: As one of the four components that constitute beer (along with malt, water and hops) and the agent responsible for converting wort into beer, the physiological state of brewer's yeast Saccharomyces cerevisiae is of prime importance to the beer-making process. Yeast's role does not come to an end during primary fermentation: during secondary fermentation/conditioning it plays a

key part in carbonation and flavour development. With the growth of the craft beer market, which is dominated by bottle-conditioned beers, the role of live yeast in the development and maturation of such beers is of increasing interest. Among the standard methods whereby the viability of yeast is measured are: plate counting; methylene blue staining and assays such as the acidification power test. These methods have the disadvantages of being slow, laborious and subjective. Flow cytometry holds promise as a rapid, multiparametric method which can yield an insight into yeast heterogeneity within a sample. This study examines the use of cytometry to measure the viability and cell-cycle stage of the yeast in bottle-conditioned beers. The viabilities and cell cycle stages of yeast from different beer styles were compared, as were these parameters under different storage conditions and for beers at different distances from their best before dates.

Methods: Yeast was sampled from bottle-conditioned beer by careful decanting of the beer, and removal of the sediment by resuspending in PBS. After washing and centrifuging, the yeast cells were resuspended to 1.0 x 106 cells ml-1 in PBS for viability staining or fixed using ice-cold 70 % ethanol for cell cycle analysis. For viability staining the dyes PI (5 µg ml-1), DAPI (20 µg ml-1), 7-AAD (2 µg ml-1) and TO-PRO 3 (0.1 µg ml-1) were used to assess membrane integrity, while PI (50 ug ml-1) was used to examine cell cycle. Following staining, samples were acquired using a flow cytometer and analysed using FlowJo v10.1r5. Results from flow cytometry assays were compared with the standard methylene blue assay for yeast viability.

Results: Differences were found between the viabilities of yeast from various styles of beer. In general, the higher the alcohol content and the darker the beer the lower the percentage of live cells. Viabilities were also reduced in beers stored for longer periods or at higher temperatures. Cell cycle patterns also reflected this: the lower the viability, the higher the percentage of cells in G1/G0 arrest.

Conclusions: To the authors' knowledge this is the first time flow cytometry has been used to examine the viability of yeast in bottle-conditioned beer. The method proved rapid, simple to use and gave clear results. Instead of the often laborious methods currently used by brewers, this study shows that flow cytometry can be used to monitor the changes in yeast viability and therefore could prove useful as part of the shelf-life and stability analyses that brewers of bottle-conditioned beers undertake.

P2460 INFLUENCE OF ABSCISIC ACID (ABA) ON TOLERANCE TO HEAT SHOCK IN THE MICROALGA "CHLAMYDOMONAS REINHARDTII"

Marta Esperanza Marta Seoane, Iria López, Carmen Rioboo

Laboratorio Microbiología, Facultade de Ciencias, Universidade da Coruña. Campus da Zapateira s/n. 15071 A Coruña, Spain.

Introduction: Currently, the main concerns of the scientific community are the consequences of global warming and climate change. In recent years, heat stress has been target of numerous investigations. Microalgae must deal with stressful situations in the environment using physiological and molecular adaptations, since they are also exposed to environmental fluctuations.
In order to prevent and repair cell damage caused by sudden changes in temperature, plants have developed a series of strategies to perceive these changes, activating appropriate defensive responses. In particular, plant hormones, such as Abscisic acid (ABA), can have an active role in the response of plants to different stressors.
The main objective is the study of the potential role of the plant hormone ABA in the response of the microalga C. reinhardtii against heat shock.

Methods: Microalgal cultures were preincubated with and without ABA (200 µM) for 24 h under controlled light, temperature and agitation conditions. Then cultures were exposed to two temperatures (20ºC and 41ºC) during 90 min. Finally microalgal cultures were kept during 24 h in the incubator with controlled conditions for doing the timely analysis. Effect of heat stress was quantified on different parameters in cultures with and without ABA using different flow cytometry (FCM) protocols: cell viability, autofluorescence of chlorophyll a, cellular metabolic activity, cell cycle and caspase activity.

Results: Results in the present study showed that exposure of the cultures of C. reinhardtii to heat shock (41ºC) after 24 h causes significant alterations on the cellular physiology of this microalga. On the

one hand, damage in the plasma and chloroplast membranes was observed, as reflected by the loss of cell viability and the appearance of chlorotic cells, respectively. A decrease in cellular metabolic activity was also perceived. On the other hand, an increment of caspase activity and the loss of nuclear DNA in microalgal cell (nucleus subG0) were detected, being these alterations frequently described in apoptotic cells.
When cells are maintained at its optimum of growth temperature (20ºC), ABA treatment had no detectable effect on the analyzed parameters. However, treatment with ABA increases the heat tolerance (41ºC) of C. reinhardtii, reducing the impact caused by this stress on all parameters analyzed in the present work.

Conclusions: Treatment with the plant hormone ABA increases the tolerance of the microalga against heat shock; this thermotolerance could be due to the induction of mechanisms for controlling the intracellular concentration of reactive oxygen species (ROS) in cells treated with ABA.

Acknowledges: M. Esperanza and M. Seoane acknowledge their pre-doctoral fellowship from the Xunta de Galicia (Spain).

P2461 REACTIVE OXYGEN SPECIES OVERPRODUCTION ON MICROALGAL CELLS EXPOSED TO DIVERSE POLLUTANTS

Marta Seoane Marta Esperanza, Concepción Herrero, Ángeles Cid

Laboratorio de Microbiología. Facultad de Ciencias. UNIVERSIDADE DA CORUÑA. Campus da Zapateira s/n 15071 A Coruña (Spain)

Introduction: In ecotoxicology, microalgae are of primary interest when addressing the potential adverse effects of pollutants, due to their characteristics and position in the aquatic food web. Although reactive oxygen species (ROS) are continuously produced in microalgae as byproducts from various pathways of the oxygen-based metabolism, microalgae may have higher ROS levels as a result of the presence of contaminants. Since ROS have a very short half-life and very low intracellular concentrations, their study requires very fast and sensitive methods for detection and quantification. Therefore, flow cytometry (FCM) is the most convenient method to characterize the generation of these substances in the cell.
The aim of this study was to evaluate the amount of ROS produced in two microalgal species after exposure to different priority and emerging pollutants, looking for a common response and a sensitive biomarker of acute cytotoxicity on microalgae against different aquatic contaminants.

Methods: Microalgal species used were the marine microalga T. suecica and the freshwater microalga C. reinhardtii. T. suecica cultures were exposed to the emerging compounds bisphenol-A, ibuprofen and omeprazole; whereas C. reinhardtii cultures were exposed to the herbicides paraquat and atrazine. The concentration tested for each compound was chosen taking into account its respective 96 h EC50 value for growth. Cultures without the contaminant were used as a control.
Oxidative stress in these microalgal species was evaluated after 24 h of exposure to the different pollutants based on FCM determinations of intracellular levels of superoxide anion radical (O2-) and hydrogen peroxide (H2O2), using the fluorochromes hydroethidine (HE) and dihydrorhodamine 123 (DHR123), respectively.

Results: All the tested compounds provoked significant overproduction of ROS in the two microalgal species after 24 h of exposure. On the one hand, bisphenol-A, ibuprofen and omeprazole caused a significant increase in hydrogen peroxide levels on T. suecica cells; while superoxide anion levels also increased in cells exposed to omeprazole. On the other hand, paraquat caused a significant increase in superoxide anion levels on C. reinhardtii; whereas atrazine caused high levels of hydrogen peroxide.

Conclusions: The environmental stress produced by the pollutants triggers the overproduction and accumulation of hydrogen peroxide or superoxide anion. Therefore, the ability to provoke oxidative stress in microalgal cells was shared by all the tested pollutants regardless of their chemical nature, although the primary ROS formed and its quantity varied from one compound to another. Oxidative stress is a common response and a highly sensitive biomarker in unicellular algae exposed to different pollutants, being very useful for the characterization of possible contaminating hazards in the aquatic environment.

Acknowledgements: M. Seoane and M. Esperanza acknowledge their pre-doctoral fellowship from the Xunta de Galicia (Spain).

P2470 FLOW CYTOMETRY BASED APPROACHES FOR NANOTOXICITY STUDIES

H.Oliveira1,2, C. Roxo1, C. Menezes1, A.Mendes1, Lucian Farcal3, Christina Ziemann4, Sabina Burla3, Otto Creutzenberg4, and F. Gonçalves1

1 Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal;
2 CICECO-Aveiro Institute of Materials, University of Aveiro, Portugal;
3 BIOTOX Srl, Cluj-Napoca, Romania
4 Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

holiveira@ua.pt

Nanomaterials (NM), due to their unique physicochemical properties, are used in various applications in the industrial, electrical, pharmaceutical and biomedical fields and are being included in several consumer products such as cosmetics and food, or specially designed for bio-imaging or drug delivery applications. The nano-scale dimension of NMs, together with other specific physicochemical properties may determine their varying biological effects. The consequent increase of human exposure to NM advises urgency on a proper risk assessment by thorough studies that cover the evaluation of their toxicity.
In the present study, a flow cytometric based approach to evaluate the toxicity of graphene family of nanomaterials (GFNs) to mammalian cells will be presented. Briefly, to evaluate cell-nanoparticles interactions, several cell models were exposed to different concentrations of GFNs and analysed by flow cytometry after 24h or 48h. Endpoints include nanoparticle cell uptake (FSC/SSC), intracellular ROS formation (DCFH2-DA), inflammatory potential by cytokine estimation using cytometric bead arrays, induction of apoptosis using FITC Annexin V Apoptosis Detection Kit and evaluation of cell cycle dynamics. Flow cytometric analysis provide a rapid and accurate screening of biological responses and, although other non-flow cytometric techniques should be used, (eg microscopy, gene expression, etc), our studies indicate that flow cytometric analysis are an extremely helpful tool for in vitro nanotoxicology studies.

P2473 APPLYING R AUTOMATED ANALYSIS TO CYTOMETRY DATA FROM A PROSPECTIVE COHORT OF HIV-INFECTED PATIENTS

Rita Rb-Silva1,2, Edward Lee3, Claudia Nobrega1,2, Ana Horta1,2,4, Andrew Yates3, Margarida Correia-Neves1,2
1 Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho (ECS-UM), Braga, Portugal.
2 ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal.
3 Institute of Infection, Immunity & Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, United Kingdom.
4 Infectious Diseases Service of the Centro Hospitalar do Porto, Hospital Joaquim Urbano Unit, Porto, Portugal.

Keywords: Flow cytometry, Bioinformatics, Automated gating, R languagr
Flow cytometry (FC) has contributed to major advances in immunology, oncobiology and many other areas in the biological sciences. Conversely, FC has benefited remarkably from the technological growth in recent years, expanding its applications and developing novel extensions such has cytometry by time-of-flight (CyTOF), imaging FC and spectral FC. However, perhaps the most important obstacle to new advances in these areas is the increasing size and complexity of the data FC can generate. The solution lies not only in the creation and optimization of computational algorithms, but also in their widespread adoption. Automated FC analysis methods can be faster and more reproducible than manual gating strategies, and are almost certainly destined to become ubiquitous, but some limitations that render automated FC not commonly used at present. Here we report our experience with automated analysis using R packages from Bioconductor, an open source software project, applied to FC data from HIV-infected patients enrolled in a prospective cohort study.

P2474 FLOW CYTOMETRIC OSMOTIC FRAGILITY TEST FOR THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

Maria Luís Queirós1,6, Cátia Morais1, Paulo C. Costa2, Luciana Pinho3, Jorge Coutinho3, Emília Costa4, Esmeralda Cleto4, Inês Freitas5,6, Margarida Lima1,6.

1Laboratório de Citometria, Serviço de Hematologia Clínica, Hospital de Santo António (HSA), Centro Hospitalar do Porto (CHP), Porto, Portugal
2UCBIO-Requimte, Faculdade de Farmácia, Universidade do Porto (FFUP), Porto, Portugal
3Serviço de Hematologia Clínica, Hospital de Santo António (HSA), Centro Hospitalar do Porto (CHP), Porto, Portugal
4Serviço de Pediatria, Centro Materno-Infantil do Norte (CMIN), Centro Hospitalar do Porto (CHP), Porto, Portugal
5Serviço de Hematologia Laboratorial, Hospital de Santo António (HSA), Centro Hospitalar do Porto (CHP), Porto, Portugal
6Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas da Universidade do Porto (UMIB/ICBAS/UP), Porto, Portugal

Introduction: Hereditary spherocytosis (HS) is the most common cause of inherited chronic hemolysis and presents with diverse clinical features and laboratory results. The conventional osmotic fragility (OF) test of red blood cells (RBC) is the most widely used diagnostic method, however it is no longer recommended for routine testing in HS. Osmotic fragility testing based on flow cytometry (FCM-OF) was recently introduced for the screening of HS. The purpose of this study was to evaluate the FCM-OF for the diagnosis of HS.

Methods: One hundred thirty six peripheral blood (PB) samples collected into EDTA-K3 containing tubes, from 136 individuals were studied: 103 healthy adults (blood donors), 13 HS patients, 6 patients with thalassemia and 14 patients with anemia of other causes. The diagnosis of HS was made based on the clinical features and laboratory tests. These included complete blood cell counts, with RBC indices and reticulocyte counts, PB smears with RBC morphology, serum lactate dehydrogenase and bilirubin levels, conventional OF (without and with incubation), cryohemolysis tests, and flow cytometric eosin-5-maleimide (EMA) binding tests. FCM-OF was performed, according to the method described in 2009 by Won and Suh (doi: 10.1002/cyto.b.20448), modified (the RBC were suspended in phosphate buffer saline (PBS) instead of isotonic normal saline). Briefly, a RBC suspension was spiked with deionized water during acquisition and the count of residual RBC was measured sequentially in real-time using FCM. Sample acquisition and analysis were made in a NaviosTM flow cytometer (Beckman Coulter). Statistical analyses were performed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA). The cut-off value was established, and the sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy were calculated.

Results: The HS group showed significantly decreased percentages of residual RBC (median 29.5%, minimum 2.6%, maximum 70.0%), as compared to controls (median 82.3%, minimum 31.5%, maximum 98.2) ($p<0.001$), as well as compared with patients with thalassemia (median 97.4%, minimum 90.0%, maximum 98.0) ($p<0.001$) and with patients with anemia of other causes (median 79.3%, minimum 34.7%, maximum 98.8) ($p<0.006$). The ROC area of the HS/NHS (non-HS patients) was of 0.958. Using a cut off value of 65.8%, the sensitivity and specificity of the FCM-OF test for the diagnosis of HS were of 92.3% and 82.1%, respectively and the NPV, PPV, and accuracy were of 99.0%, 35.3% and 83.1%, respectively. By decreasing the cut off value to 50.1%, the sensitivity and specificity were of 84.6% and 93.5%, respectively, and the VPP, VPN and accuracy were of 57.9%, 98.3% and 92.6%, respectively.

Conclusions: The FCM-OF test showed a high sensitivity and NPV for the diagnosis of HS, although the specificity and PPV were somewhat lower, and these values depended on the cut off used. Associated with the fact that is a quantitative and objective, user-friendly, labor-saving, time-efficient, inexpensive method (requiring only PBS and deionized water), this make FCM-OF an effective approach for HS screening in Hematology laboratories where a flow cytometer is available.

P2502 YELLOW-GREEN LASER NEXT GENERATION-BASED FLOW CYTOMETRY FOR CD34+ PROGENITOR CELL COUNTING

Laura G. Rico1, Jordi Juncà1,2, Mike D. Ward3, Jolene Bradford3 and Jordi Petriz1
1 Josep Carreras Leukaemia Research Institute, Badalona, Barcelona, Spain, 2 Institut Català

d'Oncologia, Badalona, Barcelona, Spain, 3 Thermo Fisher Scientific, Eugene, Oregon, USA.

P2503 ACOUSTOPHORETIC ORIENTATION OF RED BLOOD CELLS FOR DIAGNOSIS OF RED CELL HEALTH AND PATHOLOGY

Laura G. Rico1, Jordi Juncà1,2, Mike D. Ward3, Jolene Bradford3 and Jordi Petriz1
1 Josep Carreras Leukaemia Research Institute, Badalona, Barcelona, Spain, 2 Institut Català d'Oncologia, Badalona, Barcelona, Spain. 3 Thermo Fisher Scientific, Eugene, Oregon, USA.

P2504 Proposal of 10-color panels for clinical diagnosis in next generation flow cytometry

José Antonio Delgado García1, Carlos Salvador Osuna2

1 Universidad de Navarra,
2 Servicio de Hematología y Hemoterapia, Hospital Universitario Miguel Servet

Introduction: Flow cytometry is a crucial tool for diagnosis, prognosis and follow-up of hematologic malignancies. During the last years we assisted great advances in flow cytometry: increased number of fluorescent channels, new fluorochromes, new monoclonal antibodies and innovative sample preparations are available. Thanks to its terrific evolution, flow cytometry has being applied to more and more applications. Although such a rapid evolution, still difficulties are present and new challenges arose. Firstly, the increased number of fluorescences and the high number of available markers make panel design a complex task where a significant know how and time investment are required, especially in clinical cytometry. Secondly, there are multiple alternatives for cytometer settings and staining protocols which result into a non-standard work.
Currently, there are several international validated proposals for panels and protocols. Examples are: Leukemia Net, the European Group for the Immunological Characterization of Leukemias (EGIL), or the Euroflow scientific consortium. Since the present flow cytometry instrument generation expands its limit offering to detect up to 10 fluorescences in the same tube, more specific and simultaneous information for our studies are possible; still a broader solid panel proposal should be proposed to cover the wide range of hematological disease.

Methods: We identified the most frequently groups of pathologies studied in clinical laboratories. Afterwards a thorough review of the literature was carried out to identify the main markers for each group of diseases. For reagent combination we identify and avoid potential conflict objectives, pairing markers with appropriate fluorochromes. For practical approach 5 samples from Hospital Universitario Miguel Servet was used; 4 healthy donor peripheral blood and 1 bone marrow diagnosed as follicular lymphoma without marrow infiltration. All samples were acquired in a Navios flow cytometer (Beckman Coulter). Kaluza v1.5a and Infinicyt v1.7 were used for data analysis.

Results: We have defined 11 panels including screening, mature and acute neoplasm analysis, according to the clinical situation and the laboratory resources. For each tube we have studied the individual staining of all marker as well as all possible fluorescence confrontations, to identify possible compensation issues due to bad combinations.

Conclusions: Next generation flow cytometry makes panel design a complex task where a significant time investment and know how are required, especially in clinical cytometry. We defined a set of optimized panels for clinical diagnosis in 10-color flow cytometry. Marker selection and combination were based on WHO, Euroflow, LeukemiaNet, EGIL recommendations. Moreover the most recent papers, reviews, manuals and scientific researches on hematological diagnosis by flow cytometry were also considered.
In addition, for each panel, the quality of individual staining has been tested, as well as the identification of possible compensation conflicts between the most commonly confronted or most relevant markers in the diagnosis.
Using predefined panels in hematological cytometry not only improves the workflow, but also allows data interchange between laboratories. Additionally panels have been flexibly structured into sections so that they can fit to each laboratory needs and to the specific characteristics of each patient.
Here we show our preliminary results. More samples must be analyzed, especially pathological samples specific of each panel.

Award BD Biosciences for Best Oral Communication

P2442 Identification and enumeration of distinct subsets of monocyte-macrophages in different human tissues throughout life using 9-color flow cytometry.

First Author - Daniela Damasceno, Universidad de Salamanca, Spain

Introduction: Mononuclear phagocytes include circulating monocytes (e.g. classical (CD14+/CD16-) monocytes (cMo), intermediate (CD14+/CD16+) monocytes (iMo), and non-classical (CD14het/CD16+) monocytes (ncMo), and tissue macrophages (TiMas). Despite extensive research on the monocyte-macrophage system, the precise nature of maturational links between populations, functional differences, and age-related distribution are not well-known.
Here we have analysed 5 different monocytic subpopulations -from cord blood (CB) to peripheral blood (PB) specimens throughout life-, to determine age-reference intervals for those subsets; in addition, we evaluated the distribution of monocytic subpopulations in spleen, lymph node (LN) and PB paired samples.

Methods: A total of 11 CB and 171 PB, 4 spleen and 4 LN from healthy individuals, were investigated for the distribution of CD62L+ cMo, CD62L- cMo, iMo, and SLAN- ncMo and SLAN+ ncMo. PB samples were stratified according to the following age groups: newborns=4; 1 to 6 months=14; ≥6m<1y=10; ≥1<2y=13; ≥2<5y =18; ≥5<8y=18; ≥8<13y=17; ≥13<16y=6; ≥16<20y=5; ≥20<30y=13; ≥30<50y=14; ≥50<60y=12; ≥60<70y=9; ≥70<80y=9; ≥80y=9. Tissues other than CB and PB were collected from 4 adult donors (mean age of 69y), together with their paired PB samples.
For the multiparametric flow-cytometry analysis, more than 5x106 leukocytes were stained with a single nine-color combination of fluorochrome conjugated monoclonal antibodies against: CD14, CD16, CD36, CD45, CD62L, CD64, CD300e (IREM2), HLADR and Slan. Data acquisition was performed in a LSR FORTESSA X-20 cytometer (BD), and analysed using the Infinicyt software (Cytognos).

Results: The absolute number of PB cMo reached a peak in CB specimens (932 cells/μL), decreasing hereafter (273 cells/μL) until poverty (>16≤20y), when another peak of cMo was identified (though not so high as in CB: 365 cells/μL), mainly at the expenses of CD62L- cMo. Then, the absolute number of PB cMo decreased and remained stable until 50y-old subjects (197 cells/μL), increasing again in the elderly (329 cells/μ). No significant gender-related changes were found. In turn, PB iMo, and ncMo showed an age-related kinetics similar to cMo, except for CB and newborn groups: the higher number of iMo (53 cells/μL) and ncMo (68 cells/μL) was registered days after the delivery, remaining constant until 50 years old and coming up again in the second half of life, mainly due to an increase in the Slan- compartment. Of note, the distribution of the different monocytic subsets were different in the three tissues analysed in paired subjects: accordingly, in PB the great majority of cMo expressed CD62L, while the CD62L+/CD62L- ratio was inverted both in LN and spleen. Moreover, iMo were increased in LN, and nearly no ncMo were detected, while spleen showed higher frequency of ncMo (both Slan- and Slan+) vs both PB and LN.

Conclusions: Our study shows that the circulating monocyte pool dynamically changes during ageing and among different tissues in humans, which can be useful to establish a frame of their distribution, to be used as reference for pathological conditions. Further, the finding that the different subsets here studied are differentially distributed in tissues may give light on the dynamic trafficking of these cells and therefore in their specific functional roles.

Award Enzifarma for Best Poster

Abstract 2473 Applying R automated analysis to cytometry data from a prospective cohort of HIV-infected patients.

Rita Rb-Silva1,2, Edward Lee3, Claudia Nobrega1,2, Ana Horta1,2,4, Andrew Yates3, Margarida Correia-Neves1,2

1 Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho (ECS-UM), Braga, Portugal.
2 ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal.
3 Institute of Infection, Immunity & Inflammation, Glasgow Biomedical Research Centre, University of

Glasgow, Glasgow, United Kingdom.
4 Infectious Diseases Service of the Centro Hospitalar do Porto, Hospital Joaquim Urbano Unit, Porto, Portugal.

Keywords: Flow cytometry, Bioinformatics, Automated gating, R languagr

Flow cytometry (FC) has contributed to major advances in immunology, oncobiology and many other areas in the biological sciences. Conversely, FC has benefited remarkably from the technological growth in recent years, expanding its applications and developing novel extensions such has cytometry by time-of-flight (CyTOF), imaging FC and spectral FC. However, perhaps the most important obstacle to new advances in these areas is the increasing size and complexity of the data FC can generate. The solution lies not only in the creation and optimization of computational algorithms, but also in their widespread adoption. Automated FC analysis methods can be faster and more reproducible than manual gating strategies, and are almost certainly destined to become ubiquitous, but some limitations that render automated FC not commonly used at present. Here we report our experience with automated analysis using R packages from Bioconductor, an open source software project, applied to FC data from HIV-infected patients enrolled in a prospective cohort study.

Award SIC for Best Poster presented by a Student

Abstract 2444 How to reliably choose settings for optimal resolution in a multicolor flow experiment.

Tania López1, Ultan P.Cronin1, Miguel Ángel Sánchez-Luengo1, Lola Martínez1.

1.Flow Cytometry Core Unit. Spanish National Cancer Research Center (CNIO). Madrid-Spain.

Multicolor flow cytometry rapidly reveals a large amount of biological information about a single sample (1). Over the past few years, the number of parameters simultaneously analyzable in typical flow cytometry experiments has increased from the typical 2-4 color experiments to 8+ color experiments routinely run on the labs these days (2). Not only has this increased the power of flow cytometry, but it has also increased the importance of a proper experimental setup to ensure accurate and meaningful results (1). Certain factors such as fluorochromes spillover and the selection of the proper voltage for each detector have a direct influence on populations spread, which in turn, have a clear impact on population resolution, and a loss of accuracy and significance of the data.

The selection of the proper voltage for each detector, it's essential in order to obtain the minimum spread and the maximum resolution of our populations during acquisition (3). Instrument resolution is dependent on the electronic noise, which can be defined as the background signal caused by electronics. An increase on electronic noise will result in a decreased resolution sensitivity of our populations of interest (4). Currently, there are three different approaches that people use to select the optimal voltage for the experiment.

Conventionally, many users set up the voltage “by eye”, increasing it until they get the best separation between the positive and negative populations.
Manufacturers have developed over the years different approaches, such as the CST (Cytometer Setup Tracking) BDTM method, which looks at background and detector efficiency to determine a minimum PMT voltage setting for each detector, ensuring the populations will be set over the electronic noise. This method is based on the use of CST beads that contains dim, mid-range and bright emission intensities. These beads are only valid on BD DIVA instruments.
Another approach, described here, involves the assessment of the electronic noise of each PMT in your instrument and to employ the voltage required to obtain approximately 2.5 times the rSDEN (Robust Standard Deviation of the Electronic Noise) in each detector. This should ensure that we use a voltage where the electronic noise does not interfere with the measurements at the low end of the scale. This approach could be used for all cytometers and will employ your unstained beads or cells to perform such calculations.
Our goal here is to compare these methods and determine which is both convenient and best able to establish optimal, consistent and reproducible cytometer settings for multicolor immunophenotyping applications, while ensuring that the voltage settings will be sufficient to resolve various lymphocyte subpopulations present in mouse spleen using an 8-color inmunophenotyping panel and a FACS CANTO II flow cytometer equipped with 488nm, 640nm and 407nm lines.

References
1.BD Technical Bulletin. 2009. An Introduction to Compensation for Multicolor Assays on Digital Flow Cytometers. BD Biosciences.
2.Perfetto, S.P., Chattopadhyay, P.K. and M. Roederer. 2004. Seventeen-colour flow cytometry: unravelling the immune system. Nat Rev Immunol.4:648-655.
3.Maecker, H.T. and J. Trotter. 2006. Flow Cytometry Controls, Instrument Setup, and the Determination of Positivity. Cytometry Part A. 69A:1037-1042.
4.Kukuruga, M. Using the BDTM Cytometer Setup and Tracking (CS&T) System for Instrument Characterization and Performance Tracking. BD Biosciences.

Turning Cytometry Data Into Results

SPONSORS

enzifarma

BD
BD Biosciences

cytognos | Flow Cytometry Solutions

immunostep
we know how

alfagene

FLOWJO, LLC

MACS
Miltenyi Biotec

DIAGNÓSTICA
LONGWOOD
25 ANIVERSARIO

sysmex

PROGRAM

XV CONGRESS SIC - Lisbon

25th to 27th MAY 2017
LISBON, PORTUGAL
Centro Cultural de Belém

Sociedade Ibérica de Citometría

ORGANIZAÇÃO

Eventos By Transalpino