Summer brings about - besides some minor thoughts of holidays and little hope for a more relaxed state of affairs - schools that bear its name: ‘Summer Schools’ which according to Wikipedia is ‘a type of conference, [where] typically, established academics will give presentations on advanced topics in a field to postgraduate students’ bring me to attentions that I would like to share with you.

Summer Schools have a longstanding history in H&I and I think, with some nostalgia, of the one I attended in Greece at the beginning of the nineties, and then organised by the Council of Europe. The experience made me convinced that the H&I field is highly interesting and worthy to stay in. In the realm of EFI, Frans Claas accomplished a joint international annual Summer School, together with ASHI and APHIA (then ASEATA) and was the organizer of the first one which was held in Sevilla.

The school is coordinated in turn by one of the three societies; this year it will take place in Stintino, Sardinia, and EFI’s Education Committee has the lead. I have been informed that the programme has attracted more participants than there are places which confirms the attractiveness of these meetings.

Recently, several meetings took place that could also fall under the term Immunogenetics Summer School. They have been organised, however, on a national (or bi-national) level. One was arranged by the Italian Association of Immunogenetics and Transplant Biology (AIBT) in Monopoly, Italy, another by the German Society for Immunogenetics (DGI) jointly with the Turkish Society for Transplantation Immunology and Genetics in Cesme, Turkey; and a third in St. Petersburg, Russian Federation, the local Institute of Haematology and Blood Transfusion being the organiser.

All events were very well attended and received. The main conference language was the national one.

The fact that in Europe we have such a diversity of regions, languages, nations, all with different needs stresses the importance of the national societies (or individual initiatives). And luckily, as observed in the example of the summer schools, the various societies take initiatives and are perfectly able to cover the needs of their membership or colleagues. EFI has always been supportive as can be seen on the various bursaries that are offered. But I would also argue that we should aim to put the (very amicable) relationships with the national societies on a more formal level. This could be achieved by agreements that would express the supportive attitude of EFI as well as the autonomy of the national societies; the principle of subsidiarity should be implemented; and above all the willingness to cooperate should be formally expressed.

Just a little afterthought to the last members General Assembly held at the Maastricht conference. The Accreditation Committee report traditionally includes the number of accredited laboratories, a number that seemed to have reached a plateau ~ 250 over the last years. It was explained, however, by Andrea Harmer that this number rather reflects a dynamic status, where newly accredited laboratories are counteracted by others who are lost from the accreditation statistics because they become merged with other units. Being myself in a department where the organisational chart is currently under review and the future of the H&I laboratory is all of a sudden open for discussion, I know rather precisely that the
WELCOME TO THE 28TH EUROPEAN IMMUNOGENETICS AND HISTOCOMPATIBILITY CONFERENCE (EFI)

It is a great pleasure to invite you to participate in the 28th European Immunogenetics and Histocompatibility Conference (EFI) “From Genes to Therapy” in Stockholm June 25-28, 2014. The program will contain state of the art sessions on immunogenetics in organ and stem cell transplantation as well sessions on HLA-linked autoimmune diseases, NK-cell impact on transplantation and methodological and diagnostic developments.

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Confirmed speakers
Marta E. Alarcon-Riquelme, Spain  Robert I. Lechler, UK
Andrew Brooks, Australia       Kalle Malmberg, Norway
Menna Clatworthy, UK           Svante Pääbo, Germany
Andrew Fontenot, USA           Steven H. Sacks, UK
Olle Korsgren, Sweden          Michael Uhlin, Sweden
Klas Kärre, Sweden             Kathryn Wood, UK

Registration and Abstract submission opening November 25th 2013

Conference venue
Stockholm Waterfront Congress Centre, which opened in January 2011, is located between the Central Station and the City Hall. www.stockholmwaterfront.com

Conference Secretariat
MCI Stockholm, Sweden. confirmation@mci-group.com Phone: + 46 8 5465 1500

Mats Bengtsson, Marie Schaffer, Ann-Charlotte Wikström
Local Organizers
It is a great pleasure to invite you to participate in the 28th European Immunogenetics and Histocompatibility Conference (EFI) “From Genes to Therapy” in Stockholm June 25-28, 2014. The program will contain state-of-the-art sessions on autoimmune diseases, NK-cell impact on transplantation and methodological and diagnostic developments.

Well located with most of Europe within three hours reach. www.visitsstockholm.com

A big thank you from Maastricht

Furthermore, several reports on meetings elsewhere in Europe and announcements of new meetings are included in this Newsletter showing once more how active and vivid our society is.

Also the international summer school on Immunogenetics, organized by the educational committee together with our sister societies ASHI and APHIA which takes place in September in Stintino, Sardinia, is very popular considering the very high number of applications.

I hope that this Newsletter provides you with a lot of useful information and I am looking forward to your contributions to the next one.

Frans Claas

Deadline for contributions to EFI Newsletter 72 is December 15, 2013.
Please send your contribution by e-mail to fhjclaas@lumc.nl

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(Dr Mike Bunce, CSO Biofortuna)
Obituary Art Robbins

On June 15, Art Robbins died at the age of 81. Art was the owner and founder of several companies including Robbins Scientific, a company which produced all kind of tools and instruments for HLA laboratories. He was well known in the HLA community and a regular visitor of our EFI meetings. Art was a great inventor, who listened carefully to the needs of the people in the labs and came up even with solutions for problems we did not realize yet that we had them. His main aim was to facilitate the daily work in the labs and to develop instruments for new technologies. He was a friend of many people in the HLA community and many of us remember the very good wines he served at several ASHI and EFI meetings. Even if he was not able to attend a meeting, he arranged that some Californian wines arrived in order to not to disappoint his HLA friends.

We will miss him, both as a colleague but even more as a friend!

Frans Claas

Membership update

Since the last issue of the EFI Newsletter we received a lot of applications forms from new members. Hereby we would like to welcome the following new EFI members:

Dr. H. Senturk-Ciftci, Istanbul, Turkey
Ms. M. Sabah, Haifa, Israel
Ms. S. Lydon, Dublin, Ireland
Ms. S. Stokes, Dublin, Ireland
Dr. S. Guidotti, Piacenza, Italy
Dr. A. Bradley, London, UK
Dr. F. Ingrassia, Palermo, Italy
Ms. U.M. Impola, Helsinki, Finland
Ms. K. Andrews, Cambridge, UK
Ms. V.E. Iveson, Manchester, UK
Dr. B. Schmid-Horch, Tuebingen, Germany
Dr. A. Bermejo-Becerro, San Sebastian, Spain
Ms. B. Grumbt, Martinsried, Germany
Mr. R. Segers, Mechelen, Belgium
Dr. P. Koefoed-Nielsen, Aarhus, Denmark
Ms. G. Bierfass, Buenos Aires, Argentina
Dr. K. Detari, Vienna, Austria
Dr. A. Pospeiov, Moscow, Russia
Dr. O. Buturca, Bucharest, Romania
Dr. J. Dmitrovic, Belgrade, Serbia
Dr. B. Biderman, Moscow, Russia
Dr. L. Petrova, Moscow, Russia
Dr. L. Jurgausskiene, Vilnious, Lithuania
Dr. O. Guidova, Moscow, Russia
Prof. G. Clive, Cape Town, South Africa
Dr. P. Bohacova, Praha, Czech Republic
Dr. S. Kandilarova, Sofia, Bulgaria
Dr. M. Sobhani, Tehran, Iran
Mr. D. Roodenburg, Leuven, Belgium
Dr. O. Shragina, Moscow, Russia
Dr. P. Moskovchenko, Lyon, France
Dr. A. Inal, Istanbul, Turkey
Mrs. S. Dzurec, Lyon, France
Dr. S. Ferrari, Geneva, Switzerland
Dr. G. Hörger, Basel, Switzerland
Dr. V. Renac, Rennes, France
Dr. S. Alhsyek, Tripoli, Libya
Dr. I. Thuess, Lund, Sweden
Dr. G. Guarda, Lausanne, Switzerland
Dr. F. da Silva – Nardi, Essen, Germany
Ms. L. Powley, London, UK
Dr. Z. Drace, Turin, Italy
Dr. A. Karakoli, Thessaloniki, Greece
Dr. R. Hod-Dvorai, Haifa, Israel
Dr. A. Domouchtsidou, Essen, Germany
Dr. S. Benko, Budapest, Hungary
Ms. C. Wilson, Glasgow, UK
Dr. A. Stavridou, Athens, Greece
Dr. B. Omazic, Stockholm, Sweden
Dr. B. Wagner, Essen, Germany
Dr. S. Tafulo, Porto, Portugal
Dr. M. Cocco, Nuoro, Italy
Dr. A. Kennel, Nancy, France
Dr. S. Gregori, Milan, Italy
Dr. A. Berces, Budapest, Hungary
DR. K. Tsalimalma, Athens, Greece
Dr. M. De Donno, Firenze, Italy
Dr. P. Gombos, Heidelberg, Germany
Ms. E. Ntaountaki, Athens, Greece
Dr. M. Lopez-Hoyos, Santander, Spain
Dr. L. Crucitti, Milan, Italy
Dr. S. Tokic, Bizovac, Croatia
Dr. S. Marczi, Bizovac, Croatia
Dr. G. Yanikkaya Demirel, Istanbul, Turkey
Dr. L. Roda, Papeete, Tahiti, French Polynesia
Dr. A. Pavlova, St. Petersburg, Russia
Dr. C. Sabia, Naples, Italy
Dr. A. Picascia, Naples, Italy
Dr. V. Grimaldi, Naples, Italy
Dr. T.H.R.M. Habets, Maastricht, The Netherlands
Mr. J. Visentin, Bordeaux, France
Dr. M. Bernheiden, Freiburg, Germany
Diagnosis in Transplantation

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1. Opening
The EFI President, Gottfried Fischer, opened the General Assembly meeting at 16:30 pm and welcomed all EFI members present. (Approximately 100 EFI members in attendance).

2. Minutes General Assembly 2012
The minutes of the General Assembly, held June 2nd 2012 during the EFI annual meeting in Liverpool, United Kingdom, which were published in the EFI Newsletter Issue 68, September 2012 were approved.

The EFI President, Gottfried Fischer reported on the following:
The EFI Executive Committee; Accreditation; Education; EPT and Standards Committee held their autumn meeting in Leiden. The autumn meeting is now the main time all committee members get together as there is insufficient time for extended meetings during our annual EFI conference.
Interactions with other societies is of benefit to EFI and the EFI membership and the EC are exploring their relationship with EBMT, WMDA and ESOT. One option is to sponsor EFI sessions at these organisations meetings and also to ensure representation from these organisations are included when appropriate at EFI meetings.
The three presidents of ASHI, APHIA and EFI had a joint meeting at the 2012 ASHI meeting and all agreed to continue with agreements that are in place including shared discount registration for members of each society to attend the other societies annual conference.
Plans to develop the EFI website are in place and money has been allocated for doing this in the EFI budget.
Thanks were given to Professor Ronald Bontrop, who steps down as chair of the EFI Scientific Committee. Ronald has been a member of the scientific committee since 2005 and has been a valuable chair since 2008 providing much support to the EFI Executive Committee and all organisers of EFI conferences. We are all very appreciative of the support Ronald has given to EFI.
There were no questions or comments from the membership

4. Report of the Secretary
The EFI Secretary, Ann-Margaret Little gave an update on the following:
EFI Elections:
There were four vacancies arising on the Executive Committee: Secretary to replace Ann-Margaret Little; Treasurer to replace Valérie Dubois and two Councillors to replace Katharina Fleischhauer and Joannis Mytilineos. The Executive Committee nominated Ann-Margaret Little for Secretary (2nd term); Valérie Dubois for Treasurer (2nd term) and Carlo Carcassi and Peter Horn for the positions of Councillor. No other nominations were received from the EFI membership.

These nominations were accepted unopposed by the General Assembly.

Bursaries:
1. Education and Training Bursaries
3 bursaries have been awarded: Luminita Loga and Lucia Dican from Romania to visit Evangelismos General Hospital Athens and Lisa Creary from London to visit Stanford University. Reports arising from the first two bursaries were published in the EFI newsletter no.69.

2. Annual Meeting Bursaries: Fifteen bursaries were awarded bursaries to members to aid attendance at the annual EFI Conference in Maastricht.

3. Additional Bursaries
In addition to awarding bursaries to support members attending the annual EFI Conference, 4 bursaries were given to members to aid attendance at the 2012 summerschool
There were no questions or comments from the membership

5. Report of the EFI Treasurer
The EFI Treasurer, Valerie Dubois, presented the accounts for 2012. The EFI annual meeting in Liverpool has provided a profit, but this has not yet been paid to EFI as one year needs to pass before the LOC can close the accounts. Therefore for 2012 we currently are showing a deficit of €119,620 which is less than the estimated – €152,700. This difference is due to increased income from commercial advertisements in the EFI newsletter and no money yet spent on the Website redevelopment.

Despite the negative figures, the 2013 budget proposal was presented with a proposed profit of €12,300. This proposed profit is due to the receipt of profits from both Liverpool and Maastricht conferences in one year and also the income received from the final accounts of the POSEIDON project and we are very grateful to Anne Cambon-Thomson for her work regarding this on EFI’s behalf. However the budget proposal for 2013 does not include money required for the website development.

This budget was accepted by the membership. The EFI accreditation budget was presented and approved by the membership: €54,150 in profit for 2012 and €59,840 proposed for 2013.
Overall at the end of 2012 EFI had the following equity: €261,692 (Lyon) and €408,978 (Leiden).

There was one question regarding the budget of the EFI office. Currently €60,000 is budgeted but less is spent. Is this likely to stay the same? The answer was yes, we do not anticipate any major changes to this.

6. Report of the EFI committees

6.1 Accreditation, Chair: Andrea Harmer

Accredited Laboratories:
As of May 2013, 261 laboratories are accredited. The increase in the number of accredited labs that has been observed over the last 10 years has now slowed down with around 259 for the last four years.

The Accreditation Committee held a workshop in Leiden in October 2012 for the training of eight new inspectors.

Extra meeting
A special ad hoc meeting was held in Leiden in February 2014, attended by members from the Accreditation, Standards, Education and EPT Committees and also representatives from the Executive Committee. The following matters were discussed with particular reference to how they will impact on the function of the committees:
- Director Qualifications
- ESHI Diploma
- Working with National Accrediting Bodies

Accreditation Website:
The software for the new accreditation website has been developed and testing of it has started. The aim is to go live with this early 2014.

Committee Membership:
The following changes in membership of the Accreditation Committee (AC) have taken place:
- New Commissioner for region 8 from May 2013 is Elissaveta Naumova
- The following four Commissioners will leave the committee: Thomas Eiermann; Katharina Fleischhauer; Luca Mascaretti and Chryssa Papasteriades
- The new Commissioners are:
  - Region 9-10: José Luis Vicario
  - Region 4: Christian Seidl
  - Region 5: Ingrid Faé
  - Region 7: Andrea Bontadini and Elisabetta Zino
  - Region 8: Amal Bishara

Poster
Andrea invited all EFI members to view the poster describing the accreditation activity, which was on display at this meeting: “Activity of Accredited Laboratories in 2011: A Preliminary Analysis”, Mascaretti L, Geelhoed S et al. Poster/Abstract 220

There were no questions or comments from the membership.

6.2 Education, Chair: Joannis Mytilineos

Joannis Mytilineos presented a list of members of the Education Committee (EdC) with their assigned areas of responsibility. The members cover all of the EFI regions as defined for accreditation. M Drouet and A Martinho will join the Committee and will take on responsibility for ETHIQ Curriculum and Regional Workshops respectively.

The Education Committee met at this conference and during their meeting they discussed the following:

H&I Curriculum (European Specialisation in H&I, ESHI-Diploma)
The ESHI syllabus is now standing as Version 1.7 incorporating changes made after December 2012 and approved by the EBTI assembly, the EBTI EC, and the EFI Educational Committee on the 10th May 2013. The next step will be to put the period with details where to send applications, pay the fee, etc.

The first exams and first honorary diplomas will be given in Leiden in October 2013.

Annual EFI-Meeting Teaching Sessions
The programmes for Maastricht and Stockholm were discussed and the production of guidelines for teaching sessions were derived.

Technical Curriculum
The syllabus for the European Technical H&I Qualification (ETHIQ Diploma), was discussed with the first draft to be presented in Stockholm 2014.

EFI/ASHI/APHIA Summer School
This important event, hosted by EFI this year will be held 15th-18th September 2013 at Stintino, Sardinia, Italy.

The faculty for this summerschool includes: Marco Andreani (EFI); Carlo Carcassi (EFI); Dianne De Santis (APHIA); Paul Dunn (APHIA); Gottfried Fischer (EFI); Katharina Fleischhauer (EFI); Amy Hahn (ASHI); Joannis Mytilineos (EFI) and Marilyn Pollack (ASHI)

In addition the committee has been active reviewing EFI Education and Training bursaries applications, and has also been involved in the organisation of regional educational activities.

There were no questions or comments from the membership.

6.3 External Proficiency Testing, Chair: Falko Heinemann

The EPT committee met over two days, preceding the Maastricht Conference. The main agenda items were:

Update committee membership
Susan Corbin (Co-chair) will be replaced with Deborah Singleton; Chantal Gautreau will be replaced with Pascale Loiseau and Elissaveta Naumova will be replaced with Anastaysia Mikaylova

Registration of EPT Providers
The aim is to document the real number of Providers in Europe using Registration form (Oct. 2012). The registration process started in January 2013 and has been supported by the EFI Office (Ingrid Abelman). The first analysis of responses was performed during the EFI meeting Maastricht 2013. Fifteen out of seventeen providers responded within two months. There is some need for clarification of the registration process. Further work has to be undertaken together with other committees regarding the scoring system; certification and coverage.
Mission statement
In response to a request from the Executive Committee, the EPT Committee has proposed the following mission statement:

The EFI EPT Committee:
- Collects information on external proficiency testing schemes operational in H & I laboratories affiliated to EFI and works to ensure their homogeneity and comparability.
- Is responsible for defining and revising EPT Standards for Laboratories
- Makes recommendations concerning the organisation of EPT schemes and provides help and advice to new EPT schemes when requested.
- Defines and revises the Standards for Providers of EPT schemes in concordance with national and international standards
- Identifies regions lacking EPT schemes and develops strategies to serve these areas.

There were no questions or comments from the membership

6.4 Scientific Committee, Chair: Katherina Fleischhauer

Committee Members:
No Changes other than Katherina taking over as chair from Ronald Bontrop. Membership: Rainer Blasczyk, Ronald Bontrop, Katharina Fleischhauer, Frits Koning, Ludvig Sollid, Jean-Marie Tierry, John Trowsdale, Junior Lardy (LOC)

Abstract review
308 Abstracts were reviewed of which 87 were for oral presentation and 221 for poster presentation.

Scientific Programme for future EFI meetings
The scientific committee met during the Maastricht meeting and approved the scientific programme for Stockholm 2014.

Working Groups
In addition the organisation of EFI Scientific Committee “working groups” to focus on particular areas of scientific development (collaborative projects) related to the interests of EFI was discussed. The goals of such projects would be:
- To promote scientific collaborations within Europe
- Scientific Production
- Technical Outputs (Generation of databases etc.)
- Funding opportunities
- To establish contacts with relevant Scientific Societies
- Speakers/Joint Sessions at EFI Meetings
- To attract young scientists in their field
- JBA
- To help scoring abstracts of the Annual EFI meeting if needed

The first proposed working group is the “EFI SC Working Group for Population Genetics” On the lead of the EU-funded HLA-NET program. The coordinators of this group are Jean-Marie Tierry, who is a member of the Scientific Committee and Alicia Sanchez-Mazas (University Geneva). The objectives of the group are:
- To provide guidelines for population data reporting
- To provide new informative tools for population data analysis
- To characterise the diversity of European populations

6.5 Standards Committee, Chair: Kay Poulton

Membership News
Chantal Gautreau (Paris) has joined the committee and Benedetta Mazzi (Milan) will join in Leiden (October 2013)

Links with ASHI
A member of the EFI Standards Committee sits on ASHI’s QAS Committee and there is now a reciprocal arrangement to attend each others’ meetings every other year. This year Don Constantino (Chair, ASHI QAS) attended this meeting.

Standards v6.0
Copyright has been obtained for these standards.

Standards v6.1
This version of the standards has been circulated to the membership and these will be active from October 2013 Future Revisions of the standards will include reference to the following:
- ESHI Diploma
- Null Alleles / High Resolution Typing
- Re-arrangement of Section C to facilitate joint inspections
- Next Generation Sequencing

There were no questions or comments from the membership

7. Next EFI conference
The 28th European Immunogenetics and Histocompatibility Conference will be held in Stockholm, Sweden June 25-28th. The theme of this conference is “From Genes to Therapy”. The 2015 meeting will be held in Geneva, Switzerland and the 2016 meeting will be held in Kos, Greece. Applications to host the 2017 EFI meeting are formally invited in this newsletter.

8. EFI medals
The EFI medal is awarded annually by the EFI Executive Committee to recognise the achievements of individuals who during the course of their career, have made a significant contribution to EFI. This year the EFI medal was awarded to Maria Edvige Fasano and Ciaran Dunne in recognition of their contribution to the activities of EFI.

9. Installation of new officers and Councillors
The President thanked the outgoing members of the EFI Executive Committee, Ilias Doxiadis – Past President; Katharina Fleischhauer – Councillor and Joannis Mytilineos - Councillor. Carlo Carcassi and Peter Horn were welcomed as new councillors to the EFI Executive Committee.

The General Assembly was closed at approximately 18.00 hrs.
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At our EFI Standards meeting in Maastricht, we considered comments received from EFI members to our proposed changes to the Standards v6.0. These final revisions have now been approved by the EFI Board. Standards v6.1 will become active from 1st October 2013. They will be circulated to all members by email, and published on the EFI website for easy access.

I am currently collecting together comments on problematic standards for our next revision. At the moment I have received requests to review resolving ambiguities observed, and to review the requirements for defining Null alleles. If you have any suggestions or comments on how we may improve the EFI Standards, please contact me. We will discuss all requests when we meet in Leiden in October. As always, comments are welcomed in any language and do not have to be in English.

Kay Poulton, Manchester UK
Kay.Poulton@cmft.nhs.uk
Chair of the EFI Standards and Quality Assurance Committee

### NEWS FROM THE EFI COMMITTEE FOR EXTERNAL PROFICIENCY TESTING (EPTC)

1.) During the EFI conference in Maastricht this year some personnel changes in the composition of the EPTC have been approved by the EFI EC: Deborah Singleton (Region 3), Anastasiya Mihaylova (Region 8), and Pascale Loiseau (Region 6+11) were welcomed as new members of the EPTC. Elissaveta Nau-mova and Chantal Gautreau served the EPTC for many years and will now leave. Thanks a lot to them for their continuous contributions!

The function of the EPT Regional Coordinators is to liaise with their respective Regional Commissioners to ensure conformance with the EPT Standards. EPT Coordinators shall advise and support EFI accredited laboratories, those laboratories actively seeking EFI accreditation and EPT Organisers to meet these standards.

#### EPT Regional Coordinators (2013):

- **Region 1** - Scandinavia - Helle Bruunsgaard
- **Region 2** - Benelux - Ilias Doxiadis
- **Region 3** - UK & Ireland - Susan Corbin
- **Region 4** - Germany - Falko Heinemann
- **Region 5** - Central Europe - Martin Petrek
- **Region 6 + 11** - France + Switzerland - Pascale Loiseau
- **Region 7** - Italy - Francesca Quintieri
- **Region 8** - Balkans + Israel - Anastasiya Mihaylova
- **Region 9 + 10** - Iberia - Jose Vicario
- **Region 99** - South Africa - Susan Corbin

2.) The EPTC refined a Mission Statement outlining what the EPTC stands for:

- The EPTC
- 1. Collects information on external proficiency testing schemes operational in H & I laboratories affiliated to EFI and works to ensure their homogeneity and comparability
- 2. Is responsible for defining and revising EPT Standards for Laboratories
- 3. Makes recommendations concerning the organisation of EPT schemes and provides help and advice to new EPT schemes when requested
- 4. Defines and revises the Standards for Providers of EPT schemes in concordance with national and international standards
- 5. Identifies regions lacking EPT schemes and develops strategies to serve these areas

3.) The EPTC has started a register of EPT programs in January 2013. This registration aims to establish a list giving an overview of EPT programs for H&I laboratories and the EFI EPTC and to subsequently provide assistance and education for developing EPT programs. A special focus will be laid on the regions lacking necessary EPT schemes. Strategies will be developed together with other EFI Committees and Regional EFI Commissioners to adequately serve these areas.

H&I EPT Providers were requested to register on the EFI database. For this purpose, a survey document of a few pages was sent via e-mail, filled in by the EPT Providers, and subsequently sent back to the EFI office. Almost all Providers (15/17) did so until the end of February 2013. The EPTC would like to express its thanks to Ingrid Abelman from the EFI Office having summarized the data! The reported information was analysed in detail during the two EPTC meetings in Maastricht. In general, the analysis revealed a great variety in coverage of different H&I EPT categories, sample numbers, assessment of the results, etc., which results in a heterogeneity in certification of EPT results within the EFI regions. This important topic will be addressed by the EPTC in collaboration with the EFI Accreditation Committee and the EFI Commissioners.

4.) The EFI EC approved the EFI EPT Standards for Laboratories (version 5.1) and Standards for Providers (version 5.1), which can be downloaded from the EFI Website.

5.) List of EFI EPT Regions, Regional Coordinators and local EPT Providers (Update June 2013):

- **EFI Region 1**, Scandinavia: Helle Bruunsgaard, Copenhagen (helle.bruunsgaard@rh.regionh.dk)  
  - no Providers
- **EFI Region 2**, Benelux: Ilias Doxiadis, Leiden (i.i.n.doxiadis@lumc.nl)  
  - Eurotransplant (ET), Leiden – Doxiadis/Zoet (etrl@eurotransplant.org) www.etrl.org  
  - High Resolution EPT, Maastricht – Voorter/Tilanus (c.voorter@mumc.nl)
- **EFI Region 3**, UK & Ireland: Susan Corbin, Pontyclun (susann.corbin@wales.nhs.uk)  
  - UK NEQAS for H&I, Pontyclun –
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Development of a Higher Training Qualification within Europe; The European Specialisation in H&I (ESHI) Diploma

Background

Over the course of the last two years the EFI Education Committee has been working with the European Union of Medical Specialists (UEMS) to develop a training qualification for senior H&I staff within Europe. It has been a long term aim of EFI to develop a consistent training approach for members working at the level of Lab Directors or co-Directors or for individuals who wish to attain this level. Working with UEMS has allowed EFI to engage with a structure that already provides training for all areas contained within the Division of Transplantation of the Section of Surgery of the UEMS. This is the most logical place to site the Training Syllabus and the Examination and thereby all areas of H&I are covered (solid organ, HSCT, transfusion, disease association etc) and not just those that pertain to solid organ transplantation. The EBTI has responsibility for the syllabus and they will have to have evidence of completion of the Training Syllabus and the Examination. There is no aspiration to replace existing qualifications in those countries that already have relevant qualifications, but the drive has been to create something suitable for the many European countries that have no specific training in H&I at this level.

European Board of Transplant Immunology (EBTI)

The first stage in this process has been to create a European Board of Transplant Immunology (EBTI) within the UEMS structure. This body will work closely with EFI to oversee the training and examination process. The EBTI has members from each European Country (that has EFI members), and is located within the Division of Transplantation of the Section of Surgery of the UEMS. This is the most logical place to site the EBTI Committee, but the EBTI and EFI have full control of the content of the Training Syllabus and the Examinations attended and therefore all areas of H&I are covered (solid organ, HSCT, transfusion, disease association etc) and not just those that pertain to solid organ transplantation.

The EBTI met for the first time in Leiden in October 2012 and again at the recent EFI meeting in Maastricht. Representatives from 20 different European countries attended meetings and discussed the training syllabus and examination format. The Executive of the group has been created as follows; Joannis Mytilineos (Germany) is the Chair, Marco Andreani (Italy) is Deputy Chair, Tony Slavicek (Czech Republic) is Senior Secretary and David Turner (UK), the Junior Secretary. Mireille Drouet (France) is Treasurer and Martin Petrek (Czech Republic) is the representative of the EFI Board on the EBTI Executive Board.

European Specialisation in H&I (ESHI) Diploma

The EBTI has responsibility for the development and maintenance of a syllabus or curriculum for ‘higher’ training in H&I throughout Europe. There is no aspiration to replace existing qualifications in those countries that already have relevant qualifications, but the drive has been to create something suitable for the many European countries that have no specific training in H&I at this level.

The qualification obtained will be known as the European Specialisation in H&I (ESHI) Diploma. There will be two parts to the process; Part I involves providing evidence of eligibility to be examined and Part II is the examination itself. To show proof of eligibility a candidate will have to show evidence of completion of training for all areas contained within the syllabus and they will have to have worked within H&I for at least 5 years...
Dear participants, organisers and sponsors of EFI 2013 Maastricht

Thank you all for participating interactively at the EFI annual meeting in Maastricht 2013 and for the many compliments and feedback we received on the scientific, educational, social and commercial programme. The newly introduced master classes and meet the speakers sessions have been well appreciated and I am happy to hear that the EFI executive committee recommended to continue this in the next years’ EFI annual meetings; a compliment to all presenters at the master classes and meet the speaker sessions. The EFI annual meeting app has been introduced and used by many participants either by tablet and iPhone, a new tool that will be around in next annual meetings. Although the programme was quite full, according to some comments, the provided joint lunch gave many possibilities to interact and to relax. The positive feedback on the joint lunch in the exhibition area and have it separated from the industrial symposia enabled sponsors to focus on their symposia. Looking at the photo’s I see many happy faces enjoying the events. It was worth putting so much effort in the organisation of the meeting and I like to thank especially the members of the steering committee, all co-organisers of the HLA working group in the Netherlands, the EFI scientific and education committees and the executive committee for their support and suggestions. We are making up the balance now and will give feedback on our experience and the comments received to the EFI executive committee later this year. A special thank-you to the co-workers of the Dutch HLA laboratories, who took the immense task for preparing the conference bag, guiding participants to the conference rooms, taking audio tasks, supporting the social events and equipped the registration desk. All of you made the EFI annual meeting in Maastricht a success.

A big thank you to all

Marcel G.J. Tilanus

Representatives from the Dutch HLA laboratories supporting the EFI 2013 meeting in Maastricht.
Each year at the annual EFI Conference, a scientist who has made substantial contributions to the field of Immunogenetics is honored by the Society and invited to present their work in the form of the Ceppellini Lecture. The Lecture is named in honor of Ruggero Ceppellini (1917-1988), the Italian geneticist who greatly influenced the HLA field. The first Ceppellini Lecture was delivered in 1988 by the founder of EFI, Jon van Rood. Over the past five years, it has been held by Gerhard Opelz (2012), Erik Thorsby (2011), Emil Unanue (2010), Cees Melief (2009) and Hans-Georg Rammensee (2008). A complete list of Ceppellini Lecture Awardees can be found on the EFI website (http://www.efiweb.eu/index.php?id=26#c222).

This year’s Ceppellini Lecture was delivered by Professor James McCluskey from the University of Melbourne, Australia, at the Annual EFI Conference in Maastricht on May 11, 2013. Jim is a well-known personality in the EFI Community and beyond, not only for the enlightening lectures he has made us used to during Plenary Sessions at previous EFI meetings (Florence 2010, Liverpool 2012), but also as organizer of the very successful 14th Histocompatibility and Immunogenetics Workshop in Melbourne 2006, and last but not least as editor-in-chief of the official journal of the Society, Tissue Antigens, which he runs with the help of his charming wife, Eva Strinovich who works behind the scenes as senior editorial assistant.

Jim received his MD from the University of Western Australia where he trained as a pathologist before spending four years at the National Institutes of Health. On returning to Australia in 1987, he worked at Monash University until 1991 before joining Flinders University and the Australian Red Cross Blood Service. In 1997, he became Professor of Microbiology and Immunology at the University of Melbourne, where he was appointed Deputy Vice Chancellor of Research in 2011.

Jim is one of the most highly recognized leaders in our field, reflecting his groundbreaking discoveries to unravel the molecular mechanisms governing T cell immunity. These include numerous milestone contributions to our understanding of the structural basis of T cell alloreactivity, and more recently of abacavir hypersensitivity association with HLA-B*57:01. Jim’s work, published in over 300 scientific articles, has previously been honored by different Awards including the Parr Prize from the Australian Rheumatism Association and the Rose Payne Medal from the American Society for Histocompatibility and Immunogenetics, and the Ceppellini Award from EFI fits well into this distinguished record.

Given Jim’s talent not only as a scientist but also as a visionary speaker who can make the most complex matters appear simple, we were looking forward to an outstanding summary of the major advances he contributed to T cell alloreactivity and pharmacogenomics. But Jim surprised us: his presentation, entitled “MHC-1 related T cell immunity - leveraging the central logic of HLA”, was focused on a new topic, mucosal-associated invariant T cells (MAIT).

MAIT are selected for by intestinal bacteria – they are not present in germ-free mice - and make up for 10% of T cells in the human gastrointestinal mucosa. MAIT are characterized by an invariant Vα7.2 T cell receptor which interacts with the MHC class I – related monomorphic ligand MR1 presenting vitamin B metabolites. Interestingly, the MAIT TCR can bind MR1 in association with a non-stimulatory or a stimulatory antigen, represented by a metabolite from folic acid and riboflavin, respectively, the latter requiring bacterial activation of a riboflavin precursor. The agonist ligand is able to make direct contact with the invariant MAIT TCR, while the non-stimulatory ligand is not, resulting in a higher affinity of interaction with the former compared to the latter. Once again, Jim in this novel work elucidated the structural basis for an important functional activity of the T cell immune system, this time innate-like pattern recognition. This is the very essence of Immunogenetics.

We were privileged to hear these data ahead of their publication in the July issue of Nature Communications. In his presentation, Jim proved not only his scientific excellence and great clarity of mind, but also his human delicacy, by acknowledging who actually had done the work in his lab with very personal words. Jim’s Ceppellini Lecture has been a treat for the audience and one of the highlights of this year’s EFI Conference.

Katharina Fleischhauer – on behalf of the EFI Scientific Committee
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This year the 27th European Immunogenetics and Histocompatibility Conference meeting was held in Maastricht, the Netherlands. The city is located on both sides of the Meuse river and it will be remembered for its picturesque squares, romantic streets and windy weather. The meeting was attended by a great number of participants from more than 35 countries, and more than 320 abstracts were submitted, both for oral or poster presentations. The general theme “Networking in Immunogenetics”, included a diverse mix of plenary sessions, abstract and education sessions covering immunogenetics, solid organ transplantation and hematopoietic stem cell transplantation, antigen presentation and recognition, biomarkers and population genetics.

Plenary session I caught my attention from the beginning. It was about immunogenetics: “At the edge of self nonself”. Prof. Mary Carrington from the Centre for Cancer Research (Bethesda, USA) started her talk about the influence of HLA class I expression levels on viral and autoimmune diseases. She presented some results which demonstrated how differential cell surface expression of some class I alleles may be influenced by variations found within them or in their proximity. Also, those data displayed how their interaction with KIR molecules can regulate NK cell activity and trigger different immune response which will end with various pathogens, tumors or autoimmune diseases being destroyed or tolerated by the immune system. Her concluding remarks focused on the need to investigate more factors that can influence HLA class I expression levels so all this knowledge could be applied in the treatment of patients who suffer from infectious or autoimmune diseases.

Next lecture in this session was held by prof. Erin Adams from University of Chicago about the Gamma/delta T cells and their ligands, MHC-TCR recognition. Gamma/delta T cells represent a small subset of T cells that possess a distinct T-cell receptor (TCR) on their surface. They reside in tissue compartments that are initial sites of infection such as the digestive and reproductive tracts. These cells perform a variety of functions ranging from cytokine release and cytotoxicity to immune modulation. Although the general signals, which activate these functions are as of yet unknown, what it is now well established is that some of this molecules can recognize nonclassical MHC class I molecules. The data about this recognition was shown during the lecture.

Last talk in this session was about the shaping of the immune repertoire by pregnancy and viruses and the consequences for transplantation. Prof. Frans Claas from Leiden explained how these conditions lead to a production of virus-specific or paternal-specific T-cells that can recognize allo-human leukocyte antigen molecules through cross-reactivity of their T-cell receptors. This can result in a production of more memory T-cells with alloreactive potential against potential donors that can have a great impact in transplantation.

Ending my report, I would like to say that my impression on the meeting was thoroughly positive. I would like to thank the EFI committee for the bursary, which allowed me to attend the 27th EFI Conference in Maastricht. Lectures, experience and chance to meet all this excellent people will definitely help me, as a young scientist, with my future work.

Derek Middleton, Liverpool, UK

I have chosen to report on Education Session 3 on Population Genetics given by Jill Hollenbach, Children’s Hospital Oakland, Paul Norman, Stanford University, and Martin Maiers, NMDP. The enthusiasm of the speakers, all from USA, although Paul is originally from UK, a long time ago, gave cause for much admiration and it is easy to see that the subjects they discussed are as much a hobby as work. The reason I attended this session was because of my on-going interest in this subject connected to www.allelefrequencies.net

Jill Hollenbach commenced the session by discussing methods for examining diversity within and between populations, a diversity brought about by historical human migration - very apparent in our field of HLA with the variation in allele frequencies. This variation results from evolutionary processes; appearance of new alleles by mutation, recombination, gene conversion, genetic drift, selection, migration and non-random mating. Population history (founder effects, bottlenecks/expansions, substructure/admixture), also require consideration in such studies. Important aspects that warrant attention are: does the population shares a single history, geography and culture and if the population sample is random and of sufficient size. Jill then proceeded to detail methods for examining diversity, ranging from the simple calculation of allele frequencies to testing for departures from neutral expectations. These included tests to fit Hardy-Weinberg equilibrium (HWE) which assumes no new mutations, no migration in or out of population, no selection, random mating and a very large population. Jill pointed out that HWE only holds if all of these are true and that this is not realistic in human populations but met approximately in many populations. Common reasons for deviations from HWE are genotyping error, population substructure, disease association and strong selection.

Next was a description of effect of selection on allele frequency distribution, ranging from the Neutral theory in which the observed allele frequency distribution is a function of random mutation/genetic drift rather than selection; to Directional selection in which one or few alleles are favoured in a population, pushing alleles towards fixation in a population over time; to Balancing selection where single alleles will not predominate, mutation can be favoured and diversity is advantageous. Examples were given of allele frequencies in each of these situations. Demographic events affect these selection processes. For example founder effects and rapid recent expansion with subsequent accumulations of new mutations on Directional, and recent bottlenecks, with not
enough time for accumulation of new mutation, on Balancing. There are various means of testing for departures from neutral expectations. This is where it starts to get complicated and the best advice is to become friendly with individuals like those giving talks at this session, in order to obtain help. Two tests were expanded upon. Ewens-Watterson which compares observed homozygosity (F) under HWE for sample size n and k alleles with F expected under neutrality. F is the sum of the squares of allele frequencies and under Balancing selection F will be lower than expectations and under Directional F will be higher. Tajima’s D considers nucleotide differences between alleles in addition to their population frequencies. If there is deviation from neutral expectations there are several explanations; selection, bottlenecks/rapid recent expansions, non-random mating extensive recombination and gene conversion. To examine differences among populations there are various analytical methods. Genetic distances give degree of similarity between populations and can be visualised using population trees, Principal Component Analysis and unsupervised clustering. Jill finished by emphasising careful collection of population samples, well advised application of statistical methods and thoughtful interpretation of results.

The next speaker was Paul Norman who described population genetics and evolution of HLA and Natural Killer (NK) Cell diversity. Paul presented diagrams of ancient divergence of modern human populations, starting 200,000 years ago, showing the deep structure and great variation that Africans exhibited. The African populations are as far away from each other as from European populations. The example used, the Ga-Adangbe population from Ghana, a single ethnic population, has a very recent expansion in population size during the last 3000 years. This divergence is large in contrast to Amerindians who have had populations of unstable size, via various stages of expansion and bottlenecks, due to migration, famine and disease. There are 4-5 times more HLA alleles in Ga-Adangbe than Yucpa from Venezuela at each of the HLA-A, B, C loci. This is further demonstrated by the many more haplotypes, both HLA and KIR, in Africans compared to Amerindians.

HLA-C*07:02 in Yucpa, a single dominant allele (freq 0.75) in response to recent disease, when the Yucpa encountered Europeans, was given as an example of positive selection.

Paul then presented data on KIR receptors present on NK cells. KIR A and B haplotypes are found in every world-wide population. Despite there being positive selection of telomeric KIR in Ghanaian population, corresponding to a large drop-off in diversity, there is an overall balance of KIR A and B haplotypes in Africans and Amerindians.

Turning to KIR alleles, it was shown how HWE departure could uncover novel features such as deleted alleles (2DL4, 3DL1/S1) or the fusion of two genes, 3DL1 and 3DL2. Use of techniques discussed by Jill (Watterson’s F and Tajima’s D) showed the balance of three divergent 3DL1/S1 lineages in the Amerindian Warao population.

Martin Maiers started by giving an introduction to haplotypes, in particular that more information was to be gained from haplotypes than alleles, linkage disequilibrium, and appropriately at EFI, the frequencies of the top 100 A, B, DRB1 haplotypes on NMDP from those of European Caucasian. Martin went on to show an Expectation Maximization Algorithm for finding maximum likelihood estimates, in existence since 1955 for blood groups from a famous European Ceppellini who termed the word “haplotype”. The method involves the initial designation to haplotype frequencies of equal values, using current haplotype frequency estimates to compute genotype (haplotype pairs) frequencies, using updated genotype frequencies to obtain new haplotype frequency estimates. This is continued until either the haplotype frequencies change very little or the number of iterations exceeds a predefined maximum. Data was shown to perform this, using the HLA-A, B types of two individuals. Martin is one of the co-leaders of the Registry Diversity Component (RDC) of the last three International HLA Workshops. Although haplotype frequency estimation algorithms have been used in previous International Workshops, this has mainly been in anthropology studies. Implementation for Registries is very different and much more difficult. Problems include dataset size, number of studied loci, and the heterogeneity of the data, resulting from HLA typings over many years using different methods, leading to different levels of resolution and incomplete data. The goal of the RDC is to address these issues unique to Stem Cell Registries and to produce a comprehensive analysis of HLA data from the 20 million donors from the Bone Marrow Donors worldwide database, at a clinical relevant level of resolution! One task was to develop a series of simulations that produced population deviations from HWE, a frequent occurrence in registry data. Two global populations were set-up using data from two registries in which either the sample population had equal numbers of haplotypes drawn from each population, or had mixed proportions.

The medical use of haplotype frequencies was touched upon i.e. to inform the search process, to guide recruitment and to reveal population structure. Visualisation of the world-wide distribution of some of the more common haplotypes using data from the registries, was presented.

If your appetite has been wetted you will find much more comprehensive detail in two publications: “16th HIW: Global analysis of registry HLA haplotypes from 20 million individuals” in International Journal of Immunogenetics 2013, 40, 66-71 and “Comparative validation of computer programs for haplotype frequency estimation from donor registry data” in Tissue Antigens 2013, 82, 93-105.

Finally one must mention Martin’s use of his own family as an example of pedigree analysis and his quote, shown beside a picture of Martin and one of his many children. “If a child looks like his father that’s genetics. If a child looks like the neighbour that’s the environment”.

Sebastiaan Heidt, Leiden, the Netherlands

This report will encompass a selection of plenary session presentations, mainly covering the environment and its impact on the immune system.

The presentation of Professor Marry Carrington was on various aspects of HLA-C. One of the subjects discussed was on the effects of HLA-C expression levels and HIV immunity. It is known that HLA-C molecules are expressed at varying levels, correlating with the allotype. Concomitantly, the strength of CTL responses has been shown to correlate with the level of HLA-C expression. Independent of the individual HLA class I
alleles, HLA-C expression levels are associated with HIV control. Whereas microRNAs (miRNAs) regulate the expression of most genes in the human genome, it was hypothesised that miRNA binding to the HLA-C 3' UTR may regulate HLA-C expression. Indeed, miRNA-148a can bind to a polymorphic binding site on some HLA-C alleles, but not all, while every HLA-B allele escapes miR-148a regulation due to lack of the polymorphism.

There is also an insertion/deletion polymorphism for miRNA-148a (-127), determining its expression levels. This -127 deletion results in high levels of miRNA-148a, causing decreased HLA-C levels of susceptible alleles, which leads to a high HIV load. Individuals who have HLA-C alleles not regulated by miRNA-148a have strong HIV control. These results nicely demonstrate the effects of microRNA regulation on the immune system, and its impact on anti-viral immunity.

Professor Frans Claas discussed the effects of the environment on the alloimmune response. Two factors potentially affecting the alloimmune response are pregnancy and viral infections. Pregnancy leads to the two-way exchange of cells between mother and child. Since mother and child are essentially haplo-type matched, the mother recognizes the inherited paternal antigens (IPA) and the child recognizes the non-inherited maternal antigens (NIMA). These types of recognition lead to opposite effects. The mother recognizing IPA leads to immunization, whereas the child recognizing NIMA leads to tolerance. Indeed, for kidney transplantation it has been shown that in the setting of haplo-identical sibling kidney transplantation, a graft with a mismatched NIMA leads to better graft survival than one with a mismatched NIPA (non-inherited paternal antigen). Also for cord blood transplantation it was shown that there is less mortality when the HLA mismatch is a NIMA. For allocation of (partially mismatched) cord bloods, it may therefore be advisable to take the mother's HLA typing into consideration as well.

The second environmental factor that may affect the (pre-existing) level of alloimmunity is the previous exposure the viruses. Transplant tolerance is easily achieved in laboratory rodents, much more difficult to achieve in non-human primates, and virtually impossible in humans. This coincides with the levels of viral exposure in these species. Indeed, is has been shown for several viruses that there is cross-reactivity of the virus-specific T cells with alloantigens. The breadth of alloimmunity caused by viruses is unknown, but currently under investigation. Knowledge on which viruses cause alloreactivity to what alloantigen in the HLA background of a the recipient may lead to the definition of non-acceptable antigens based on viral exposure.

Professor Emmanuel Wiertz provided an excellent overview on another aspect of viruses and the immune system, namely the several mechanisms used by viruses to escape immune-recognition. Different viruses use different mechanisms, mostly in the antigen presentation pathway, to avoid being sensed by T cells. These mechanisms include inhibition or blocking of the transporter associated with antigen processing (TAP), competitive peptide binding in HLA class I, and cell surface presentation of HLA-like molecules. A new aspect in viral immune escape is the production of microRNAs (miRNAs) by viruses, which is especially observed in herpesviruses. miRNAs are small non-coding RNA molecules that post-translationally regulate gene expression. Some miRNAs produced by herperviruses have been shown to be able to downregulate cell surface MHC class II expression. Current work of the group of Professor Wiertz includes the identification of which viral miRNAs are involved in this process.

The concept of viral miRNAs was further elaborated on by Professor Ofer Mandelboim, now in the context of natural killer (NK) cell recognition. Infected cells upregulate stress molecules, such as MHC class I polypeptide-related sequence A (MICA) and MICB, which are NK cell ligands. For example, HCMV miR-UL112 and EBV miR-BART2 target the 3'UTR of MICB, causing specific downregulation of MICB, which can lead to viral immune evasion. The human body fights back through. The human HAS-miR-376a has the same binding region as EBV miR-BART2, but can undergo RNA editing, leading in the alternative binding capacity to HLA-E. Upregulation of this human miRNA therefore leads to decreased expression levels of HLA-E and the subsequent NK-mediated killing of infected cells. These results, together with those of Professor Carrington and Professor Wiertz show the importance of miRNA regulation of the immune system, which is a relatively new field of investigation.

As Professor Claas already introduced, the environment plays an essential role in immunity. The lecture of Professor Frits Koning stretched important point this even more, now in light of autoimmunity. His lecture was on celiac disease, an inflammatory disease of the small intestine caused by an immune response against gluten peptides. Gluten are proteins found in foods processed from wheat and related grain species, including barley and rye. Celiac disease is both dependent on the environment, as well as on genetics (HLA). Gluten are the environmental component, whereas HLA-DQ2/8 is the HLA background which leads to celiac disease susceptibility. The disease is initiated by minimal inflammation caused by the low affinity recognition of gluten peptides in HLA-DQ2/8. Upon the minimal tissue damage caused, tissue transglutaminase is produced, which unfortunately, modifies gluten peptides to high affinity binding peptides to HLA-DQ2/8. This leads to an aggravated immune response and full-blown disease. Eliminating gluten from the diet results in rapid disease remission.

It is intriguing that 95% of patients is DQ2 positive, but 95% of DQ2 positive individuals is not a celiac disease patient. This appears to be related to the large differences in the affinity of T cell clones recognizing gluten peptides and corresponds to the concentration of gluten needed for the to respond. This also may relate to the observation that celiac disease is mostly diagnosed in early childhood and in people around the age of 50.

The abovementioned lectures together provided an excellent overview of the environment en the immune system, it’s implication for alloimmunity as well as autoimmunity. Overall, together with the many other plenary and parallel lectures of this 2013 EFI conference, the scientific program was a great success.

Laura Zito, Milan, Italy
Plenary Session V was dedicated to the Aging of the Immune System, which is today an important scientific, clinical and social issue. Indeed, while scientific progress and successful
Innovation and New Horizons

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treatment of human diseases helped to extend human life expectancy, at the same time they resulted in an increase of the elderly population. One of the major causes of morbidity and mortality in this population is the enhanced sensitivity to infectious diseases due to the senescence of the Immune System. Immune aging determines not only inadequate immune responses to infectious agents but also refractoriness to preventive strategies such as vaccines. For this reason, understanding of the “factors influencing immune aging” is important to extend healthy life expectancy.

Prof. Rob de Boer, Professor of Theoretical Immunology at University of Utrecht, held the first presentation illustrating the fundamental difference between aging of mouse and man, and giving important insights into human T cell dynamics. He showed that application of complex mathematical models to data obtained by different methods, such as TREC analyses and deuterium labeling, were useful to quantify the contribution of thymic output and peripheral T cell division on the global maintenance of naïve T cell pool in human and mice. His results demonstrated that in healthy human adults only about 10% of naïve T cells were actually produced by thymus and, despite the functional decline of this organ with age, its contribution remained constant over the years. As consequence, about 90% of these cells were maintained by peripheral division. In contrast, analyses of the naïve T cell pool in mice underwent to thymectomy revealed that in this species, naïve T cells are almost exclusively derived from thymus production even at old age. This difference was further underlined by the different turnover rate observed for human and murine naïve CD4+ T cells which was estimated as about 0.0005 division per day in human and 0.021 division per day in mouse, corresponding to life span of 1,517 and 47 days, respectively.

These results showed that mouse and man have different mechanisms to maintain the naïve T cell pool, which are likely to be differently affected by aging. Starting from the very first observation of decline of the thymus function with age, made by Steinman et al in 1985, progressive reduction of the thymic production can differently impact on T cell dynamics and immune aging of mouse and man; making difficult any extrapolation from the animal model to humans. Nevertheless, an important question arose from the audience: What about wild-type mice? Indeed, results presented by Prof. de Boer were obtained from mice kept in pathogen-free environment. This environment cannot replicate the natural daily exposition to antigenic stimuli from pathogens; leading to the final consideration that these mice are not the good models for investigation of T cell dynamics of normal and aged human immune system.

Prof. Lenhard Rudolph, Scientific Director of the Leibniz Institute for Age Research/Fritz Lipmann Institute (FLI) in Jena, was invited to hold the second presentation on the molecular mechanisms underlying the aging of hematopoietic stem cells (HSC) revealed by investigation in mouse models. In his presentation, he showed that immune aging could be due to the progressive functional decline of HSC compartment characterized by impairment of lymphopoiesis and enhanced myelopoiesis. This functional decline was linked to the activation of a differentiation checkpoint responding to DNA damage or telomere shortening, typical hallmarks of aging cells. Cell differentiation could lead HSCs to limit their self-renewal potential because of their commitment, but also prevent DNA damage accumulation and consequent development of stem cell-derived cancer.

In particular, Prof. Rudolph described the role of basic leucine zipper transcription factor, ATF-like (BATF) as a major component of this mechanism. He demonstrated that BATF is upregulated in response to DNA damage and telomere shortening by G-CSF/STAT3-dependent signaling. Upregulation of BATF resulted in induction of gene for lymphoid differentiation while BATF knockdown reverted the lymphoid commitment and re-established self-renewal and function of HSCs, although resulting in accumulation of DNA damage. Finally, the observation that lymphoid competent HSCs (CD34+, cKit+, Sca1+, Lineage-, CD150lo) were more sensitive to DNA damage-induced differentiation than myeloid competent cells (CD34+, cKit+, Sca1+, Lineage+, CD150hi) supported the hypothesis that DNA damage can lead to the progressive exhaustion of the lymphoid differentiation potential of HSC compartment in favor of the expansion of myeloid competent cells explaining the functional decline described above.

Data presented by both speakers provide new insights into the molecular mechanisms and cell dynamics underlying immune aging. These data are promising to be translated in improvement for the cure of elderly population by definition of molecular targets for the Immune System rejuvenation and, moreover, they will be useful to improve management of immune reconstitution in lymphopenic patients.

Heleen van den Heuvel, Leiden, the Netherlands

In this report my attention goes out to the 3rd Abstract Session: Solid Organ Transplantation I. The presentations given in this session were highly captivating and of substantial importance for the field of transplantation.

The first speaker, Kirstin Heutinck from the AMC hospital in Amsterdam, The Netherlands, introduced the audience to a new tool to identify alloreactive responses mediated by virus-specific CD8+ T cells. Building on the basis of a mixed-lymphocyte reaction, a resting period was introduced followed by a re-stimulation episode. This technique increases the sensitivity of identifying cross-reactivity in vitro, and provides a helpful tool for determining alloreactive responses.

Second, Paula Xavier from the Portuguese Institute of Blood and Transplantation in Porto, Portugal, presented her work on the identification of TLRs in renal transplantation biopsies. TLRs are associated with ischemic reperfusion injury and acute/chronic rejection, and her results showed that especially TLR2 may represent an important link in ischemia reperfusion injury.

The next speaker was Eva Zillian from the Hannover Medical School in Hannover, Germany. As the mechanism by which anti-HLA antibodies affect transplantation outcome is incompletely understood, she proposed that VCAM-1, a cell-adhesion molecule expressed on the vascular endothelium, may be involved. She showed that anti-HLA antibodies increase the adhesion of THP-1 monocytes to endothelial cells, and this is indeed facilitated by VCAM-1.

The succeeding speaker was Ilias Doxiadis, from the Leiden University Medical Center in Leiden, The Netherlands. The former EFI president questioned why HLA-C molecules are generally less immunogenic than other HLA-I antigens, and...
he revealed a hierarchy of the immunogenicity of different HLA-C molecules. His take home message taught us that both the level of expression and the immunogenicity of an individual HLA molecule are of great importance for the generation of an alloresponse.

Eric Spierings from the University Medical Center Utrecht, Utrecht, The Netherlands, presented his research on indirect recognition of HLA epitopes. Peptides derived from mismatched donor HLA-I molecules that are presented in recipient HLA-II molecules, can be recognized by CD4+ T cells, leading to donor-specific antibody formation. Aim of the study was to investigate the effect of indirect recognition on isotype switching, and he showed that the presentation of donor-derived HLA-I peptides by recipient HLA-II molecules plays a significant role in de novo generation of donor-specific anti-HLA IgG antibodies.

Susan Fuggle, from the Oxford Transplant Centre, Oxford, UK, presented the possibility of identifying grafts at risk for rejection by using de novo donor-specific antigens as a biomarker in pancreas transplantation. This is the largest study so far to assess the association of de novo anti-HLA antibodies and graft outcome in pancreas transplantation, and a strong association was seen between donor-specific antibodies and graft failure.

Thomas Bachelet, from the Hopital Pellegrin, Bordeaux, France, continued the topic of identifying donor-specific antigens, yet his focus was on kidney transplantation. As acute humoral rejection is associated with low levels of circulating donor-specific antibodies, the antibodies could consequently be sequestered in the allograft. He showed that over time, the graft donor-specific antibodies, rather than serum donor-specific antibodies, were associated with microcirculation lesions, C4d disposition and ultimately graft failure.

The final speaker of the session was David Keegan from Beaumont Hospital, Dublin, Ireland. Patients with antibodies against DRB3*/4*/5* antigens have increased panel reactive antibodies (PRA) and have a longer time on the waiting list. By investigating the linkage disequilibrium of DRB3*/4*/5* with DRB1*, allele-specific antibody reactivity could be distinguished and taken into account in clinical decision making. Antibodies against DRB3*/4*/5* were determined in over 500 patients on the kidney transplantation waiting list, and he showed that indeed strong haplotypic associations do exist. The obtained data can help interpret positive cross-match results as well as allele-specific antibodies, resulting in a lower PRA and a decreased time for a patient on the waiting list.

To summarize, research concerning T-cell allorecognition, donor-specific antigens and the identification of biomarkers is important for our understanding of the complex mechanisms involved in allograft rejection. The research presented in this session has shone new light upon these topics, and additional research building on these results is likely to be of benefit for successful transplantation outcome.

**Thomas Kraemer, Hannover, Germany**

My report is about the role of different factors that influence immune aging. In the first presentation of this plenary session, Prof. Rob de Boer (University of Utrecht, Netherlands) explained the usage of mouse models to investigate kinetics of naive T cells in humans. For elucidating T cell dynamics in humans, the extrapolation of mice to men as a model system is widely used. For example, research on naive T cell recovery in lymphopenic humans and long-term failure of immune reconstitution after stem cell transplantation were derived following naive T cell generation from the thymus in mice. It is known that in both these species, the thymic output declines with age. There is a dramatic loss of naive CD4+ and CD8+ T cells in the age span between 30 – 75 years of age in humans and in older mice as well. Despite these similarities, naive T cell maintenance in mice is not conferrable to human naive T cell maintenance. This conclusion was proposed based on the analysis of T cell receptor excision circle (TREC) data. These TRECs are byproducts of the V(D)J recombination in the thymus and can give information if a particular T cell is derived from thymus or by peripheral T cell proliferation. The results showed that ~ 90% of the naive CD4+ T cell pool in human adults was generated by peripheral T cell proliferation, whereas in adult mice the naive T cell pool is derived from thymus output. The life span of naive CD4+ and CD8+ T cells in human and in mice was compared by quantificational analysis of the amount of Deuterium in the DNA, resulting from the incorporation of deuterated water (2H2O). The data were fitted to a mathematical model and revealed that the average life span of murine naive CD4+ and CD8+ T cells with ~ 7 and 11 weeks and is ~ 40 fold shorter than in humans. Taken together, it could be impressively demonstrated that the contribution of the thymus in naive T cell production in human adults is much smaller than is widely assumed. Since the basis of this assumption were mouse models, the take home message is that one can not freely derive naive T cell kinetics in human and mice because the source of naive T cell production differs qualitatively between mice and men.

The focus of the second talk, given by Prof. Lenhard Rudolph (Leibniz Institute for Age Research, Germany) was on how the shortening of telomers affects somatic stem cell function and accumulation of DNA damage in adult stem cells during aging. The main function of telomers is chromosomal capping. The enzyme telomerase is responsible for the elongation of telomers by synthesizing six-nucleotide repeats. At the end of every cell division the end replication problem of DNA polymerase and telomere processing are responsible for the shortening of telomers. By reaching a critically short length, DNA damage checkpoints are activated and telomers lose their capping function. The telomerase activity is repressed in most somatic cells, except a subset including stem cells, progenitor cells and germ cells. The importance of telomerase activity in these cells was highlighted by utilization of a telomerase model system with mice that lack telomerase activity. The mice in the 3rd – 6th generation showed a shortened life span and impaired maintenance of organ systems with high rates of cell turnover. Another interesting fact was that DNA damage checkpoints are induced when telomers reach a critically short length, which leads to cell cycle arrest (senescence). The induction of senescence is caused by the cyclin-dependent kinase inhibitor p21. The deletion of p21 elongated the life span of telomere dysfunctional mice, but did not affect apoptosis checkpoints. It has also been shown that p21 impairs self-renewal and functionality of telomere dysfunctional stem cells. As a second factor, the B cell activating transcription factor (Batif) was also pointed out as a core factor limiting self-renewal of haematopoietic stem cells (HSCs) in response to DNA damage and as well leads to the
induction of lymphoid differentiation related genes in HSCs. This finding could be an explanation for the fact that immune function decreases during aging, since depletion of lymphoid-competent HSCs during human aging results in impaired lymphopoiesis. A correlation between the activation of Batf and activation of p21 and telomere shortening could be found in patients with myelodysplastic syndromes, assuming the presence of Batf-dependent checkpoints in HSCs.

The current data obtained from mouse models serves as a rational basis to understand molecular causes of ageing but are strongly context dependent. However, the development of a cell kinetic model, based on humanized mouse models will help to understand tissue ageing and impaired immunity in ageing humans.

**Anastassia Mihaylova, Sofia, Bulgaria**

Many interesting topics were presented at the scientific sessions during the conference. One of them was the abstract session “Non-HLA Polymorphism and Miscellaneous”, that took place on 14th May from 8.30 to 10.00 AM. The session included 9 presentations. The first speaker, Viviana Fromont-Racine, presented the work on the blood levels of HLA-G and pregnancy-associated protein A in normal pregnancies. The authors monitored the blood levels of soluble HLA-G and PAPP-A in 51 women with a normal pregnancy, at the first, second trimester and at the delivery. Their results showed that the mean levels of soluble HLA-G decreased progressively with advancing pregnancy, while the mean levels of serum PAPP-A increased progressively. Association between the exon 14 polymorphism of PAPP-A gene and protein serum levels was observed. The second speaker, Milena Ivanova, presented an abstract entitled “Rapid quantitative identification of IDH1 and IDH2 mutations using a novel bead-based liquid array with LNA-modified oligonucleotide probes”. She presented development of a novel luminex-based assay for analysis of 5 IDH1 and 2 IDH2 mutations commonly associated with hematological disorders. The method was validated on artificial plasmid standards and 114 patients with AML and MPNs. The assay was sensitive, multiplex, quantitative and could be successfully implemented in the diagnostic work-up of patients with myeloid malignancies. The third presentation was made by Peter Novota and discussed the association of polymorphism in HSPA1B gene with idiopathic inflammatory myopathy. A total of 6 genetic polymorphisms in 3 HSP70 genes were analyzed by direct sequencing. The frequency of the “INS” allele in HSPA1B increased in patients group and was strongly associated with HLA-DRB1*03 allele. The next speaker, Yunping Xu presented an approach for analysis of genomic full length sequence of HLA class I genes in Chinese Han individuals. In their study, the authors elucidated for the first time full-length sequences of 70 alleles including 22 novel alleles and 47 alleles with unknown promoter, 3’UTR or introns. The fifth speaker, Bouke Hekema presented HLA associations with Hodgkin lymphoma. Associations with several HLA allele groups were observed and these were dependent on patient age indicating existence of multiple etiological entities. Data on distribution of HLA and MICA alleles and haplotypes in Maori and Polynesians was presented by Paul Dunn. Restricted HLA class I allele set compared to other well defined populations was observed. In contrast HLA-DRB1 locus seemed to be more diverse in these two ethnic groups. Data provided support the theory for hybrid origin of Maori and Polynesians. The next abstract entitled “Conservation of HLA-A and HLA-G haplotypes in European and African populations” was presented by Julie Di Cristofaro. Regulatory and coding regions of HLA-A and HLA-G alleles were genotyped by SNAPSHOT and luminex techniques. Distribution of HLA-A and HLA-G alleles and haplotypes differed significantly between Malian and French populations. The data strongly support a high conservation of HLA-A and HLA-G haplotype structure in different populations. Next speaker Natasjia de Groot presented transcriptional levels of different MHC alleles in orangutans. The results showed that A locus is most abundantly transcribed, while the B locus presented different transcriptional patterns that seemed to be dependent on the B allotype present in animal. C locus, if present, was also poorly transcribed. The last presentation on HLA-Net was given by Alicia Sanchez-Mazas. She presented a collaborative study within the European COST action involving 22 countries. The main achievements were establishment of HLA molecular diversity map of Europe; recommendation from the working groups on: anthropological criteria in population and disease association studies; guidelines for reporting ambiguities; data format, storage validation and statistics; ethical aspects of HLA studies in Europe; education and training of young researchers.

Participation at this EPI annual meeting was extremely useful to me as I could update my knowledge on immunogenetics and have opportunity to meet interesting people working in this field.

**Sarah Maxfield, Cambridge, UK**

The educational session on kidney and liver transplantation compared and contrasted indications for transplantation, donor source, and factors influencing outcome such as immunosuppression and post-transplant complications. Finally, the session ended with a discussion of future considerations.

The indications for kidney transplant include end stage renal failure caused by glomerular nephritis, polycystic kidney disease, diabetes mellitus and renovascular disease. The key indications for liver transplantation include cirrhosis, cancer and cholangitis. Unlike for the kidney, liver failure does not benefit from a dialysis equivalent and consequently preemptive transplantation is necessary to improve survival outcome and quality of life.

The best renal graft outcome results are seen in live donations, as such donors are healthy at the point of donation, followed by donation after brainstem death. Worse outcomes are seen in donation after cardiac death partly due to ischaemia reperfusion injury, increasing age of donors and reduction in health at point of donation. Despite little recent improvement in the number of deceased organs available, the kidney donor pool is expanding with the increasing number of living donors, and the improvement in outcomes for ABO and HLA incompatible transplantation as a result of immunosorption, plasma exchange, IVig and Rituximab. This, in addition to the adoption of the pooled/paired donor scheme and altruistic donations (of which the Netherlands has the highest number) has enabled highly sensitised patients to find suitable donors. However, as the numbers of couples enter-
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CD is closely linked to DQ2 and DQ8. However, those genotypes are much more common in the population than the actual incidence of CD. It seems that the heavier the genetic burden (from a single DQ2 or DQ8 gene to DQ2 or DQ8 homozygosis), the more chances a given individual has to develop CD.

Another puzzling fact: DQ2 and DQ8 present to T cells acidic peptides bound to their peptide binding site, but gluten has no acidic residues. The research of the Leiden group demonstrated that peptides derived from gluten digestion in the intestine, can be transformed to show a glutamic acid residue. These peptides readily link to DQ2 and can elicit a T response. This can happen as a result of transglutaminase, an enzyme capable of turning glutamine into glutamic acid.

Then, one could ask why patients have a T cell repertoire ready to react with gluten digestion products. The hypothesis is that these patients have T cell populations that are capable of recognizing - albeit with a low affinity - gluten residues presented by DQ2 or DQ8. These T cell populations would then damage the gluten presenting cells, and cause tissue damage. Transglutaminase (an intracellular enzyme) would escape the cell and transform the glutamine residues found in gluten digestion peptides into glutamic acid, thus increasing the ability of DQ2/DQ8 to present them to T cells. These acidified peptides can arise from gluten derived from wheat, and have been obtained from other cereals: barley, rye or oats.

Thus, a gradient is formed, between DQ genetic charge, the presence and affinity of gluten reactive T cells, and other environmental factors (amount of gluten in the diet, infections...) that increase or decrease the chances of developing CD. These results, have been obtained working with DQ2 cells, and work with DQ8 cells is expected.

Margarita Pritfi-Kurti, Tirana, Albania
For me it was one of the impressive participation of my professional experience. The organizing of the conference was very good. Choice of theme was meant for us as young doctors in the field of immunogenetic also for them who work everyday. Each day was rich with themes that were stood in two auditoriums. The topics of interest to me were most valuable for the young doctors. The coffee Break was also interesting. In that time we met colleagues from different countries and exchanged valuable experience, also we become familiar with the new
The most common complications after transplant surgeries are infections (20% viruses, 50% bacterial), hypertension, osteoporosis and carcinoma. Among carcinoma the most frequent is skin cancer. Besides that, it is crucial to take into consideration a degree of HLA matching between donor and patient.

Many other intensive and interesting lectures and new working techniques gave impetus to my further work.

**Nadia N. Bagheri, Tehran, Iran**

Attending this Conference was a good opportunity to meet people with the same interest and to build potential relationships with experts. It was a key to know application of new methods especially Next Generation Sequencing in HLA typing and keep abreast with the current and emerging challenges. An interesting speech was on Monday 13th May. It was about one of the challenges in HLA genotyping with Next Generation Sequencing that presented by Julie Lane under the title of “Generation of HLA Diversity In Vitro from Hybrid Amplicon”.

Lane and her colleagues had started Nest Generation based HLA genotyping 3 years ago. They observed some never seen rare HLA alleles while searching for some probable association between DRB3 locus and Type I Diabetes.

**DRB Loci** (DRB1, DRB3, DRB4 and DRB5) were genotyped with Roche 454 technology and interpreted with Conexio and Score software. 131 DRB1*03:01 homozygous samples were selected; 59 of these samples were previously genotyped as DRB1*03:01, DRB1*03:01 with SSO. They found that 110 of the 131 samples were genotyped as DRB1*03:01 and DRB1*03:42 by Roche 454. They had never seen this rare allele of DRB1*03:43 and was wondering what is going on.

Their observation raised the question if some of thousand newly known HLA alleles could be the result of jumping PCR artifacts; a process that partial PCR product is separated from the DNA template and annealed to another HLA with a very similar sequence in the reaction and whether in this case DRB1*03:42 could be a hybrid PCR product coming from jumping PCR of DRB1*03:42 rather than actually distinct HLA allele.

It’s known that if there is DRB1*03:01 on a chromosome, there is DRB3 locus on the same chromosome with DRB1*03:01 and it is common that DRB3 allele to be either DRB3*01:01 or DRB3*02:02 so some allele specific primer pairs for DRB1*03:01, DRB1*03:42, DRB3*01:01 and DRB3*02:02 were designed and a series of DNA amplification had done to investigate if there was a DRB1*03:42 allele there but they missed it and it is possible to amplify DRB1*03:42 directly through genomic DNA. They used DRB1*03:01 and DRB3*01:01 homozygous sample as control. However, DRB1*03:42 couldn’t be amplified directly from genomic DNA. This strong evidence confirmed the hypothesis that “DRB1*03:42 produced in vitro”.

They found lots of evidence of such hybrid DNA products
and alleles (DRB3*, DRB1*16:01 and DRB1*16:09) from combination of different loci depend on where the particular product crashes and sits in next run of PCR.

According to the Lane observation, although such a hybrid amplicons have been always produced in HLA amplification but clonal amplification nature of Next Generation Sequencing and Software design in order to look for heterozygous genotype calls, allows detection of all sequences including the minor ones. Hybrid amplicons correspond to named and common alleles can result inaccurate HLA genotyping, however most of hybrid amplicons don’t correspond to a normal alleles and don’t interfere in genotyping. The appearance of rare allele only in specific genotype combination, deviation of Hardy-Weinberg and deep disparity between allele sequences can be a warning sign for detecting hybrid amplicons. Knowledge of population can be helpful.

Finally they found that better polymerase processivity (phusion>Amplitaq Gold> FastStat), PCR cycling condition like additional cycling steps (3steps>2 step) and reduced cycling number (28 cycles >35 cycles) can minimize hybrid alleles formation while increasing primer concentration has no effect on reducing their production.

Although lots of people are starting to do complete genomic sequencing of the HLA loci by Next Generation Sequencing (NGS) to eliminate ambiguities by sequencing previously uncharacterized segments (i.e. additional exons and/or introns), this experiment showed that we must be vigilant for hybrid alleles and may be other new emerging phenomena.

Kuzminova Elena, Chelyabinsk, Russia
During the abstract and education sessions there were a lot of interesting and useful reports devoted to some aspects of immunogenetics, antigen presentation and recognition, organ transplantation. I would like to note the bioinformatics session, which held on second day of the conference. It was devoted mainly to next generation sequencing, software for NGS, issues that require resolution, including the agreement of appropriate standards. Dr. Hoffman presented his report about a software designed for human leukocyte antigen typing based on short amplicon NGS data from Illumina MiSeq systems, calling “neXtype”. Reporter outlined the main advantages and workflow of this software, which basic principle is to assigns each individual read to alleles from the HLA reference library instead of comparing the consensus sequence to the library. In addition my attention was attracted the report of Dr. Shmidt about the probability that registered donors who are 10/10 allele-level matches to patients in need of a transplant are not identified in the search process and that the non-identification of HLA-matched registered donors with incomplete HLA typing information is of practical relevance. This session was very interesting and useful to me, because our laboratory is going to get Ion Torrent to make high-resolution HLA typing for bone marrow transplantation in the near future. That’s why report of Dr. Bialozynski from Life Technologies, who are developing an HLA typing method based on the technology that enables read lengths of 400 bases on the PGM platform, in Immunogenetics session also caught my attention. Her report was about evaluation of two different library preparation workflows.

Also I’d like to mention a poster session. More than two hundred posters were presented at the exhibition area of MECC foyer. Me and my colleagues have found many interesting information in submitted posters, especially about HLA allele frequencies and it’s role in disease predisposition in different populations. And it was extremely interesting to discuss the results with the authors.

All bursary recipients expressed their sincere gratitude to EFI for the financial support.
AN IMPRESSION IN PICTURES OF THE EFI MEETING IN MAASRICHT

by Jeanine Opreij (jeanine@jeanineopreij.com)
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Next Generation Sequencing (NGS) is a technique that allows the determination of clonal sequences of PCR products or genomic DNA in a “massively parallel” manner; this means that in one run the sequence of millions of DNA molecules is determined. Initially this method was developed for ‘whole’ genome sequencing, where complex loci, however, were not addressed thoroughly.

Only recently this technique has been applied for the characterisation of HLA alleles. Proof of principle analyses have been published that show NGS can be used at different scales either for high-throughput typing at intermediate resolution or medium throughput characterisation of the whole HLA gene at allele level resolution. Using the whole gene strategy the NGS technology has the potential to solve ambiguities, determining intron sequences and resolving “null” alleles.

Starting with NGS for HLA typing was a challenge, because of the large polymorphism and the high number of homologous loci so far not tackled in whole genome sequencing approaches. However the technology has improved in the meantime and kits for HLA typing by NGS as well as HLA NGS-typing software packages are offered by several companies. Since two years EFI conferences host teaching sessions and other lectures dedicated to this theme. A few laboratories are already using NGS for routine typing and have already received EFI accreditation. So far the accreditation requirements stress the validation of the new technique and the compliance with existing more general molecular typing and quality assurance standards.

Validation of new techniques is performed by testing of an adequate amount of samples for each locus parallel to the golden standard, the Sanger sequencing technique. Validation of the method must provide evidence of analytical sensitivity and analytical specificity of the technique. A summary and interpretation of the validation and SOPs must be made available. Training and competence evaluation of technical personnel must be amended. Dedicated external proficiency testing must be carried out to show compliance with typing results of other participants. All the other current relevant standards related to DNA typing must be followed.

Analysis of NGS data presents a new challenge for both, laboratories and standards. The ‘software’ for NGS is equally important as the reagents and instrumentation, and probably more important than in current sequencing strategies, where data could, theoretically, be analysed by eye. The amount of data produced by the NGS devices can realistically only be handled by computer assisted analyses and they have to prove their ability to cope with the huge amount of data. Different strategies for checking and approving the result must be established. Quality checks have been performed by the particular commercial available analysis programmes, nevertheless users must be able to interpret and understand the outcome.

The Standards Committee is working on new standards dedicated to NGS which are expected to be proposed for the 2014 standard revision cycle. Members who are already working with different NGS systems and who wish to contribute are cordially invited to communicate with myself (ingrid.fae@meduniwien.ac.at), or any member of the Standards Committee. Ingrid Fae, Vienna

Report from the “First EFI Training & Educational Histocompatibility & Immunogenetics Meeting in Cyprus”

The above Meeting, co-organized by the Histocompatibility & Immunogenetics Laboratory at the Nicosia General Hospital, the Karaiskakio Foundation and the Medical and Public Health Services of the Ministry of Health, took place in Nicosia, Cyprus on April 6th, 2013. Even though Cyprus is currently undergoing a big economical crisis, which turned out about two weeks before the Meeting, we were able to pool it through thanks to the financial support from EFI and our local sponsors; we are grateful to all. The Cyprus Minister of Health was present at the opening and addressed the Meeting.

The scientific program was approved by the EFI Educational Committee.

The local organizing committee (Dr Agathi Varnavidou-Nicolaidou, Dr Paul Costeas and Dr Dia Voniatis), thank Dr Joannis Mytilineos and Dr Ilias Doxiadis for their valuable contribution in organizing the scientific part of the Meeting. We thank both of them as well as the other guest speakers of the Meeting for accepting our invitation to take time from their busy schedules to travel to Nicosia and present their interesting topics. We would also like to thank Dr Katie Stavropoulos, for her participation and contribution to the discussions following each session.

Dr Chryssa Papasteriades gave the 1st lecture of Session I describing in a very analytical way the organizational structure of EFI, its role in the field of Histocompatibility & Immunogenetics, the cumbersome procedures of EFI accreditation aiming at high quality practices in the field and most importantly the benefits of accreditation to the laboratory and the clinicians. Dr Papasteriades stated that, what was more important was the learning process and effort put into attaining accreditation. She likened it to Odysseus’s 10 year journey back home to Ithaca and his heroic travails as he tried to reach his destination (accreditation) after the Trojan War.

Dr Joannis Mytilineos followed with a detailed lecture on HLA-typing techniques. He mentioned the advantages
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and disadvantages of serological and molecular typing methods, as well as the principles of the various HLA-typing techniques. He concentrated on Sequence Based Typing (SBT), which is more specific than PCR-SSP but gives more ambiguities, which increase as the number of newly discovered alleles increases. He said that these ambiguities are expected to be resolved by the “Next Generation Sequencing” otherwise known as “Massive Parallel Sequencing” which is highly automated and less expensive than the SBT techniques. This new procedure involves the generation of a single-stranded template DNA library, emulsion-based clonal amplification of the library, data generation via sequencing-by-synthesis and data analysis.

Dr Ilias Doxiadis gave the 3rd lecture of Session I on HLA-specific antibody screening techniques. He referred to all currently available methodologies for the detection and characterization of HLA-specific antibodies mentioning their principles and their sensitivity. He listed them in order of sensitivity (CDC, AHG, FCM, ELISA, and BEAD ARRAY -luminex technology) and he raised the question whether we need such high sensitivity tests as luminex or not? He emphasized that the antibody isotypes detected only by luminex are important as risk factors but are not always contraindication for transplantation. Currently there is no consensus on the clinical relevance of Solid Phase Immunoassays (SPI) – detected antibodies such as ELISA and luminex. As he said, it is very important to perform antibody testing by at least two different techniques, one cell-based and one SPI. He pointed out that each laboratory had to establish its own cut offs for each technique, based on their experience and requirements.

Dr Alicia Sanchez – Mazas, the last presenter of Session I, talked about the HLA-NET 2012 Recommendations on strategies how to work with HLA population data. She mentioned ethical issues such as anonymized molecular data and the need for informed consent. Recommendations on population sampling were mentioned so that allele frequencies calculations are valid. One should avoid mentioning absence/presence of an allele if the sample population studied is small. There are very useful tools available for estimating allele frequencies and for translating old HLA-nomenclature data to the new nomenclature, all available in the HLA-NET website.

The Session II began with a lecture of Dr Ilias Doxiadis on the challenging issue of “Alloimmune Response in Renal Transplantation”. He emphasized that it is best to prevent rejection than having to treat it. The number of HLA-DR mismatched antigens on the graft is an independent risk factor for transplants with early acute rejection, and therefore, at least one DR antigen compatibility match is recommended for organ allocation. Recipient age, donor age, donor gender, and CREG mismatches were presented as independent risk factors for late acute rejection. There are controversial data on the IgM donor-specific-antibody (IgM-DSA) presence pre-Tx and its effect on the graft outcome, while the absence of IgG-DSA allows for transplantation. IgA antibody presence is not thought to be protective anymore. Prevention for rejection could be achieved by the “Hygiene Model”, which refers to avoiding incompatible epitopes on mismatched graft antigens, which can lead to alloantibody production. The epitopes are the main targets for antibody binding/response. Eurotransplant is considering starting typing for HLA-Class I epitopes instead for antigens. i.e. reducing allosensitization by careful selection of the donors. Within Eurotransplant only 1% of highly sensitized patients are present on the waiting lists by applying specific programs to help such patients, like the Acceptable Mismatch Program. It was also mentioned that KIR ligand matched kidneys have a better survival.

Dr Alici Iniotaki gave a lecture on “The role of HLA-Class II antibodies in renal transplantation”. She emphasized that production of de-novo HLA-Class II DSA post-Tx is a sign of beginning of rejection. In a post-renal-Tx alloantibody monitoring study carried out at Dr Iniotaki’s centre it was found that the recipients of HLA-Class II incompatible grafts developed DSA more frequently than recipients of HLA-Class II compatible grafts. The significantly higher incidence of HLA-DQ DSA observed in this study compared to antibodies of other HLA-loci was attributed to the high number of polymorphic epitopes expressed on both α and β chains of the HLA-DQ molecule. HLA-Class II incompatible grafts should be avoided in high immunological risk transplants.

Session II ended with a presentation on the “Clinical Relevance of anti-HLA antibodies in renal transplantation” by Dr Ilias Doxiadis, on behalf of Dr Dennis Glotz, who at the last minute was unable to attend due to an unexpected problem. We thank Dr Glotz who was kind enough to send his slides to Dr Doxiadis and the latter who undertook to give a 3rd lecture in the Meeting. At the beginning of the lecture it was mentioned that anti-HLA immunization is the 1st cause of graft loss in renal transplantation and therefore it is recommended to HLA-match for transplants if possible. In France 80% of patients who are listed in the wait-
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ing lists for re-Tx are sensitized. A study was done to calculate the relative risk of antibody mediated rejection (AMR) according to the MFI of pre-Tx DSA. It was found that the relative risk for graft loss in patients with DSA of MFI>3000 was 3.8. They found that DSA affected the graft survival irrespective of complement fixation. According to Dr Glotz the "Classical Allocation System" must be revisited. The following were listed in increasing order as risk factors for AMR: Positive CDC crossmatch, positive flow cytometry crossmatch, and DSA presence. At the end of the presentation several desensitization protocols were described.

The afternoon session focused on population genetics and haematopoietic stem cell transplantation. The first speaker of this session, Dr Jean-Marie Tiercy, spoke about unrelated donors. He suggested that this is because of the high degree of HLA diversity (7843 alleles, 5764 proteins, 252 null alleles), and as an example he mentioned the HLA-B44 serotype, which has over 200 alleles, but only 10 of these are identified in the clinical laboratory. There are rare alleles which are not found in all populations and the frequencies vary from each population. Matching at HLA-C locus for units matched at HLA-A,B,DRB1* or with one mismatch should be included to minimize mortality risks. Interesting was that 3-7% of the patients have no ABDR matched donor on BMDW, while >90% have 1 potential donor, 40-50% have more or one 10/10 matched donor, and 20-30% a 9/10 matched donor.

Dr Joannis Mytilineos shared his knowledge on HLA matching, hematopoietic stem cell transplantation in Germany. Patients diagnosed with AML, and those with MDS, require a median of 15 days from the diagnosis to the decision to proceed to BMT. Emphasis was given on the indications for Bone Marrow Transplantation (BMT). Emphasis was given on the improvement of immunological treatment strategies and immunotherapy after BMT. These would essentially provide vulnerable patients with a protective response to acute infection. Dr Spyridonidis mentioned T cell therapy, whereby multi viral specific T cells are transferred to help treat infections, and provide memory responses to persistent or potential viral infections.

Dr Agathi Varnavidou-Nicolaidou on behalf of the Organizers

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**ONE LAMBDA EUROPEAN CLINICAL HISTOCOMPATIBILITY WORKSHOP WARSAW, POLAND**

Dr. Brendan Clarke - St. James Hospital - Leeds, United Kingdom

The One Lambda European Clinical Histocompatibility Workshop was held at the Sheraton Hotel in Warsaw, Poland from the 24-27th June 2013. The meeting was attended by 50 invited HLA and Transplant professional from across Europe and further afield. Representatives of One Lambda and their European partners were also in attendance.

The meeting format was based around a core structure of invited presentations by opinion leaders in the field supplemented by short presentations made by delegates. The daily programme comprised a morning session of two invited presentations followed by short presentations into the early afternoon, a break for lunch and a social event and then a further invited presentation before dinner. This structure allowed plenty of opportunity for discussion both within and outside of the conference hall and permitted the valuable interchange of ideas to continue as delegates interacted informally. Discussions over coffee and at mealtimes were certainly equally important components of the programme as the more formal Q&A sessions following presentations.

The purpose of this report is to provide a précis of the invited presentations to the meeting.

Prof. Caner Susal (University of Heidelberg) opened the meeting by providing a detailed overview of the development of the recently published consensus guidelines on antibody testing. This multinational, collaborative work was contributed to by twenty seven expert authors. Financial support into the project was provided through the various European transplantation societies.

The project initiation meeting was held in November 2011. This established three working groups - technical, pre and post-transplant which were tasked with creating the evidence bases for derivation of guidelines. These groups came together at the consensus conference in May 2012 at which each presented their summary of recommendations for discussion. These were then collated into one of three categories, should or must, beneficial or opinion of panel, reflecting the level of supporting evidence. Key recommendations were as follows. The technical group considered that best practice as supported by the literature required a mixed methodological approach to testing involving the use of cell and solid phase based assay systems. Prof. Susal provided data from his own centre which demonstrated high levels of apparent false positivity when sera are tested by SAB alone providing a caution against stand-alone use of the method. Conversely the exquisite sensitivity of SAB allows its confident use as a reflex test in determination of unacceptable HLA mismatches or in spotting windows of opportunity for transplantation in HSP. The pre-transplant group advised establishment of ‘risk categories’ based on profiles of results obtained on screening and crossmatch results. Guidance for avoidance of a prospective crossmatch based on SAB testing was also formulated. Based on evidence of a higher incidence of DSA in patients with graft loss the post-transplant group advised that a risk-stratified management plan should be implemented which sets a schedule for DSA monitoring based on time of transplant immunological risk.

Dr Alexandre Loupy (Necker Hospital,
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Ambiguities resolved by incl. the last 10 bases.

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Extensive primer site analysis, updated with every new release of Assign™ SBT 3.6+ references, is available for download on the Conexio Genomics home page: www.conexio-genomics.com. Please click on the DOWNLOADS bar and thereafter go to Assign™ SBT 3.6+ – IMGT/HLA References and download the latest references.

For In-Vitro Diagnostic Use.
Dr Loupy explained that as a clinician he needed to address three questions in diagnostic decision making - what process is operating? what is the risk? and what is the appropriate treatment? In regard to the first of these he pointed to the ‘circle of confusion’ in defining ABMR arising from the background ‘noise’ against which the process must be detected. The question raised was do we need new tools to refine our definitions? In this respect Dr Loupy considered that gene expression and ‘omics’ studies held significant promise. In respect of the second he identified that his personal experience led him to conclude that ABMR was a portent of poor outcome. The third question is presently debated.

The model developed by Dr Loupy regarded ABMR as a dynamic and evolving process rather than a discrete clinical episode. The situation described involved a complex interplay of factors and the end-to-end process was not the same in each case. Indeed, whilst C4d positivity is regarded as a canonical finding in ABMR C4d negative ABMR is an emerging entity.

By eschewing established methods for classification of rejection and adopting the principles of John Snow in constructing a ‘heat map’ of findings linked to vascular rejection Dr Loupy has provided an entirely fresh insight to the process and made several novel observations including the fact that in terms of its significance for outcome the presence of donor specific antibody is secondary to the level at which it occurs. This work will shortly be published in the Lancet.

Reflecting the complex diagnostic problem it represents Dr Tomasz Kozlowski (University of North Carolina) gave a presentation entitled ‘AMR in Liver Transplantation: Hard to define but we know it when we see it’. He began by identifying the conflict which exists in the literature between studies that indicate increased rates of graft loss in patients with ABMR and those which don’t support this association. Exploring the basis of the confusion which exists he stated that this had principally resulted from a lack of uniformity of investigational approach between studies which had been conducted. He also explained that since no specific diagnostic criteria exist this may have contributed to an under-reporting of AMR in general. In the face of this lack of consensus or basis for consensus Dr Kozlowski’s group performed a prospective analysis of 200 liver transplant recipients aiming to confidently identify AMR by exclusion of all other causes of cholestasis in those with circulating antibody and then to institute treatment to reduce antibody levels and monitor clinical outcomes of this intervention. Clinical investigations included ERCP, Doppler ultrasonography, MRI, angiography and biopsy. Laboratory investigations included liver enzymes, HLA antibody screening/specifc analysis and T and B cell crossmatching. Patients were maintained on a standard immunosuppressive regimen of tacrolimus/MMF/steroids and treated for AMR by plasma exchange, IVlg infusion and administration of rituximab. Dr Kozlowski reported that under this management plan graft function improved in those with donor HLA specific antibody as levels were reduced. In a final analysis Dr Kozlowski offered the audience the benefit of his experience, being that we cannot predict which patients will develop AMR but those with sustained post-transplant positive crossmatches are at greatest risk and that patients with positive crossmatch and cholestasis should therefore be investigated for AMR. Professor Duska Dragun’s (University of Berlin) presentation ‘Non HLA antibodies and their clinical relevance’ began with the observations of Opelz concerning influence of PRA’s to outcome in identical siblings, which pointed to an involvement of non-HLA antibodies. She then went on to show the audience the rich variety of non-HLA targets which exist before moving to the main theme of her talk - the role of the angiotensin type 1 receptor (AT1R) as a target for clinically relevant humoral immune responses. Pointing to an extensive literature concerning immune reactivity vs cell surface receptors and the clinical consequences of this Prof Dragun explained firstly the biology of the AT1R, the development of a rat cardiomycocyte based bioassay for identification of AT1R agonistic IgG, and the intracellular signalling pathways which were engaged upon binding. In the context of transplantation she then detailed how antibody mediated stimulation of the receptor could lead to downstream processes of graft vasculopathy and HLA-DSA negative alloreactivity. Moving her research into the clinical environment she had unequivocally demonstrated that targeted therapy improved graft survival in those with AT1R antibody. Seeking to improve the method of detection of AT1R antibodies Prof Dragun’s group had developed a diagnostic solid phase assay system. Technical difficulties had been experienced arising out of the need to maintain AT1R in native conformation to achieve acceptable assay sensitivity. These had however been overcome and the assay had been deployed in investigation of the value of AT1R antibodies as biomarkers. Using the assay investigations in renal patients performed by the Cedars-Sinai group have shown that AMR occurs in HLA/MICA negative patients with high AT1R antibody levels. The more recent Nantes study, currently in press, has demonstrated that AT1R antibodies are an independent risk factor for inferior allograft survival. A unifying theory of how HLA-DSA and AT1R antibodies might collaborate as graft damaging mediators of humoral immunity was described by Prof Dragun. Based on the observation that incubation of human microvascular endothelial cells with AT1R antibody induces HLA-DR expression the ‘zipper hypothesis’ leads to an enhanced risk stratification in which the co-existence of AT1R and HLA-DSA marks an individual as higher immunological risk for AMR than HLA-DSA alone. Dr Robert Liwski (QEII Health Sciences Centre, Halifax, Canada) gave a highly detailed technical presentation entitled ‘Optimisation and standardisation of HLA antibody testing to improve detection of clinically significant HLA antibodies’. This talk exemplified the value of maintaining critical oversight and willingness to dissect apart even our most established assay systems in driving service improvements. The re-configuration of the flow crossmatch as a more rapid method and the optimisation of the C1q assay provided the basis for discussion. Noting that the red cell crossmatch performed for purposes of transfusion takes approximately 25 min-
utes to perform Dr Liwski had revisited the protocol for flow crossmatching which in his centre had a performance time of around 2h with the aim of developing a more expeditious assay. Every aspect of the procedure was systematically evaluated for potential to release time savings and changes then carefully worked through to ensure that confidence in data outputs was not reduced. In this way Dr Liwski was able to reduce the time from first incubation stage to end of final wash from 1h 25min to just 35min without compromise to assay sensitivity. Having successfully re-configured the assay locally Dr Liwski then sought to investigate whether the method could be rolled-out to other centres with similar benefit. This roll-out was performed as part of a broader National HLA Proficiency Testing Committee backed exercise looking into variability in flow crossmatch procedure and data outputs across Canada. Key findings of this work were that between centre standardisation of method improved concordance of flow crossmatch results and that the ‘Halifax protocol’ offered advantages of improved signal/noise ratio, improved precision and decreased TAT. In terms of clinical governance these benefits translate as reduced lab contribution to organ cold ischaemia time and improved equity of organ sharing. Referring to the evidence base for correlation of C1q DSA with adverse outcomes of transplantation including AMR, transplant glomerulopathy and graft failure Dr Liwski shared the experience of his group in establishing the C1q screen assay. In common with the reported experience of others initial findings were of a lack of concordance between IgG SAB and C1q in terms of detected antibody profiles and strength of reactivity. These had led the group to question whether the manufacturer defined method of assay performance resulted in suboptimal sensitivity. Modified assay conditions were therefore employed involving the introduction of wash steps or AHG incubation. Both modifications increased MFI values of reactive beads and revealed additional specificities within the profile of serum reactivity. Additional studies were then performed using sera from ten highly sensitised patients, nine with failed transplants. These experiments again confirmed the superiority of the modified over the standard protocol and further work was able to demonstrate an improved correlation of results with those of AHG-CDC crossmatches. Prof. Georg Bohmig (University of Vienna) developed Dr Liwski’s theme further in his talk ‘Classical complement activation on HLA beads’. Beginning with a rehearsal of the evidence for the humoral theory of allograft rejection Prof Bohmig came to the conclusion that improved defining criteria were necessary to improve our management of the process. In agreement with Dr Liwski he showed data from his own group which indicated that donor specific antibodies which trigger complement activation are the most deleterious in terms of graft outcome. Stating the need for a diagnostic assay system providing a surrogate of AMR he then pointed to the potential utility of solid phase C4d staining and went on to describe the impressive efforts of his group in establishment of a robust method. The experimental approach taken was detection of C4 activation at the surface of HLA coated beads and this was neatly accomplished in a single antigen bead system with multi-colour reagents. An extensive programme of assay validation was performed and good correlation with C1q binding assay results was found to exist, moreover data outputs could be shown to be predictive for CDCXM results. The assay was then evaluated for its predictive value in respect of clinical outcomes after kidney transplantation. Persistent or de-novo complement fixing DSA were found to correlate closely with inferior graft outcomes establishing the value of the method in the laboratory test repertoire.

Dr Stefan Schaub (University Hospital Basel) moved proceedings slightly away from the core theme of histocompatibility with his talk ‘Polyomavirus BK-nephropathy: Current diagnostic and therapeutic concepts’. For non-clinicians this provided a fascinating insight into the problems of differential diagnosis and clinical management in situations where conflicting therapeutic needs exist. Following discussion on general aspects of BK virus biology Dr Schaub described various approaches to diagnosis beginning with the histopathological finding of polyomavirus-associated nephropathy (PyVAN). Albeit that PyVAN is the cause of allograft loss in 7% of cases the utility of this approach is reduced by the patchy involvement of tissues meaning that on biopsy the findings can be missed. Greater diagnostic certainty is afforded by detection of decoy cell, virally infected epithelial cells which can be found in the urine. PCR techniques serve to enable detection and quantification of viral load in plasma and urine and represent a useful adjunctive approach in monitoring. With a diagnosis established the therapeutic goal is to clear the viraemia. Decreased immunosuppression is the principle treatment but carries a risk for graft loss of around 10%. Dr Schaub described the particular dilemma which occurs where rejection is suspected in a patient with active BK virus infection. Using an illustrative case he showed how, through guidance of treatment using functional measures it is possible to target the leading process (viraemia vs rejection) and walk the tightrope between under and over-immunosuppression to successfully manage the patient.

The final invited presentation of the meeting was given by Dr Sian Griffin (University Hospital of Wales) who shared her extensive experience of quantitative determination of DSA levels in patient serum in her talk ‘Measurement of donor specific antibodies in serial serum dilution: A guide to desensitisation’. This provided a useful overview of activity in UK deceased donor renal transplant programme leading into discussion on options for progression of sensitised patients to transplantation. Returning to activity in the UK programme Dr Griffin then showed how use of HLA antibody incompatible donors had increased from 2006-2010 and introduced the concept of stratification of immunological risk based on profiles of laboratory test results. In refining this stratagem the Cardiff team sought to achieve a semi-quantitative analysis of HLA antibody levels in patients and in reference to three illustrative cases Dr Griffin went on to explain how this had been accomplished using a high-confidence Luminex based approach combined with serum dilution. This approach overcomes issues of certainty of analysis arising from technical factors affecting bead performance and allows improved clinical decision making around programme desensitisation and delisting of ‘antibody defined’ unacceptable antigens from patient records. Those who were lucky enough to have attended this meeting or who have attended previous such meetings would recognise them to be exceptionally well organised with truly leading edge, high value content. Thanks are due to One Lambda for their continued investment in education and development of scientific and medical staff involved in delivery of Histocompatibility and Immunogenetics services.
ANNOUNCEMENT BSHI MEETING

The 24th annual meeting of the British Society for Histocompatibility and Immunogenetics will be held at Chancellor’s Conference Centre, Manchester on 4th and 5th September, 2013. For more information go to www.bshi2013.org.uk

ANNOUNCEMENT GERMAN SOCIETY FOR IMMUNOLOGY

The 43rd Annual Meeting of German Society for Immunology (DGfI) 2013 will take place on the University’s campus of Mainz, Germany from 11 – 14 September, 2013, bringing together about 1,000 students and professionals from academia, industry and government organizations. It starts with the Presidential Symposium on 11 September, 2013. Furthermore there are plenary sessions and workshops, where an international and national delegation of renowned scientists will present and discuss the stated main topics of basic and translational immunological research: Tolerance, T cell plasticity (transcriptional regulation of immune cells), APC/T-cell interaction, Signalling, Pattern recognition, Controlling immune responses, Autoimmunity, B cells, Immune organogenesis, Innate effector cells, Tumor immunology, Gut (mucosal immunity).

Up-to-date and any necessary information regarding scientific topics, registration and organisational remarks can be found on the conference website www.immunology-conference.de.
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