The 17. European Histocompatibility Conference in Baden-Baden has just finished. Let me first thank the two chairman of the local organizing committee, Gregor Bein and Ralf Wassmuth, for a most successful meeting. The program reflected, on a very high quality level, the extensive developments that are currently taken place in our field. Further, immunogenetics at large was very well covered, which you know I consider should be the focus of our organization. For the very same reason the General Assembly decided that future EFI conferences should be named European Immunogenetics and Histocompatibility Conferences.

We had a constructive General Assembly during the conference. I refer to the minutes published elsewhere in this Newsletter. I would like again to thank the members of the Executive Committee (EC) as well as the members of all other EFI committees for their extensive and devoted work for our organization. All of them use quite a lot of time, for free, for the benefits of EFI, which is the reason for our strength. Let me in particular thank the outgoing EC members Mogens Thomsen, our treasurer for two full terms, as well as the councillors Andrezj Lange and Antonio Nunez-Roldan. And let me welcome our new president elect Federico Garrido, our new treasurer Colette Raffoux, and the two new councillors Katalin Rajczy and Marcel Tilanus.

We had set aside ample time for a “Question and Answers” session at the end of the General Assembly. Even though participation was at times lively when issues on the agenda were discussed, the actual Q and A session was very silent! Perhaps it was a sign of exhaustion and/or that we were all looking forward to the quickly approaching Conference Dinner (which also was most successful!). In any case, let me repeat that the work of the executive committee and the other committees of EFI are very dependent on a continuous interaction with you, the members.

An important issue which was discussed at the General Assembly was the relationship between the main aims of EFI and the national immunogenetics and/or histocompatibility societies (see the minutes). I am very happy that it seemed to be almost generally accepted that EFI should primarily remain an independent organization of immunogenetecists in Europe and not just a federation of national societies, as our name may indicate. I see no reason to change our name, however. Further, a close collaboration with national societies with similar aims should be further sought. There are several ways that we may pursue this. One is to invite the national societies to use our Newsletter also as a vehicle for their communications with their membership. Our most efficient editor, Frans Claas, will be happy to receive contributions from all national societies. In this way we may be better able to serve both European immunogeneticists at large as well as our national societies. Our goal is the same: Promotion and advancement of immunogenetics, both inside and outside our borders.

Erik Thorsby
June 2003
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....FROM THE EDITOR’S DESK

Dear EFI members,

The current Newsletter gives you a nice impression of the successful meeting in Baden-Baden. Gregor Bein and Ralf Wassmuth should be congratulated with the excellent way they have organised this meeting in a beautiful resort. Both scientifically and socially the meeting was a big success. Most of us felt very young after leaving Baden-Baden, not only because of the healthy baths but also because of the difference between our age and that of the local population. For those of you who were not able to attend the meeting, the recipients of an EFI travel award have written reports on the different sessions. Furthermore, Ieke Schreuder and Steven Marsh have provided some nice pictures reflecting the typical EFI atmosphere in Baden-Baden. In the meantime, Ellisaveta Naumova has started with the preparations for the next “European Immunogenetics and Histocompatibility Congress” (note this new name for our EFI meeting!) in Sophia, for which the scientific committee has been active already as well. I am sure that you will enjoy the meeting in Bulgaria at least to the same extent as the one in Baden-Baden. Another meeting which is in the pipeline, is a summer school on Immunogenetics that will be organised by EFI, ASHI and ASEATTA next year in Seville. This will hopefully be the start of a closer collaboration between the three societies, From a very sunny Holland (where is the rain?), I wish you pleasant holidays and I am looking forward to your contributions for the next Newsletter.

Frans Claas

Copy date for EFI Newsletter no. 41 is October 1, 2003. Please send your contribution to Frans Claas, Leiden, the Netherlands, preferentially by e-mail: fhjclaas@lumc.nl

IMPORTANT ANNOUNCEMENT TO EFI MEMBERS

For security reasons we need to change the username password for the EFI website on a regular basis. Changes to the password will be announced in the EFI Newsletter and will apply from the publication date of the Newsletter in which the change is announced.

The new username and password from July 2003 are:

Username:  efimember  
Password:  Sofia  
URL:  http://www.efiweb.org/members/

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The General Assembly was held on Thursday May 8, 2003 in the Kongresshaus, Baden-Baden, Germany, during the 17th European Histocompatibility Conference. The EFI president, Erik Thorsby, chaired the meeting.

1. Opening
Erik Thorsby opened the General Assembly and welcomed those present. He asked for special attention to item #10 on the agenda and invited those present to ask questions and make comments to the Executive Committee, in order to improve its function for EFI.

2. Minutes of General Assembly
The minutes, which had been published in Newsletter # 36, were approved.

3. Report of the EFI Executive Committee meeting
A report of the Executive Committee meeting in Leiden in October 2002 has been published in Newsletter#38. The Executive Committee met on May 6 and May 8 and discussed a number of important items. It was proposed to restructure the scientific committee to consist of three ex-officio members (the EFI president, the chairmen of the local organising committees of the immediate past and the next EFI conference) and 5 selected members (F. Claas, G.B. Ferrara, J. Trowsdale, D. Charron and M. Colonna). Frans Claas is willing to chair the committee until after the EFI conference in Istanbul in 2005. The General Assembly agreed with this proposal. The EFI Newsletter has been restructured and improved since Frans Claas took over as editor. It now is a useful medium for communication for EFI members. Claas has offered to continue as editor for a few more years, which was gratefully accepted by the General Assembly.

The abstracts for the Baden-Baden conference have been printed in Genes and Immunity. It has been a good opportunity for EFI to have its abstracts published in this relatively new but high quality journal. Presently the Executive Committee is negotiating reduced subscription of this journal for EFI members.

As Erik Thorsby has emphasized previously, EFI will need to widen its scope of interest into immunogenetics in general besides histocompatibility. It is therefore proposed to change the name of the annual EFI conferences into “European Immunogenetics and Histocompatibility Conference”. Although several General Assembly members suggested to skip “Histocompatibility” from the name, the majority was in favour to keep both, at least for a few more years.

4. Report of the EFI treasurer
Mogens Thomsen presented his report for the last time as EFI treasurer.

The Julia Bodmer Young Scientist Award: Through some large donations a fund has been created which is sufficient to provide annual awards of 1,000 euro for the coming years. This year’s candidate is to be announced on Friday May 9.

Conference travel bursaries: 19 applications were received of which 14 were granted.

Education bursaries: No bursaries were given in 2002, but several will be processed soon. It is surprising that so few members apply for travel and education bursaries.

State of the account, budget 2002 and 2003: The figures show a large positive balance in 2002 (see table) because of increased income and lower expenses. There were large benefits from the Strasbourg conference 2002. Several expense items were lower than usual:

- The card machine, used previously for payment of EFI subscription, is no longer needed.
- The webmaster spent less hours on the website in 2002, but the sum will increase for 2003 due to programmer expenses.
- No auditing was made in 2002, but in 2003 Deloitte and Touche will do auditing for two years.
- Newsletter costs have been less in 2002 (no editor’s fee). An advance for the congress budget was made in 2003 for the Sofia congress.

The prospective budget included an increased income from the membership fees. The support of the IMGT-HLA Sequence Database will be continued. The contribution to the central office will be increased.

Payment via the website: This function was finally installed with the help of the webmaster and a French computer technologist. Presently some 20% of the members use this facility.

The figures of the budget for the accreditation office will also be published in the EFI newsletter.

5. Reports of the EFI committees
Standards and Quality Assurance, chairman Jean-Marie Tiercy.

The Standards version 5.3 was accepted as per April 22, 2003. The central office received over 75 votes, all in favour. In section C some modifications have been implemented to better agree with ISO norms. The new rules for 4 digits typing in related (I1.120) and unrelated (I1.220/230) bone marrow transplantation were shown. A few minor modifications have also been implemented in sections M and N. Version 5.3 will become effective as of January 1, 2004. This latest version, including minor modifications approved by the committee on May 6, will become available on the website.

For the future the committee will work on minor revisions for the Flow and ELISA sections. Working groups have been formed to prepare major revisions of the DNA section, and to compare the ISO17025/15189 and CPA norms in relation to EFI standards.

Craig Taylor asked why the requirement of 4 digit class I typing was abandoned. Tiercy answered that because of the great diversity in transplant protocols EFI standards should reflect the best practice of today.

External Proficiency Testing (EPT), chairman Ilias Doxiadis.

The minimal number of samples for molecular typing has been reduced to 10. The rules for EPT organisations have been published and resulted in several applications for official EFI-EPT recognition. Six were awarded, while 1 is pending. This means that over 150 laboratories take part in EFI approved EPT schemes.

The following problems will be addressed in the near future:

- Improvement of the translation from molecular to serological names.
- Acceptable ambiguities need to be defined with the help of specialists.
- The allele list in the package will be removed.

Cristina Navarrete asked how the application of the EPT schemes was defined. Doxiadis answered that the applications will be further formalised.

Accreditation, Thorsby mentioned that SWEDAC, the Swedish accreditation organisation, has asked if EFI could take care of the accreditation for H&I in Sweden. An agreement for this will soon be signed. Such an agreement may serve as a template for similar agreements of EFI with national accreditation organisations in other countries. A working group (P. Reekers, C. Gautreau, M. Bengtsson) has investigated accreditation require-
The initiative, to organise an international summer school as a joint venture between EFI, ASHI and ASEATTA, was previously accepted by the Executive Committee. As a result a plan was presented by Frans Claas to hold a 5-day course, early September 2004, in Sevilla - Spain, organised by EFI and ASHI. It is meant for 25-30 students and postgraduates with 10 faculty teachers. Each day one topic will be addressed. The General Assembly approved the education activities.

Web, chairman Steven Marsh Marsh reported that about 20% of the members now pay online. The numbers of people visiting the EFI web site continued to increase, and details of the EFI meetings such as presentations of the education sessions and the abstracts were very popular. He will negotiate with Genes and Immunity to have this years’ abstracts also available as .pdf files. All EFI members were encouraged to submit information to be included on the web site.

Scientific Affairs, chairman Frans Claas. Claas thanked all committee members for their help and cooperation.

Erik Thorsby thanked all the chairmen and their committee members for their dedication and support of EFI. The General Assembly applauded their activities.

6. Membership of EFI in European Laboratory Medicine (ELM)

Erik Thorsby showed part of the ELM bylaws and its goals. A proposed membership would allow EFI to get more widely known and have influence in ELM activities. Membership will cost EFI only 200 euro. The General Assembly agreed with the proposal that EFI will become members of ELM.

7. Relationship between EFI and national societies

Erik Thorsby explained why he had proposed this year not to organise a separate meeting with the national representatives. The main reasons were to avoid collision with other meetings and that the issues discussed would be of general interest to the EFI members and might as well be discussed at the General Assembly.

In the past there have been proposals to change EFI into a federation of national societies. Although several countries have very active societies for immunogenetics and/or histocompatibility this is not the case everywhere. Moreover it should be realized that the “F” in EFI originally stood for ‘foundation’. This had to be changed, however, as it was not acceptable to French legislation. To keep the “F” the name ‘federation’ was chosen instead, but not implicating that EFI should become a federation of different societies. Thorsby suggested that EFI should remain an independent European “society” of immunogeneticians, but with strong relations to national societies with similar aims. This was generally approved by the General Assembly.

8. Future EFI conferences

The 18th European Immunogenetics and Histocompatibility Conference will be held in Sofia, Bulgaria. May 8-11, 2004. Elissaveta Naumove gave a short presentation about the plans for the conference. The meeting will be over the weekend, starting on Saturday. Most communication will go via internet and a website has been established at (www.efi2004.bg).

The 19th European Immunogenetics and Histocompatibility Conference will be held in Istanbul, April 23-26, 2005. Mahmut Carin presented his plans.

The 20th European Immunogenetics and Histocompatibility Conference will be held in Oslo, June 8-11, 2006. Frode Vartdal, who will be the chairman of the local organising committee, presented some preliminary plans.

The General Assembly was satisfied with the planning of the future conferences.

9. Installation of new Officers and Councilors

Several executive committee members finished their term of office. Mogens Thomsen, who has served two full terms as treasurer was thanked by Thorsby for the very good job he has done for EFI. Andrzej Lange and Antonio Nunez-Roldan will end their terms as councillor and were both thanked for their constructive work for the Executive Committee. The following members were installed as new officers: Federico Garrido had been elected as president elect; Colette Raffoux, previously deputy treasurer, as new treasurer; Katalin Rajczy and Marcel Tilanus as new councilors.

10. Other topics

No further topics were raised, nor were there any questions and remarks from the EFI members present. Thorsby then closed the General Assembly and thanked for the contributions from those present.

Ieke Schreuder
EFI Secretary
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REPORT OF THE
STANDARDS
AND QA
COMMITTEE
MEETING OF
MAY 6TH 2003
(BADEN-BADEN)

Following the EFI Committee coordinators meeting that took place on 5 October 2002 in Leiden, the Standards Committee was asked by the Executive Committee to reconsider the issue of 4-digit class I typing and make it clear that it was mandatory only when required by the local transplant protocol. The modified version 5.3 of the Standards was submitted for a vote by the EFI membership and accepted on April 22. During the vote the Standards Committee received a few suggestions that were discussed in the Baden-Baden meeting. Minor modifications were thus included in Version 5.3: among those it was also stated that high resolution class I and II typing for intra-familial potential donors who are not HLA-identical siblings was mandatory only when required by the local transplant protocol. A few items was also simplified in sections M and N. The final version 5.3 will thus be available on the web site and will become effective starting January 1, 2004. The checklist will be modified accordingly by the Accreditation Committee.

In Baden-Baden Standards Committee has initiated two tasks:

a) a small working group, headed by Mats Bengtsson comprising 2 members of the Committee and an expert from outside, has initiated a revision of the DNA standards, trying to simplify this section,
b) an extensive comparison of the ISO17025/15189 norms with the EFI Standards.

One of the pioneering members of the Committee, Jean-Denis Bignon has resigned, and we would like to acknowledge his commitment in improving the Standards. He will be succeeded by Dominique Masson from Grenoble who was recently elected by the Committee.

FROM THE EFI TREASURER

PROFIT AND LOSS ACCOUNT in Euro

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ASSETS JANUARY 2003, in Euro

- Current account: 10757
- Savings account: 1160
- Stocks and Shares: 155814
- Equipment: 487
- Total: 168218

EFI ACCREDITATION OFFICE

PROFIT AND LOSS ACCOUNT FOR 2002.

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Total operating expenses: 82.482 euro
Operating result: 7.626 euro
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The 2003 Ruggero Ceppelini Lecture was given by Dr. Peter Parham. As mentioned by the chairman in the introduction, this famous immunologist has published over 40 scientific articles in Nature. After having extensively studied the structure and the functions of the HLA class I molecules between 1980 to 1998, Peter Parham’s work is now mainly focused on the structure, polymorphism and functions of NK receptors (NK-R). His talk started with the presentation of the NK cell functions. In fact, these cells are involved in the cytolysis of tumoral or virus-infected cells but also in cytokine production. Moreover, NK cells play a role 1) in the control of the switch from innate to adaptive immunity by their interactions with dendritic cells, 2) in reproduction, 3) in the control of infections and 4) in the outcome of allogeneic hematopoietic stem cell transplantations by their impact on graft rejection, Graft-versus-Leukemia effect and by preventing Graft versus Host Disease. The physiological functions of NK cells are regulated by a balance between inhibitory and activating receptors. Two main classes of NK-R with different structures are known: the KIR for Killer Immunoglobulin-like Receptors and the lectin-like CD94-NKG2 receptors. Furthermore, some of these NK-R interact with specific HLA class I molecules. As an example, KIR2D molecules interact with the α-helices of the HLA-Cw molecules. Then, Peter Parham reminded us of the complexity of the NK-R repertoire. First of all, NK cells express different combinations of KIR and CD94:NKG2 receptors. Secondly, KIR haplotypes differ in gene content and organization between individuals. As also noted by Peter Parham, the distinction between different KIR alleles and different KIR genes is not always straightforward. Basically, two major KIR haplotypes have been described: “A” (including 3DL3, 2DS2, 2DL2, 2DL1, 2DL4, 3DS1, 2DL5A, 2DS5, 2DS1, 3DL2 genes) and “B” haplotypes (including 3DL3, 2DS2, 2DL2, 2DL1, 2DL4, 3DS1, 2DL5A, 2DS5, 2DS1, 3DL2 genes). Interestingly, as reported by Peter Parham, 111 different KIR genotypes have been obtained from 1000 healthy donors worldwide. At the allelic level, KIR gene polymorphism distinguishes 22 group “A” haplotypes having identical gene content. Statistically, more than 800,000 possible combinations in the group “A” haplotypes may be obtained. Different alleles have been described for specific KIR loci (3DL3, n=5; 2DL1, n=6; 3DL1, n=11; 3DL2, n=12; 2DL3, n=6; 2DL4, n=9 and 2DS4, n=4). To conclude this part, Peter Parham suggested that the diversity of KIR repertoire is generated by 1) the combination of different KIR genes in an individual, 2) the different combinations of activating and inhibitory NK-R genes observed between individuals, 3) the frequencies of gene, haplotypes, alleles, genotypes which are different within the human species, 4) the frequencies of genes, haplotypes, alleles, genotypes which are different in the primate species as compared to humans. Peter Parham also mentioned his recent studies on KIR gene polymorphism in primates. To sum up this study, the MHC-C/KIR2DL system of ligand-receptor appears simpler in Orang-Outangs than in humans. In the last part of his talk, Peter Parham reminded us of the impact of KIR gene polymorphism on the outcome of bone marrow transplantation. Up to now, few articles have been published on this subject but many laboratories are interested in this topic. Different discordant results about the importance of KIR gene polymorphism on BMT outcome were presented during the last two EFI meetings but these discrepancies may be due to the heterogeneity of the donor/recipient pairs analysed. Depending on the authors, donor/recipient KIR gene and/or haplotype disparities may predispose to GvHD or to graft rejection but also KIR ligand incompatibilities may protect against AML relapse, GvHD and rejection. Peter Parham also made reference to his results concerning the reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation which showed that KIR phenotype disparity may be correlated with clinical outcome. Peter Parham concluded his talk by showing recent data about the involvement of KIR gene in diseases (AIDS, psoriatic arthritis, RA) and especially the role of KIR3DS1-Bw4 interaction which delays the progression of AIDS. To conclude, I was very impressed by the quality and the quantity of data presented by Peter Parham and this excellent 2003 Ceppellini Lecture may encourage further work in this immunogenetic field.
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The first presentation was given by N. Wright from London on the lineage transdifferentiation and the potential of bone marrow stem cells. He showed that stem cells in bone marrow can engraft in multiple tissues. For this purpose bone marrow of male mice was engrafted into female recipients. After transplantation the male Y chromosome was found in the liver and in kidneys of female recipients in different cell types like the tubuli and the interstitium. The bone marrow cells are probably getting in the tubuli by TGF which induces the loss of epithelium adhesion, which causes damage and influx of bone marrow cells differentiating into new epithelial cells.

This engraftment was also found in other tissues like the heart where allogeneic mesenchymal stem cells integrate into infarcted rat heart when given iv. In the gut multiple engrafted Y chromosome cells were found that were differentiating into myofibroblast and helped with wound healing and in the skin these cells were located in the bulge region of the hair follicles.

Then the question raised why these bone marrow cells develop into these specific tissue cells. Evidence was found that bone marrow cells adopt the phenotype of other cells by spontaneous fusion. This was showed in vivo by finding cells with multiple X and Y chromosomes which suggest fusion. The presentation ended with the conclusion that bone marrow stem cells are a versatile tool for organ repair by regenerating failing organs.

The second presentation was given by F. Fändrich from Kiel and was about embryonic stem cells and tolerance induction. He started by referring to Medawar who was the first to show the tolerizing capacity of embryonic cells. Some experiments were performed to show his phenomenon. Isolated embryonic cells from blastocysts from WKY rats were expanded in vitro, stained and injected in the portal vein of fully mismatched DA rats. These cells stayed in the liver for days without rejection and moved to the thymus were they could be detected for over a year. This was the first evidence that these cells could mediate tolerance induction. In the next experiments they again injected WKY cells into DA rats and after 7 days they transplanted a WKY heart or a third party heart in the DA recipient. The WKY heart survived for 150 days while the third party rat was rejected immediately. The FAS ligand expression, which is high on embryonic cells and plays a role in immune privilege, could be an explanation for this tolerance induction. However, for ethical reasons embryonic cells can not be used in humans. Therefore it was analysed if tolerance induction was also possible with use of other cells. It was found that an antibody against GM-7 stained positive for embryonic as well as adult stem cells. Expanded GM-7 positive cells from Lewis rats were injected into DA rats. After 7 days the rats were given a transplant. The Lewis hearts were accepted while the third party hearts were rejected. After 60 days the GM-7 cells vanished which showed that long term graft survival does not require chimerism.

Next they performed some experiments in pigs where a single lung transplantation with SLA class I and class II differences was performed. This evoked a strong MLR reaction. GM-7 cells were given to the animals 7 days after transplantation. Without the GM-7 cells the grafts were rejected within 50 days after canceling the immunosuppression. If less than 500,000 GM-7 cells/kg bw were injected the grafts were rejected within 130 days. If more than 500,000 cells/kg bw were injected the grafts survived for more than 400 days. This shows that the cell dose is important. These experiments show that preconditioning with GM-7 does induce donor specific tolerance. A possible mechanism for this tolerance induction could be that GM-7 cells have the ability to induce regulatory T cells by direct contact with T cells and upregulation of Foxp3 which is a master regulatory gene for cell lineage commitment or developmental differentiation of regulatory T cells.

The next presentation was given by V. Rocha about hematopoietic stem-cell transplants using umbilical cord blood cells. First the advantages of cord blood (CB) transplants over bone marrow (BM) transplants which are the easy access, the direct availability and the low infectious disease contamination were shown. At the moment 120,000 cord bloods are available for transplantation and the number of unrelated CB transplants that are performed is still increasing. It was still unclear if there were differences in GVHD after CB or BM transplants in adults and if the engraftment was adequate. It was shown that neutrophil and platelet recovery was delayed after CB transplantation, there was decreased GVHD and there was no difference in the transplant related mortality and relapse incidence. It was concluded that CB is an alternative source of stem cell transplantation in adults with high risk acute leukemia. However, patients receiving a CB transplant with more HLA disparities showed less engraftment, more severe GVHD and an increase relapse incidence whereas receiving an HLA class I mismatch is even worse than an HLA class I mismatch. Even more important than HLA matching are the number of cells that are given. A minimum of 2.107 cells/kg is needed for a successful transplantation.

The last presentation in this session was given by E. Petersdorf about allogeneic stem cell transplantation. The goal is to increase the safety, efficacy and availability of unrelated bone marrow or stem cell transplantation. It is important to look for permissible mismatches because for some patients it is impossible to find a perfect match. Unrelated donors matched for only HLA-A, -B and -DR showed increased GVHD and decreased survival therefore it was hypothesized that matched donors are not really matched and HLA loci other than A, B or DR or loci other than HLA are important. New matching data showed that a mismatch for HLA-C negatively affects risk of graft failure, GVHD and death. The risk of failure or GVHD is higher with a mismatch for one antigen compared to a mismatch for one allele and mismatches for multiple alleles compounds the risk. It was also shown that HLA class II matching is more important for GVHD although class I matching is still important. In conclusion, DNA typing for HLA-C is important and if no matched donor is found, the mismatches should be limited to one or two loci preferentially on allele rather than on antigen level.
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We learned about Einstein’s law of interesting presentation by J. Neefjes. An audacious title, and an by MHC Class I molecules, and how to improve it. The ratio of immunoproteasome to proteasome was shown to vary during the different stages of a viral infection and even differed in the particular cell type studied. Although the upregulation of the immunoproteasome seemed to peak after the immune response had been initiated. One of the more important points made was that different epitopes were produced dependant on whether a proteasome or immunoproteasome degraded the same peptide from the same pathogen.

This has important implications for both immunotherapy and vaccine design. An interesting and more contentious presentation followed, entitled “On the efficiency of antigen presentation by MHC Class I molecules, and how to improve it”. An audacious title, and an interesting presentation by J. Neefjes. We learned about Einstein’s law of diffusion and how this related to the efficiency of peptide transport, simply put free peptides travel faster compared to those associated with molecules such as Erp or the proteasome. Some elegant experimental data provided information on the cleavage sites of peptides as they were degraded prior to processing by the proteasome.

The final presentation of the session was by G. Ogg on The clinical use of Tetramer technology. This was specifically aimed at monitoring HIV patient’s post antiretroviral therapy. Tetramers of HLA-A*0201 with pol or gag peptides were used to detect levels of HIV specific CD8+ CTLs. Post therapy a reduction in the levels of the HIV specific CD8+ CTLs was evident. Non compliance by patients resulted in viremia and elevated levels of HIV specific CD8+ CTLs. Disadvantages of the technology are apparent with the restriction of patients that are HLA-A*0201 and that most immune responses are polyclonal.

Having recovered from the night before the morning commenced with an excellent plenary session on Autoimmunity. A presentation by Holmdahl entitled “Is it possible to understand rheumatoid arthritis through genetics?” provided an insight into The Of particular interest was the plenary session on “Innate Immunity”. Ljunggren presented NK cells in Immune surveillance. A phenotype comparison was made between NK, NKT and T-cells. In mice activated CD8 T-cells express the NKT cell marker NK1.1. In vitro studies with CD8 T-cells post stimulation developed expression of NK receptors such as CD94, NK1.1, Ly49, and 2B4. 2B4 is a CD2 like receptor that has 10 times the affinity for CD48 than CD2. Cross linking of 2B4 enhances cytotoxicity in NK cells, however this was not the case for activated memory CD8 cells, where cross linking 2B4 did not enhance cytotoxicity, cytokine production, or even proliferation. Through in vitro studies it was shown that 2B4+CD8+ cells were capable of enhancing the proliferation of naïve T-cells. This was possibly due to 2B4 ligation to CD48 present on the naïve T-cells. CD8 memory activated cells were also shown to contribute to innate immunity via secretion of IFNα. The kinetics of this response was similar to the production of IFNα by NK and NKT-cells. Further study determined that these activated CD8+ cells are CD44high prior to stimulation and that CD44 low cells prior to stimulation do not produce IFNα. In conclusion it was proposed that LPS activates bone marrow derived Monocytes/DCs these produce IFNα/β, IL-12, and IL-18 that stimulates CD8+ memory cells to produce IFNα, and aid proliferation of naïve cells via 2B4.

M Uhrberg presented the Expression and regulation of killer cell Ig-like receptors on cytotoxic lymphocytes. KIR expression was explained to be oligoclonal and self compatible. But the question remained on how KIR expression was regulated? Investigation had revealed a 65bp fragment containing weak promoter activity in KIR2DL3. However this did not explain the differential expression of KIRs on NK cells, and the lack of knowledge of a binding factor for the promoter region. The answer is that KIR genes are epigenetically regulated. Methylation status correlates with KIR gene expression, thus de-methylation results in gene expression. NKG2A expression was also shown to be epigenetically controlled, but in this case expression was permitted by histone acetylation which opens the chromatin structure. Reindl presented Toll-like receptors and Disease. This presentation covered the various receptors, how they are differentiated depending whether they interact with Gram negative or Gram positive bacteria. The speaker also presented the existence of polymorphism in these receptors, how this could affect their responsiveness to LPS and possible increased/decreased risk of a particular disease.
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The session began with Dr Lucienne Chatenoud from Paris presenting “The autoimmune origin of human type I diabetes mellitus (IDDM)”. Dr Chatenoud gave a comprehensive synopsis on the development of IDDM stating that there are two stages in disease development. The first stage begins with mononuclear cells that infiltrate the periphery of the pancreas and the second stage progresses with cellular infiltration of the islets of Langerhans and destruction of the insulin producing β-cells. Experimental models have shown that IDDM can be induced in immuno-incompetent mice by adoptive transfer of T-cells from diabetic animals and the presence of both CD4+ and CD8+ T-cells are required to cause β-cell atrophy. Furthermore, the transfer of regulatory T-cells in the early stage of disease confers disease protection that is IL-4 and IL-10 independent but TGF-dependent.

Although the aetiology of IDDM remains unclear its origins are multi-factorial with both genetic and environmental components. Dr Chatenoud highlighted the most recent thinking that IDDM may be triggered by Coxsackie virus infection. Certain Coxsackie viral proteins have been found to resemble the structure of glutamic acid decarboxylase (GAD65) which is known to be one of the major autoantigens in human IDDM. This finding therefore suggests that IDDM may arise due to molecular mimicry in genetically susceptible individuals. Epidemiological studies have shown that countries with a high prevalence of IDDM also have high levels of infection and second generation migrants from geographical regions with a low prevalence of IDDM who move to areas with a high prevalence acquire the same risk as the indigenous population. The presentation concluded with a brief overview of novel treatments for IDDM such as autoantigen based immunotherapy and CAMPath3.

The contribution by dr. Sollid from Oslo concerned Celiac Disease (CD), which is a complex autoimmune disorder characterised by chronic inflammation of intestinal mucosa because of intolerance to ingested wheat gluten or related proteins from rye and barley. This disease serves as an instructive model for complex autoimmune diseases since the environmental factor that precipitates the disease (gluten), the HLA molecules that confer predisposition to the disease have been identified and access to affected organ is very simple allowing in situ studies and the isolation of disease relevant cell populations. The sibling relative risk (∆s 20-60) and the high concordance between monozygotic twins (75%) indicate a strong genetic component to CD. The primary HLA association in most of celiac disease patients is with DQ2 (DQA1*05/DQB1*02) and a minority with DQ8 (DQA1*0301/DQB1*0302). Nevertheless, it seems that the overall contribution of non-HLA linked genes is greater than the HLA genes, but much less is known about the implication of non-HLA genes in CD. Gluten consists of a complex mixture of peptides like gliadins and glutenins. Some peptides of the gluten molecule are resistant to digestion by luminal and brush-border enzymes. These peptides are able to cross the mucosal epithelium and are deaminated by tissue transglutaminase (TG2), then the deaminated peptides are presented by HLA-DQ2 or HLA-DQ8 molecules on the surface of APCs, and recognised by lamina propria CD4+ T cells which secret a profile of inflammatory cytokines dominated by IFN-γ. There is much evidence that mechanisms facilitated by TH 1 cytokines are involved in lesion formation.

Gluten T cell epitopes are characterised by heterogeneity, but despite this absence of a single pathogenic motif, the gluten epitopes that are recognised by intestinal T cells are very rich in glutenine and proline residues. The enzyme tissue transglutaminase (TG2) plays an important role by deamination of gluten peptides. Very recently a gluten peptide, a 33-mer has been indentified, which is very rich in proline residues, resists degradation by all intestinal proteases. This peptide is a very potent T cell stimulator and contains three distinct gluten-specific T cell epitopes. The proline residues might influence the immunogenicity of gluten peptides in different ways:

-Resistance to proteolysis: protecting peptides from proteolytic degradation during digestion.

-Epitope selection by TG2: proline has a dominant role in the specificity of TG2 and guides which glutamine residues can be deaminated.

-Oligomerized epitopes can be particularly efficient for T-cell stimulation and they provide multivalent TG2 substrates (i.e. 33-mer)

-Proline can facilitate the binding to HLA-DQ2 molecule by acting as anchor residue or by inducing a type-II polyproline helical conformation.

The altered affinity of gliadin peptides for DQ2 seems to be an important factor involved in the loss of tolerance. Deamination increases this affinity and the increased levels of TG2 in the mucosa of celiac patients seems to be related with inflammation, since the promoter region of the TG2 gene contains response elements to IL-6 and TNF-γ. Inflammation induced as a response to an infection, might in addition breach the epithelial barrier and leads the influx of gluten peptides into the lamina propria. This circumstance, together with the increased expression of TG2, would promote the generation of deaminated gluten peptides.

Indeed infection and inflammation are thought to be risks factors for celiac disease.
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Plenary session: Autoimmunity

Katharina Stirling (University Hospital Zagreb, Croatia)

Dr. Holmdahl discussed the genetics of rheumatoid arthritis (RA). Both phenotypic and genetic heterogeneity add to the genetic complexity of this disease. Signs and symptoms, which are established as criteria for diagnosis of RA, could be the outcome of many different pathogenic pathways; for that reason RA can even be considered to be a syndrome composed of several distinct diseases. Full genome screens showed that only markers in the MHC region show a significant linkage with the disease. Animal models are of particular value in efforts to localize susceptibility genes. This approach minimizes the linked region by producing congenic animals where a linked region from one strain is bred into a background strain, permitting a phenotype analysis of each linked region separately. Pristine-induced arthritis in rats is an animal model for RA, which was used for identification of genes involved in the control of chronic arthritis. The study showed that different arthritis phenotypes are associated with different chromosomal loci. Locus on chromosome 12 (Pla4) is associated with severity and joint erosion. Positional cloning of Pla4 revealed that the disease related allele of Ncf1 (component of the NADPH oxidase complex) has reduced oxidative burst response and promotes activation of arthritogenic T cells. This discovery led to the development of treatment, which would activate NADPH oxidase complex. Such treatment was shown to ameliorate arthritis.

The use of MHC class II tetramers in studies on autoimmune diseases was discussed by H. Reijonen. Highly specific interactions between MHC/peptide complex and T cell receptor direct the specificity of the T-cell response. Monoclonal antibodies recognizing peptides in polymorphic proteins but there are other ways by which cells can generate mHags: they can arise from differential patterns of expression (tissue-specific or ubiquitous), or from differential proteosomal cleavage, or from forms of genetic variation that lead to gene deletion in some individuals. mHags are the key players for GvHD and GV},and in HLA identical transplantation. Mutis showed the impact of disparity for HA-1, a mHag with hemagglutinin activity. The incidence of relapse is reduced by the presence of GvHD, and this effect was shown by Mutis to be significantly more pronounced when patient and donor are mismatched for HA-1.

For this reason mHags are considered useful tools for adoptive immunotherapy: anti-HA-1 specific Cytotoxic T Lymphocytes (CTLs) can be generated ex vivo from a HLA-A2+/HA-1+ donor and infused into a HLA-A2+/HA-1+ patient. Due to their tissue-specificity, they allow to separate the beneficial GvL effect from the deleterious GvH effect. Clinical trials based on this strategy are ongoing.

Another more recent strategy uses non-self-HLA-A2-restricted CTLs: HA-1+ HLA-A2 restricted CTLs can be isolated ex vivo from a HLA-A2- donor by generation of a polyclonal allogeneic T cell line specific for HLA-A2, which is subsequently sorted for T cells recognizing the HA-1 peptide by tetramer staining. These HA-1 specific, allo-restricted T cells can then be infused into a HLA-A2+/HA-1+ patient. In this setting, immunotherapy can be used both in matched and mismatched transplantation, allowing a larger number of patients to benefit from this procedure.

Another useful tool is the skin explant assay which has been used to monitor the potential of anti-HA-1 specific CTLs to induce GvHD. Mutis laid great stress on the time of administration of the CTLs to patients: if the immunotherapy is performed immediately after transplantation, GvHD can be induced; if CTLs are infused later on (90days) there is no GvHD induction probably because donor Antigen Presenting Cells (APCs) have replaced patient APCs. The next speaker, Dr. Velardi (Perugia, Italy) reported on the role of Natural Killer (NK) alloreactivity in haploididentical HSCT. 30 % of patients have a matched sibling donor, 30 % have a unrelated
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donor but nearly all patients have a haploidentical donor. Engraftment is critically dependent on thorough T cell depletion and on infusion of high doses of CD34+ Stem Cells (SC). Therefore, one important difference between conventional and haploidentical transplants is the graft composition (T cells > CD34+ cells, CD34+ > T cells, respectively). However, the reactivity of NK cells plays the key functional role. NK cells are lymphocytes characterised by a cytotoxic activity against target cells that do not express specific HLA antigens. This property is due to the fact that NK cells express Killer-cell Immunoglobulin-like Receptors (KIRs), membrane receptors for HLA ligands, whose engagement is responsible for NK lytic activity inhibition.

In the transplant setting, donor NK cells specific for one HLA allele, missing on the patient haploidentical cells, kill recipient cells. This situation is called NK alloreactivity in GvH direction. In the murine system, it has been shown that infusion of host-specific alloreactive NK cells neither causes GvHD nor organ damage (100% survival in mice with a Total Body Irradiation (TBI) based conditioning). In NOD-SCID mice in Chronic Myelogenous Leukemia (CML) blast crisis it has been shown that NK cells are able to provide clearance of leukemic cells. NK alloreactivity in GvH direction has been shown to provide significant clinical advantages also in the setting of haploidentical HSCT in humans. The presence of host-specific NK alloreactivity allows full engraftment by infusion of relatively low doses of SCs from a haploidentical donor (mini-transplant). Importantly, the presence of NK alloreactivity in GvH direction not only does not induce GvHD, but actually prevents it; it also gives protection against infection as shown by experiments of challenge with candida and aspergillus.

The other very important feature of this setting of transplantation is that post-transplant immune suppression is not necessary, avoiding the risks that such therapy implies.

Velardi presented the retrospective data on 119 haploidentical transplants performed in the last 9 years: impressive were the data showing that, in the presence of NK alloreactivity in GvH direction, the percentage of both rejection and GvHD was consistently lower as compared to the situation in which NK alloreactivity was absent. It was also shown that leukemia targets that fail to be lysed by NK cells, such as acute lymphocytic leukemia (ALL) blasts, do not express LFA-1; for this reason haploidentical transplantation is more efficient with myeloid leukemia.

Finally, Velardi presented data on NK alloreactivity in partially matched unrelated HSCT. In this setting, NK alloreactivity in GvH direction initially was thought not to give advantage. It has been found that this initial finding was due to the fact these studies were performed with unmanipulated bone marrow containing T cells. These T cells antagonise and precede NK engraftment; and therefore the numbers of NK cells are not sufficiently high. Moreover, the relatively low rate of stem cells infused in conventional grafts and the immunosuppression treatment post-transplant are additional factors preventing the beneficial effect of alloreactive NK.

Velardi presented a recent study of 130 unrelated transplants performed with T cell depletion; in these transplants, an impressive correlation of NK alloreactivity in GvH direction was observed with survival; while NK alloreactivity in rejection direction was not associated with graft rejection. This latter observation may be due to the conditioning regimen of those transplants. From these data, it actually seems desirable to prefer an unrelated donor mismatched for KIR ligands in GvH direction over a perfectly matched donor.

I would like to thank the EFI bursary selection committee for having awarded me a bursary in order to participate in the Conference.

**PleNARY SESSION: Transplantation**

**Cigdem KEKIK (Istanbul University, Medical Biology Department, Turkey)**

Dr. Hans Dieter Volk from the Humboldt details monitoring of organ transplantation by immunologic means. He suggested that several parameters have to be evaluated in donor, organ and recipient for successful transplantation. The first step is the organ quality. Age of donor, ischemic/ reperfusion (I/R) injury, brain death and hypertension are the most important factors that influence the long-term transplant outcome. Hypertension, atherosclerosis, age of donor, Systemic Inflammatory Response Syndrome (SIRS), brain death, I/R injury and allogeneic immune attack increase the risk of chronic graft dysfunction respectively. When tissue is damaged by bacterial endotoxin, this leads to activation of macrophages, and IL-1beta and TNF increase in blood, urine and bile within hours and activated non immune cells in tissue. IL-6, IL-8 and Procalcitonin (PCT) increase in blood, urine and bile within hours or days. If PCT is below 2 ng/ml, graft survival is greater at 1 year. If PCT is above 2 ng/ml, graft failure develops within 1 year. The so-called inflammatory index is higher in brain death donors than the living donors. Gene expression profiling marks disease progression and therapeutic response. In experimental models, HO-1 and its subunite CO have been shown to protect the allograft from the I/R injury. Urine IL-8 level is an indicator of acute rejection as early as at post-tx 1 day. If IL-8 is below 50 pg/ml, acute rejection disappears within 3 months. If IL-8 is above 75 pg/ml, on the other hand, predicts acute rejection. The second step is to predict the patients who are at risk of rejection. High panel reactivity (PRA), genetic predisposition and repeated tx are the most important factors. Genetic mapping determines genetic predisposition and certain gene polymorphisms are associated with the clinical course. Acute rejection has been previously shown, especially by Hutchinson et al., to be more prevalent in high producers of TNF and IL-10 and low producer of TGFBeta, and chronic rejection has been claimed to be more prevalent in high producers of TGFBeta and TNF. However, all these studies were performed in small study populations, and the results need to be evaluated in larger collectives. Dr. Volk stated that the presence of donor reactive anti-HLA antibodies of the IgG1 isotype at post-tx day 5, as a reflector of a Th1-type humoral immune response, is a predictor of later graft loss. The third step is early diagnosis of rejection. CD3, granzym B, perforin and granulolysine levels were evaluated by Volk HD, Suthanthiran M et al by RT-PCR in rejection biopsy samples. While increased FasL and granzym B level marks acute rejection, augmented levels of IL-10, TNF indicate chronic rejection. The fourth step is prediction and management of
infection. Dose of Immunesuppressant (IS), microbiological tests and clinical course are important in this step. If the dose of IS is high, risk of the infection increases where as low doses cause increased risk of rejection. For this reason, regulation of IS dose is quite important. An increase in the number of HLA-DR+CD14+ monocytes after high dose IS treatment is indicative of infection. Furthermore, there is a balance between viral load and T cell response. If the viral load is increased, the risk of the rejection increases, too. The fifth step is to predict the success and damage of tolerance. Tolerance is associated with the presence of certain genes. DDRT-18 gene expression below 1 AU at post-tx day 2 is an indicator of a developing allorejection. Allorejection develops also when 1AG gene expression is above 1 AU at post-tx day 10.

**ABSTRACT SESSION IV – TRANSPLANTATION**

ZORANA ANDRIC (NATIONAL BLOOD TRANSFUSION INSTITUTE BELGRADE, SERBIA)

Spriewald et al. evaluated whether intrathymic application of a plasmid encoding a single donor MHC class I alloantigen in combination with anti-CD4 antibody prolongs the survival of subsequently transplanted fully mismatched cardiac allografts, and whether this effects was dependent on the cell surface expression of tolerance. Plasmids encoding the wildtype MHC class I molecule Kb (pSVKb), plasmids encoded a truncated form in which the signal sequence for translocation into endoplasmatic reticulum was deleted (pSV-SDEL Kb) and empty plasmids as a negative control were used. H2k recipients received 10µg plasmid intrathymically, two doses of depleting anti-CD4 antibody and 28 days later a fully mismatched H2b cardiac graft. In contrast to the empty plasmid, pSVKb as well as pSV-SDEL Kb were able to induce long term survival of fully mismatched H2b cardiac allografts. Reverse-transcriptase PCR detected expression of the injected Kb genes for up to 28 days in the thymus and the spleen of the pretreated recipients.

Next three abstracts were presented by Kotch K. In the first one, it was shown that intragraft upregulation of the stress protein H0-1 by donor pretreatment with cobalt protoporphyrin (CoPP) protects the allograft from the ischemia/reperfusion related accelerated chronic graft injury. Up-regulation of stress related genes by ischemia/reperfusion is not abolished by CoPP pretreatment, whereas induction of several inflammation related markers are prevented. The second report concerned non-invasive monitoring of granulysin as a marker for acute rejection diagnosis in kidney transplantation. Granulysin, the cytolytic molecule released by NK and CTL cells, has been shown to be upregulated during acute allograft rejection. The improvement of the method was the implementation of linear mRNA amplification technique and the real-time RT-PCR. Urine samples from renal-allograft recipients were collected 3 times a week over a time period of 3 months after transplantation. Results were correlated with obtained biopsy and creatinine data from patients with acute rejection. The data suggest that the non-invasive mRNA expression measurement of defined markers in urinary cells of the renal-allograft recipients allows early detection of the kidney graft rejection. The third abstract concerned the diagnosis of acute rejection based on quantification of donor-derived DNA in serum by real-time PCR. Kidneys from fully MHC-mismatched male DA rats were transplanted to female Lewis rats and, as control, syneric kidneys from male to female DA rats. Rat SRY-specific primers were designed for donor DNA quantification purposes by real-time PCR. SRY-specific DNA expression in the sera of all recipients was not detectible within the first 4 days but then significantly increased prior to acute rejection on day 5. In contrast, there were no measurable levels of SRY-specific DNA in isograft control group during the whole screening period of 7 days. These results suggest that donor-derived DNA is present in sera of renal transplanted recipients prior to rejection leading to the establishment of an alternative fast and reliable monitoring strategy for diagnosis of acute rejection.

Dankers et al. discussed the correlation between humoral response against HLA class I antigens and the HLA-DR phenotype of the responder. They analyzed the antibody profiles of all HLA-DR homozygous Dutch patients on the Eurotransplant waiting list between 1967 and 2000 (N=1392). The results show that HLA-DR5 positive patients produce antibodies more frequently against HLA-A11, HLA-DR3 positive patients produce antibodies less frequently against HLA-B5 and HLA-B35, HLA-DR4 positive patients produce antibodies more frequently against HLA-B7 and HLA-B12 compared to other HLA-DR homozygous patients. So, correlation can be found between the HLA-DR phenotype of the patient and the specific antibody response against HLA class I antigens. We can conclude that it is possible to predict risk of humoral rejection due to patient’s phenotype, especially in homozygotes.

The next two presentations concerned the revision of the allocation criteria for cadaveric renal transplantation due to the role of HLA mismatches and immunesuppressive therapy. It is known that well HLA matched kidney transplants are associated with longer graft and patient survival. However, because of imperative of matching, patients with rare HLA phenotypes tend to wait longer for suitable organ. Doxiadis et al. retrospectively analyzed first kidney transplants performed between 1985 and 2000, selected from the Eurotransplant and Leiden data base (N=38286 and 491 respectively). Transplantation pairs were divided into a group of 0 and a group of 1 HLA-DR mismatches, after which the additional influence of HLA-A,-B compatibility on graft loss and acute rejection was analysed. The group with 1DRMM had a significantly higher incidence of acute rejection episodes and HLAA,-B matching did not influence the relative risk. Within the 0DRMM group the relative risk increased with the number of HLAA,-B MM but was lower compared with the relative risk of the 1DRMM group. These results show that aiming for full HLA-DR compatibility confers more benefit than current matching procedure and will result in shorter waiting times for patients with rare HLA phenotypes. Magistroni reported on a single center study of renal transplantations (more than 1400) with the aim to investigate the interactions between immunesuppression and HLA compatibility between donors and recipients. A significant influence of immunization, immunesuppressive therapy,
donor age and HLA-DR mismatch. The significant interaction between therapy and HLA-A, B, DR matching suggests the need for a review of kidney allocation criteria, especially concerning the role of the HLA class I matching.

Aguilera showed that a glutathione S-transferase T1 (GSTT1) polymorphism represents a histocompatibility barrier in liver and kidney transplantation. GSTT1 is preferentially expressed in liver and kidney cells. A complete lack of protein expression due to deletion of GSTT1 gene (null/null genotype) occurs in 11.58% of individuals from different ethnic origin. GSTT1-negative liver transplant recipients who received liver graft from GSTT1-positive donor could produce antibodies against GSTT1 and develop ‘de novo’ hepatitis leading to severe liver dysfunction. With the aim to further evaluate the role of GSTT1 polymorphism in clinical transplantation, authors investigated 8 of 300 liver transplanted patients who developed ‘de novo’ immune-mediated hepatitis approximately two years after transplantation. The GSTT1 genotypes analyzing showed that all patients were GSTT1-negative and the donors were GSTT1-positive. In all cases antibodies against recombinant GSTT1 protein were present in patients’ sera. The role of GSTT1 polymorphism and significance of anti-GSTT1 antibodies in kidney transplantation are still under evaluation. Preliminary investigation has shown presence of antibodies against GSTT1 in 4 out of 8 GSTT1-negative renal patients with functioning kidney graft. These antibodies were absent before transplantation.

Müller-Steinhardt et al. showed that various IL-6 promoter polymorphisms are linked to kidney transplant outcome. Previously they showed that IL-6 -174 promoter polymorphism is associated with kidney allograft survival. As two more biallelic polymorphisms in the IL-6 promoter have been identified, they presented results of haplotype analysis in 158 primary cadaveric kidney transplant patients. Authors showed for the first time an association of IL-6 -597-572-174 genotype with 3-year kidney allograft survival. Tiercy presented the impact of HLA-C incompatibilities in unrelated BMT. In a group of 114 CML patients the most frequent single incompatibility occurred at HLA-C. Multivariate analysis in the 3 groups of patients (i.e. matched, HLA-C mismatched and mismatched for other loci) showed that HLA disparity was associated with increased overall mortality and with an increased treatment related mortality. The risk factor for patients mismatched for HLA-C only were significantly lower than in the group mismatched for loci other than HLA-C. The lower expression of HLA-C antigens may reduce their immunogenicity. Although HLA-C has a lower impact than other loci, the authors concluded that HLA-C still presents clinically relevant transplantation barrier.

Ferrara and colleagues tested the hypothesis that amino acid substitutions in specific position within class I heavy chain would have a different impact on BMT outcome. 163 pairs matched for DRB1/3/4/5/, DQA1 and DQB1 loci were typed by sequence based typing for the HLA-A, -B, and -C loci. 103 pairs had one or more mismatches at class I. Data suggest that substitution at position 116 of class I heavy chain increases the risk of acute GvHD and transplant-related mortality in unrelated donor transplant.

Müller presented an algorithm that has been designed to select potential stem cell donors for HLA-DRB1 typing on basis of their HLA-A,B-phenotype. This algorithm uses HLA haplotype frequencies to estimate absolute HLA-A,B,DR-phenotype frequencies as well as conditional distributions of HLA-DRB1 subtypes for each HLA-A,B-phenotype. Then, taking into account the current registry composition, a sequence of donors to be typed for HLA-DRB1 can be established optimizing the chance of patients to have an HLA-A,B,DRB1-matching donor initially. Their strategy can save up to 60% of typing costs over a random selection of HLA types. In HLA-identical stem cell transplantation recognition of minor histocompatibility antigen (mHA) by donor T cells is responsible for the GvHD disease. HLA-identical allogenic T cell responses can be induced in vitro using dendritic cells. Laurin demonstrated that in vitro stimulation of HLA-identical T cells with dendritic cells can result in the isolation and characterization of minor histocompatibility peptides.

In order to determine the importance of matching for NK epitopes De Santis and colleagues analyzed the outcome of one haplotype matched BMT. Potential NK alloreactivity was not associated with rejection, non-engraftment and leukemia relapse but was associated with more severe GvHD and poorer patient survival. Gagne and coauthors confirmed in a large series previously reported results of the impact of donor/recipient KIR gene mismatching on GvHD risk mainly in unrelated BMT and pointed out the critical KIR markers related to aGvHD. Mismatching for 3 activating KIR genes (2DS1, 2DS3 and 3DS1) appeared to be correlated to high GvHD rates.
TEACHING SESSION IV: DNA-BASED HLA TYPING
TECHNIQUES AND DATA ANALYSIS

WENDY SWELSEN (UNIVERSITY HOSPITAL MAASRICHT, THE NETHERLANDS).

The presentation of the first speaker Dr. Pavel Jindra (Czech Republic) focused on basic DNA typing: PCR-SSP and -SSO. For both methods a worldwide-used commercial or “home-made” format is known. For PCR-SSP the development of the basic principles seems to be definitive, whereas for SSOP there is potential for a continuous development of new modifications of the basic principle; reverse line, ELISA based and Luminex technology.

In this session the similarities and differences between the two methods are discussed. PCR-SSOP is usually based on gene specific amplification and allele discrimination during the hybridisation phase. This method has several advantages; multiple samples can be typed simultaneously, the method is easy to automate and only small amounts of DNA are necessary. However, there are also disadvantages; the method is time-consuming and has a higher ambiguity rate in comparison to PCR-SSP. In SSP allele discrimination already takes place during the amplification phase. No post-amplification specificity step is necessary, which saves time. Another advantage of SSP is the high specificity acquired, especially when both primers are targeted for unique sequence motifs. However, large numbers of PCR’s are inevitable, which make the method prone to contamination, labour intensive and difficult to automate.

To decide which method, SSP or SSO, to use, a number of factors should be considered. The specificity and sensitivity of the technique are an important factor. Data from the ‘Virtual DNA Analysis’ project of W. Helmborg confirmed that SSP seems to have more pronounced sensitivity over specificity while for SSOP this is reversed. Another factor involved in choosing the right method is the typing service (solid organ Tx, HSCT, registry typing and disease association studies) which may differ in urgency, degree of resolution needed and number of loci tested.

Finally, the number of samples to be analysed and the necessary budget have to be considered. In summary, the choice for PCR-SSP or -SSO is not so easy because both techniques have their own advantages and drawbacks for the different typing aims.

Another topic of this presentation was immortalisation of the typing data. Virtually all 4-digit typing results obtained by SSP or SSOP will eventually expire. The concept of storing typing data as allele assignments is therefore challenged. The only way to immortalise typing data is by maintaining the raw data obtained by the different HLA techniques.

The second lecture “Line probe method for HLA class II low resolution typing” was presented by Jos Drabbels (The Netherlands). Briefly, the reversed SSO method consists of three steps; amplification and gel detection, hybridisation and interpretation. The procedure is based on amino-linked probes biotiated and linked to a membrane. For low-resolution DRB- and DQB-typing 26 and 17 probes are needed respectively. Hybridisation of heat denatured biotinylated amplicons is performed, followed by critical washing. After coupling of a streptavidin-HRP conjugate, detection with ECL is possible. Advantages of the line-probe method are; re-use of the membrane for 25-30 times, high sample throughput (38 samples/day/person), DRB- together with DQB-typing (association), better hybridisation (fluid versus membrane), cheap, few equipment, home made (flexible) and easy to perform. Disadvantages are DR2 subtyping by DRB5 association, some weak probes, preparing of new membranes and labour intensivity.

Finally Dr. Erik Rozemuller (The Netherlands) discussed about sequence-based typing and data analysis. The high resolution sequence-based typing (SBT) method is based on identification of alleles by sequencing the polymorphic regions of the gene. For HLA class I, sequencing is focussed on exons 2 and 3 and for class II exon 2 is sequenced. Each baseposition is used for typing, both polymorphic and conserved positions. The sequencing reaction can be performed with universal big-dye primers or big-dye terminator chemistry. Sequence data are processed automatically and evaluated manually. The combined sequences of exon 2 and 3 are used for automatic alignment and assignment to the latest version of the IMGT sequence database. Ambiguities occur when the typing data do not result in a unique identification of the alleles. Three kinds of ambiguities can be recognised and resolved:

1) Ambiguities are noticed when alleles differ outside the region amplified and sequenced. These ambiguities can be resolved by sequencing other regions or by serology for the difference between expressed versus null alleles.

2) Another kind of ambiguity occurs when part of the sequence of an allele is unknown. Other alleles may differ in this region and cannot be discriminated, in combination with the incomplete allele. These kind of ambiguities will be resolved when the sequences are elucidated.

3) If two or more allele combinations have identical heterozygous sequences, this could lead to ambiguous typing. The ambiguities can be resolved by allele-specific amplification and sequencing. Ambiguities will always occur. They are characteristic for the typing protocol and are not caused by bad-quality data. They are predictable and can be resolved. Whether ambiguities should be resolved depends on the typing purpose.

Software for data analysis is necessary for several procedures; sequencing, determining heterozygosity, alignment with locus consensus, allele assignment, tools for manual inspection/editing and reporting. New SBT data should be submitted to the IHWG (International Histocompatibility Working Group) database together with cell information, allele assignment and sequences. In the IHWG technical handbook, SBT protocols for all HLA loci, gene- and allele-specific amplification and sequencing and sequencing of other regions are described.
NEW TECHNIQUES.

The first presentation entitled “The CTLp test in the routine HLA lab: an old-fashioned assay” was given by Dr Tiercy from Geneva. He was talking about the usefulness of the in vitro analysis of CTLp frequencies as a tool of donor selection for haematopoietic stem cell transplantation (HSCT) and as a tool to address the relative contribution of HLA class I loci (HLA-A, B, C) to the alloreponse aimed to find out the acceptable donor-recipient mismatches. The CTLp assay has been reported in several studies as either an aGVHD predictive test or an assay, which results have not correlated with BMT outcome. Moreover, there is a very good correlation between the CTLp results and HLA class I disparities. The higher CTLp frequencies are detected for HLA-A and B than HLA-C incompatibility evaluated by DNA typing. The results of a large scale CTLp testing (1990-2002) for selection of unrelated donors have shown that in a case of HLA-A, B, DR, DQ matched (HLA-A and B typed by serology and DR, DQ by high resolution DNA typing) donor recipient pairs CTLp assay is able to pick up serologically hidden A, B, C incompatibilities. The test has been also found to be useful in detecting possible sensitization against minor histocompatibility antigens (phenotypically matched family donors). However in practice, the use of DNA high resolution typing techniques decreases the need of cellular assays, especially that there are some technical problems difficult to overcome, as in example a quality of patients’ samples or an adequate control panel of frozen lymphocytes. Doctor Tiercy’s presentation concluded that with respect to donor selection, even a weakly positive CTLp test should not be a contraindication for HSCT with a donor matched at the allelic level within 5 HLA loci (HLA-A, B, C, DR, DQ).

Dr. Little from the Anthony Nolan Bone Marrow Trust in London gave the second talk on chimerism analysis. She presented an excellent overview supported by a number of very clear slides describing the principles of chimerism study. Chimerism (by definition: cells from genetically different individuals coexisting in one body) can be detected with various methods including: analysis of blood group differences, sex chromosome markers, HLA disparities and analysis of variable number of tandem repeats (VNTR) or short tandem repeats (STR). Two latter ones have been described in more detail. The VNTR 9-80 bp core repeats (minisatellites) are analysed, while in STR 2-7 bp repeats with total size < 500 bp (microsatellites) are detected. In both techniques after amplification the PCR products are run on a polyacrylamide gel. Usually, when fluorescent target primers are used for PCR amplification, the further analysis is performed with laser detection system and the software that allows to calculate peak areas (reported as %) and a ratio - % donor : % host cells. There are 4 types of chimerism: full chimerism, mixed and split chimerism or microchimerism. The first one characterizes with 100% of donor cells detected, two next ones with respectively, host cells detected in a given compartment, e.g. lymphocytes or host and donor cells detected in one or more linkage, e.g. myeloid cells 100% host while T-cells 100% donor. The latter one, microchimerism is when less than 1% of host cells are detected. Some important points in chimerism study are: (1) to identify informative loci that allow distinguishing patient and donor cells and with PCR products that do not characterise with similar size, difficult for interpretation, (2) to increase sensitivity by the linkage-specific analysis. The chimerism study is routinely used to assess kinetics and engraftment in the recipients of allogeneic HSCT. To assess the chimeric status of a HSCT patient at least 3 DNA samples are needed: one taken pre-transplant from a patient, one donor sample (taken at any time) and post-transplant patient sample(s).

Next Ms Whitelegg from the Anthony Nolan Research Institute / Royal Free Hospital, London described the methods leading to tetramers generation and they for monitoring immune responses in transplant patients. This presentation was also illustrated by a number of very nice slides, guiding all the participants of this teaching session in step-by-step preparations of tetramers. At first generation of tetrameric MHC class I complexes were described including: production of modified MHC class I and \( \beta_2 \)-microglobulin proteins in E. coli, refolding MHC/\( \beta_2 \)-microglobulin/peptide complexes, biotinylation, purification of bionylated molecules and their tetramerisation with streptavidin. Then the hints for generation of MHC class II complexes were presented.

Finally, Dr. Middleton from the Histocompatibility and Immunogenetics Laboratory in Belfast presented the technique for KIR typing. Doctor Middleton was one of the busiest persons during the EFI meeting in Baden-Baden, giving a number of oral presentations and lectures even in parallel sessions. Due to another presentation, his talk, previously scheduled as the third, was the last one of our Teaching Session III.

He started with presentation of the leukocyte receptor complex (LRC) organization and schematic representation of different kinds of killing inhibitory receptors (KIR). LRC, including the KIR genes, is located in humans on chromosome 19q. Five families of KIR receptors have been distinguished depending on a number of extracellular domains, and the length of an intracellular fragment: 2DL, 2DS, 2DL4, 3DL, 3DS.

Then Dr. Middleton described us the principals of KIR typing technology developed at his laboratory and showed the typing results obtained for Irish population. The technique is based on 4 PCR amplifications of KIR genes encoding D0, D1, D2 and TM/CYT, followed by hybridisation with over 30 SSOP.
We summarize below a part of the second teaching session given at the 17th EFI Meeting and chaired by Alicia Sanchez-Mazas and her colleagues Johan Renquin and José Manuel Nunes, who address several critical issues in HLA data analysis. For a full citation of the session and more details by the authors, consult the EFI website at http://www.efiweb.org/membert/tutorials.html.

Problem 1: The Handling of heterogeneous populations samples

The notion of population is rarely as clear as the interpretation of results would require. Indeed, population samples often result from more or less important mixtures of genetically diverse individuals (see an example on the slides available at the EFI website). Elements of answer are given by checking for Hardy Weinberg Equilibrium (HWE) and using population heterogeneity test:

- Testing for HWE aims at checking that the sample has no significant departures from necessary properties to perform models of allele transmission and calculation of expected phenotype frequencies. The Hardy-Weinberg law is applicable under conditions of general panmixia (random gametes mating and equal-probability of gametes) and sufficient population size. Stability of allelic frequencies across generations requires: no selection, no migration, and no mutation. The basic test is a Chi-square computation. A rejection of the null hypothesis (the population is in HWE) invites to consider typing error, selection effect, recruitment bias, inbred population or stratified population.

- Testing for Population heterogeneity aims at identifying a population structure. This test requires external information to design a sub-population structure. A chi-square test allows comparing distribution among the different sub-samples, null hypothesis assumes that there is no differences between these samples. Finding significant difference invites to better describe the population by a representative sample.

Problem 2: The handling of different level of HLA information in the data.

HLA typing is sometimes performed at different typing resolution, thus the information about HLA polymorphism is heterogeneous. This should be considered at two levels: the inter-population (for example, we often have higher resolution data on patient than on control populations) and the intra population (for example when older HLA typing use different HLA nomenclature than the more recent ones).

Doing conversion on Splits into broad is easy; frequencies can be obtained either prior to the conversion or after it. Here, one should remember that the sum of maximum likelihood estimates is not a maximum likelihood estimates. Thus, it is better to first convert Splits into Broad and then to perform the appropriate counting method of alleles for frequency estimation.

Broad to split conversion results from a redistribution of broad alleles observation into split classes. This redistribution is acceptable only if: 1- the sample is "large enough", 2- the split/broad ratio is high (at least two) 3- if one does so at the allelic level not the haplotypic level.

Overview: Dealing with such conversions, the investigator has to decide which from the resolution level or the accuracy of estimates is most important in his study.

Problem 3: The interpretation of genetics distances represented by trees or projections.

If we suppose that genetic distances have been computed among several populations (whatever the methods), distances can be represented using genetic trees or multidimensional analyses (projections).

- In a multi-gene system reliability of trees configurations is assessed by re sampling methods (Bootstrap or Jack’s knife). If a bootstrap value is > 95, it means that at least 95 cases out of 100 remain unchanged the nodes configuration. There are two additional important points for the interpretation of trees: -The topology of trees could be highly dependent on the data used and so very sensitive to the addition or the removal of populations. – The tree representation was primarily used to classify species. It means that tree representation assumes that no exchanges can occur between populations, which is not the case for human populations.

- An other way to analyse the relationship between populations is the projection of genetic distances according methods such as: Correspondence Analysis (CA), Principal coordinate analysis (PcoA) or Multidimensional Scaling analysis (MDS). (See references on the slides available at EFI website) Projections could lead to misunderstand the relative importance of each axis. “Percents of information by axes” assess which are the most important and sort the relative weight of each axis. Some methods are based on principal axes (PCA) whereas some are not (MDS). Projections reflect the observed genetic relationships between populations with no clustering or hierarchical structure.

Overview: Trees give simple representation of populations, but clustering and hierarchical structure should be interpreted with care as this clustering may be not reliable. On the contrary, multi-dimensional projections are complex representation of relationships between populations that do not constrain interpretation.

Problem 4: Levels of correlation with HLA.

Linkage disequilibrium is the non-random association of alleles at two (or more) physically linked loci. Gametic disequilibrium is the non-random association of alleles between two (or more) not necessarily physically linked loci. Strong linkage disequilibrium does exist in the MHC, particularly among alleles of specific haplotypes and between particular genes within the complex, even if it remains to clarify how such linkage...
disequilibrium makes sense in the light of evolution. It can be very useful to identify disease susceptibility genes for example. But one can get grips with such phenomena at two levels: genotype and phenotype levels. It can be calculated with known gametic phase data or estimated with unknown gametic phase data using estimated haplotype frequencies. Using 2x2 tables, Chi square statistics assess the significance of LD between alleles. 2x2 contingency table are also used to detect a lack or an excess of association of a HLA allele with another HLA allele or with a particular phenotype (HLA alleles association with disease) at the phenotype level. Fisher test on 2x2 contingency tables are then recommended.

Conclusion: Epidemiology and bio-statistics are the specialized fields of mathematics that have evolved to provide the mathematical representations to measurements of “the real world” of the medical sciences. Since it takes only a few minutes to teach how to find the appropriate square root, it is much harder to understand the underlying hypothesis, the exact meaning and the usefulness of the “chi squares” generally causing erroneous interpretation and misuse of statistics.

TEACHING SESSION: DATA ANALYSES IN POPULATION STUDIES

MILENA IVANOVA (CENTRAL LABORATORY OF CLINICAL IMMUNOLOGY, UNIVERSITY HOSPITAL “ALEXANDRO-VSKA”, SOFIA, BULGARIA)

The second speaker Johan Renquin (Geneva, Switzerland) talked about the Gene[VA] project. He presented a web site that provides programs for analyses of HLA data in population and disease association studies. Four programs for testing Hardy-Weinberg equilibrium, linkage disequilibrium, allelic associations and diseases associations were discussed. Examples of input and detailed result files for each program were presented. Methodological outlines implemented in the different programs were discussed.

The last presenter Jose Manuel Nunes (Porto, Geneva) talked about online tools for working with HLA data. He pointed out that working with HLA data often could result in use of codominant and non codominant data, switching between broad and split specificities, dealing with ambiguous genotypes, and use of several computer programs with distinct formats of data files. Then the author presented an algorithm for converting reactivity data to phenotype data and then estimating frequencies via web interface. Different file formats: for kit description, reactivity data and phenotypes were presented. Dr. Nunes discussed the UNIFORMAT and online tools that allows automation of common tasks. He talked about the possibilities for: conversion of data from distinct formats; recoding of UNIFORMAT data files, that is useful in case of dealing with data from multiple sources, data obtained by different typing methods and data with low frequencies specificities; generating haplotypes from UNIFORM data file and calculating frequencies. Joce Manuel Nunes ended his lecture by presenting the web interface with programs allowing online handling of HLA data files and an example of frequency estimates and their graphical representation.

TECHNICAL NOTES

How to evaluate the real quality of a DNA preparation?

Gisela Diederich, Guido Heymann, Constanze Schönemann. Tissue Typing Lab, Institute of Transfusion Medicine, Campus Virchow-Klinikum, Charité, Humboldt University, Berlin

Background: To validate our routine DNA preparation methods we compared isolation from 20 blood samples with two different protocols. Another four samples were prepared with four different methods also in parallel. Our standard quality check by measuring the purity and amount of DNA in a photometer was completed by visualization in a check gel and amplification control and brought results, that could be also interesting for other labs.

Methods: All 24 blood samples were drawn from patients (organ or marrow transplant candidates, thrombosis patients). From each of the first 20 blood samples 1 ml buffy coat of was isolated with Super Quick-GeneTM (Immucor, Rödermark, Germany). From the same 20 patient samples 350 µl of whole blood were prepared with GENOMTM-6 automate (Geno Vision, Vienna, Austria). Four blood samples were prepared with the two described testkits and additionally with Dynabeads-DNA-Direct TM (Dynal, Oslo, Norway) 300 µl whole blood and with FlexiGene DNA KitTM (Qiagen, Oslo, Norway) 500 µl buffy coat. All the preparations have been done according to the instructions of the manufacturers. GeneQuant II (Pharmacia Biotech, Cambridge, England) and the Ultraspec 2000 (Pharmacia Biotech, Cambridge, England) were used for the photometric analyses. The DNA „check gel“ was a 0,7 % Agarose gel. 8 µl genomic DNA (50ng/µl) run at 100 mA / 150V for 60 min.. DNA amplification was done with two primers, detecting HLA-Bw4 and HLA-Bw6 (Geno Vision, Vienna, Austria).

Results:

Table 1 The tables represent the photometric parameters, measured at wave lengths 260 nm and 280 nm. The GENOMTM-6 preparation was measured in the dilution 1:5, the Super Quick-GeneTM was diluted 1: 100. Preparations of all samples with both methods were of good quality concerning the DNA/protein factor and the DNA amount (notice different blood volume for both isolations).

Comparing the parallel isolation for the 4 samples, the beads techniques GENOMTM-6 and Dynabeads-DNA-Direct TM resulted all in good quality DNA at the check gel. The two salting out methods Quick-GeneTM and the FlexiGene DNA KitTM again showed degradation. 20 samples of the GENOMTM-6 DNA have been also tested with good results after five month storage at –20°C.
**advertisement**

MMD Medical Molecular Diagnostics GmbH is seeking a Director to provide the leadership and senior management for a clinical immunogenetics laboratory. The laboratory performs HLA typings for volunteer hematopoietic stem cell donors recruited by DKMS Deutsche Knochenmarkspendendatei GmbH. MMD is located in Dresden, Germany and employs technical and administrative staff of 23 employees.

The Director will be responsible for all aspects of laboratory management. He is expected to provide the operational leadership necessary to ensure MMD’s capability to deliver the highest standards of accurate reliable service that effectively support the mission of DKMS. Particularly, the Director will be responsible to ensure an accurate and efficient flow of all laboratory processes, to guide and develop all laboratory staff members to achieve a high standard of proficiency and technical skills, to prepare and review all SOPs, to review data and data reports, and to implement new technologies.

The Director must have sound knowledge of the fundamentals of immunology and genetics and must be adept in high volume HLA typing (SSO and SSP) and DNA sequencing techniques.

The Director should have an advanced degree in a biologic science and have at least three years work experience in clinical HLA testing and/or HLA research. Candidates should be certified (preferred) or eligible for certification by the American Board of Histocompatibility and Immunogenetics or by the European Federation of Immunogenetics. The candidate must have profound knowledge about the working principles and practice of using SSO, SSP, and DNA sequencing techniques for HLA typing. He/she must have an understanding of the special requirements of high volume/high throughput HLA typing and must be capable of presenting a forward looking plan for maintaining and/or improving laboratory performance proficiency. He/she must have experience in laboratory management including project and personnel management. He/she has to comply with ASHI Standards for “Director/Technical Supervisor.” A sound scientific reputation is required. DKMS would like to support the Director to set up a scientific program in the field of histocompatibility and the influence on the outcome of hematopoietic stem cell transplantation. Special staff, investment, and financial resources will be provided.

The Director will be member of the Medical Faculty of the Technical University Dresden and will keep the position of the Professorship for Immunogenetics (C3-Professor). Eligible applicants will be invited to an interview and a presentation by the Medical Faculty Dresden.

The application package should be sent to: Prof. Dr. Gerhard Ehninger Medical Faculty „Carl Gustav Carus“ Fetscherstr. 74 01307 Dresden
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Fig.1: Result of specific amplification: 4 lanes for each sample: Bw4; Bw6 primer for GENOMTM-6 (A) and for Super Quick-GeneTM (I). preparation gives PCR-products for all samples. Dilution to 25 ng/µl DNA will result in more clear lines. Quick-GeneTM preparation was very weak in single cases (i.e. Bw4 in I4, I9, I11).

Fig.2a: Check gel for the first 20 samples. Comparison of GENOMTM-6 (A). Dilution 50ng/µl.

Fig.2b: Check gel for the first 20 samples and Super Quick-GeneTM (I). Dilution 50ng/µl.

A lot of degradation fragments in this preparations were observed.

Discussion: The quality parameters obtained by photometer measurement of DNA/protein ratio give not always complete information about the real suitability of the DNA preparation for PCR. Especially the salting out methods, in our validation the QuickGeneTM and the FlexiGene DNA KitTM contain in a certain amount degraded DNA fragments, which are not defined in the standard photometer evaluation. Nevertheless, in most cases this DNA can be amplified with HLA-typing reagents. Isolation with magnet beads (by hand or by an automate) results in a very clean DNA, that often can be diluted for the PCR much more higher than other DNA preparations. It is more efficient especially for patient samples with very low cell count.
Biostatistics Teaching session of the 17th EFI meeting: crucial issues for HLA data handling and analysis

by Alicia Sanchez-Mazas

Laboratory of Genetics and Biometry, Department of Anthropology and Ecology, University of Geneva, Switzerland

The biostatistics teaching session of the 17th EFI meeting held in Baden-Baden (6-9 May 2003) was devoted to discuss a number of frequently asked questions in HLA population data analysis, among whom how to handle heterogeneous HLA data and how to assess HLA linkage disequilibrium (see the full presentation of slides at http://www.efiweb.org/members/tutorials.html and a summary of the first part of the session by P-A Gourraud below). To tackle such questions, we have developed a new website offering several programs running online to accurately handle HLA data and perform basic statistical analyses with population data: this is the the Gene[VA] project website at http://geneva.unige.ch/.

At the moment, our site is subdivided into the two following sections:
- “Data analysis tools” (http://geneva.unige.ch/tools/data_analysis/), allowing test for Hardy-Weinberg equilibrium, linkage disequilibrium, allelic association, and HLA and disease association, and
- “Data handling tools” (http://geneva.unige.ch/tools/data_handling/) allowing conversion and recoding (broad/split) data files, automatic interpretation of phenotypes, frequency estimation even with ambiguous genotypes, and other related utilities (see a description of these tools by J-M Nunes below).

The Gene[VA] website, which is still developing, will soon be linked to our local genetic databases. We encourage online registration to favour contacts between our team and interested people, to send updates announcements and to receive feedback.

THE ITALIAN QUALITY CONTROL SCHEME FOR HLA TYPING: THE NATIONAL AND REGIONAL RESULTS FOR 2002

As the years before, in 2002 the Italian National Transplant Centre assigned to the laboratory of Immunology of the Italian National Institute of Health the task of organising the serological and genomic quality controls (QC), whose original schemes have previously been described in EFI Newsletter No.14 (1995) and No.33 (2001). Both schemes include 23 laboratories comprehensive of all the Regional Reference Centres for solid organs and the Regional Registries for bone marrow transplantation. Since Italy has a high number of HLA typing laboratories, eight regional schemes were constituted to give each laboratory the chance to be included in an annual QC scheme. In contrast with the national QC scheme, which is totally organised and managed by the Italian National Institute of Health, the regional ones are organised locally under the supervision of the Italian National Institute of Health that has the task of collecting the results, checking the protocol used and elaborating the data. The national and regional QC schemes followed the same protocol and were equal in their value.

National and regional serologic QC schemes

The results of the national and regional QC schemes are shown in Table 1. The final evaluation was carried out according to the EFI Standards, which state that laboratories that do not report more than two errors for Class I and one error for Class II for compulsory specificities have an acceptable performance. EFI rules have been recently revised and will be applied for the 2003 QC scheme. The results were public and letters regarding the efficiency of the typing of the laboratories were sent to the district Council departments, to the Regional Reference Centres and to the General Managers of the hospitals.

In the national QC programme all the laboratories reached a satisfactory performance, except one that did not type the minimum number of cells required. Among the laboratories that typed successfully, only one laboratory performed one error for compulsory spe-
cificities. Figure 1 shows the improved results obtained in 2002, compared to those obtained in the period 1998-2001. The figure clearly shows the year by year increase of the percentage of laboratories with no errors for compulsory specificities, starting with 58% in 1998 and reaching the value of 91% in 2002. In the regional QC schemes (Table 1), all the 64 participant laboratories typed satisfactorily, of which 54 laboratories returned results without any error for compulsory specificities. Results have improved at regional level since the percentage of laboratories which did not reach a satisfactory performance decreased from 3.5% in both 1999 and 2000 to 1% in 2001 and 0% in 2002.

Table 1.
Serological QC results for 2002

<table>
<thead>
<tr>
<th>Laboratories</th>
<th>National Scheme</th>
<th>Regional Schemes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>No errors</td>
<td>21</td>
<td>91</td>
</tr>
<tr>
<td>Max 2 errors in Cl and/or 1 error in Cl</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>&gt;2 errors in Cl and/or 1 error in Cl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&lt;8 samples typed</td>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 1. Error distribution for compulsory specificities in the national scheme, years 1998-2002.

National and regional genomic QC schemes
The laboratories participating to the national scheme were asked, before starting, to declare which antigens and which resolution they were going to take into account (Table 2). The allele assignment was on the basis of the 75% consensus with a minimum of 9-10 typings; when the number of typings was lower, the results of the majority were considered as correct. Typing was requested for all the alleles reported by the most recent report on the Nomenclature Committee. Waiting for the application of the new general rules for external proficiency testing programmes formulated by the External Proficiency Testing Committee of EFI last year, we have taken into account the missed, extra and wrong specificities, that are reported in Table 3. In our final report we have also pointed out: 1) low resolution typing instead of high resolution (HR) for those laboratories which had previously declared to type HR; 2) ambiguities in the assignment of an allele; 3) assignment of homozigosity in absence of a family study; 4) use of an outdated nomenclature.

Table 2. Type of resolution for DNA typing in the national scheme

<table>
<thead>
<tr>
<th>Resolution</th>
<th>Class I alleles</th>
<th>Class II alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>LOW</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>HIGH</td>
<td>15</td>
<td>19</td>
</tr>
</tbody>
</table>

Since the majority of the laboratories participating to the regional QC schemes adopted a low resolution DNA typing, only low resolution typing has been analysed in these cases.


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Seventy-eight per cent of the laboratories included into the national programme performed either no errors or up to 2 errors; on the other hand, only 3 laboratories (15 %) reported more than 5 errors, as shown in Table 3. The results of 2002 seem very encouraging, since for the first time they improved remarkably; in fact, there was no significant difference between the results obtained in the QC of the first two years for the national scheme because the number of laboratories that performed from 0 to 2 errors in 2000 and 2001 was the same. The year 2002 has finally shown a positive trend towards a higher quality of genomic typing. Furthermore, this year, more laboratories have chosen to type class I alleles using high resolution techniques in comparison with the previous years. Similar results were obtained in regional schemes, where 67% of the laboratories performed 0-2 errors in 2002. The most remarkable difference with the national scheme is that a high number of laboratories did not type the minimum of 20 samples as required or did not receive all of them, due to local organisational problems which still occur. Unlike the national results, a definite improvement in the quality of typing was already observed in 2001, the second year in which the genomic regional schemes were organised. Table 4 shows the lower number of errors, which have been performed in 2001 in comparison to 2000, with a slight additional reduction of errors in 2002.


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All together, the results of year 2002 are satisfactory, especially considering the national serologic and genomic QC schemes. The gained experience and the improvement of the serologic scheme during the eight years of performance of QC programme in Italy have allowed to reach a constant improvement of the quality of typing. As regards the genomic scheme, the quality of typing has remarkably improved for the first time at national level and
TABLE 3

<table>
<thead>
<tr>
<th>Laboratories</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>No errors</td>
<td>6</td>
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<td>6</td>
</tr>
<tr>
<td>1-2 errors</td>
<td>7</td>
<td>32%</td>
<td>7</td>
</tr>
<tr>
<td>3-4 errors</td>
<td>5</td>
<td>23%</td>
<td>4</td>
</tr>
<tr>
<td>5-10 errors</td>
<td>2</td>
<td>9%</td>
<td>4</td>
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<td>&gt; 10 errors</td>
<td>2</td>
<td>9%</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 20 cells</td>
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<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100%</td>
<td>22</td>
</tr>
</tbody>
</table>

TABLE 4

<table>
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<tr>
<th>Laboratories</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
<td>Number</td>
</tr>
<tr>
<td>No errors</td>
<td>15</td>
<td>29%</td>
<td>18</td>
</tr>
<tr>
<td>1-2 errors</td>
<td>6</td>
<td>12%</td>
<td>11</td>
</tr>
<tr>
<td>3-4 errors</td>
<td>5</td>
<td>10%</td>
<td>4</td>
</tr>
<tr>
<td>5-10 errors</td>
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<td>1</td>
</tr>
<tr>
<td>&gt; 10 errors</td>
<td>1</td>
<td>2%</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 20 cells</td>
<td>19</td>
<td>37%</td>
<td>13</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0%</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100%</td>
<td>50</td>
</tr>
</tbody>
</table>

once again at regional level. Furthermore, the new EFI guidelines, which came out last year and will be applied in the 2003 QC programme, will certainly help in the future the organisers in carrying out an improved QC scheme and, above all, will give them directions on how many discrepancies for typing will be taken into account for European accreditation.

The year 2003 will see other important changes for QC schemes in Italy. The National Transplant Centre, that has the task of establishing protocols and rules for QC on immunogenetic techniques according to the law 91/99 on organ transplantation, has recently decided that the national scheme will include the EFI and ASHI accredited laboratories which were not entitled to participate to the previous QC. In addition, it was established that the regional QC programmes will be completely ran at a local level with the support of the regional authorities, since management of the Italian Health care has been recently decentralized and many tasks have been assigned to the Regions.

We thank all the participating laboratories, in particular the Regional Referring laboratories for organising the regional QC schemes. We gratefully acknowledge Ms. L. Mottola for excellent technical assistance.

Francesca Quintieri, Orsola Pugliese, Mirella Mariani*, Manuela Testi*, Alessandro Nanni Costa.

Italian National Transplantation Centre, Italian National Institute of Health, Rome;
*CRCC Tissue Typing Laboratory, A.O. San Camillo-Forlanini, Rome.

TABLE 4

<table>
<thead>
<tr>
<th>Laboratories</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Percent</td>
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<td>12%</td>
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<tr>
<td>3-4 errors</td>
<td>5</td>
<td>10%</td>
<td>4</td>
</tr>
<tr>
<td>5-10 errors</td>
<td>5</td>
<td>10%</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 10 errors</td>
<td>1</td>
<td>2%</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 20 cells</td>
<td>19</td>
<td>37%</td>
<td>13</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0%</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100%</td>
<td>50</td>
</tr>
</tbody>
</table>

Working with HLA data online
José Manuel Nunes*  
ICBAS — Universidade do Porto and LGB — Université de Genève

In a presentation made during the teaching session 4, at Baden–Baden 2003 EFI’s congress, I have shown how to use a web based system to automate several common tasks related to the manipulation of HLA data sets. Here I recall the principles underlying the system and give some additional information that, jointly with the presentation itself, explains how to use the system.

Two kinds of problems have been discussed; first, how to pass from reactivity data to frequency estimates in a few simple steps, and second, how to automate recurrent tasks faced when doing HLA data analyses. The selected choice of tasks happened to highlight some common problems, or limitations, of currently available software used to analyse HLA data. Among the difficulties HLA and related data poses, stands out the mixture or combination of codominant and non codominant loci, the conversion between broad and split definitions, the use of blank or blank–like alleles and other ambiguous genotypes, and the use of several distinct programs with their respective formats. In an attempt to minimize the respective formats. In an attempt to minimize the difficulties, a data format, UNIFORMAT, and several tools designed around it have been built, and are presented below. For those reading these lines it may be worth to point immediately the web site where the system is implemented: http://geneva.unige.ch/tools/data_handling/, a niche of the GeneVA project.*

The first problem to tackle is to transform some reactivity data into frequency estimates. For that a two–step approach is proposed: calculate the phenotypes using the reactivity data, then estimate frequencies from these phenotypes. The phenotypes can be calculated from the reactivity data using the phenotype program. The program needs two files, one with the reactivity data, and another with the kit description. Both files have similar formats; the kit description is a file where each line contains a probe information and name, and consists as many lines as probes in the kit. Every line starts with the probe name followed by the equal sign and then a colon separated list of allele names, i.e., the list of all alleles that react with the named probe. Similarly the reactivity data is described by a file, a line per each individual, where each line starts with an identifier followed by an equal sign, and then a colon separated list of probes; the probes that have been found to react with the individual with the given identifier. Once the two files, the reactivities and the kit description, exist it suffices to submit them to the phenotype program to get back the corresponding phenotypes. These calculated phenotypes can then be immediately submitted to the frequency estimator, and frequencies are done.

Two common ideas are challenged by the approach outlined above, one relates to phenotypes the other to frequency estimation. Phenotypes may be ambiguous, or have ambiguous genotype, i.e., there may have more than one possible genotype compatible with the observed reactivity data and kit. In fact the phenotype program returns the phenotypes as a list of pairs of alleles, each pair of

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For information on the GeneVA project contact Alicia.Sanchez-Mazas@anthro.unige.ch
alleles being such a possible genotype. The second challenge is to recognise that the estimation of frequencies, at least when using the EM gene-counting method, can very well deal with these ambiguous genotypes. It suffices to resort to the same principles and techniques used in haplotype frequency estimation (you may well find useful to go to http://geneva.unige.ch/tools/data_handling/ and check it by yourself). Therefore no conceptual problems exist, the only thing happening is an expansion of what is meant by phenotype and its associated genotype: a list of pairs of alleles —possibly reduced to a single pair.

The data format that connects the phenotype calculation with the frequency estimation is UNIFORMAT. This format has been conceived to represent phenotypes as lists of pairs of alleles. Hence, a HLA data file is in UNIFORMAT if each line is a case or a comment —a line starting with the # sign or a blank line—, and each case starts with an identifier followed by a tab— or tabulation mark—and then by a list of allele pairs. If several loci are to be included, followed by a tab or tabulation mark, and then by a blank line—and, each case starts with an identifier.

The basic schema set up uses UNIFORMAT either as entry or output, or both, and works over it. This is feasible because UNIFORMAT is formally defined (see bibliographic notes below). The key element is a program, UNIFORMATE, that checks if a file is or not in valid UNIFORMAT, and performs expansion of the abridged form. Another utility calculates utility calculates the number of valid multi-locus files. These haplotyped files can be hand edited to incorporate additional information, such as provenience from family data or other sources. A further tool in text replays in UNIFORMAT the automatic substitution of an allele name by another one or an expression. This transliterate tool is mainly thought to convert between broad and specific forms, and provides a versatile and non error prone way to convert large quantities of files. Finally there should be mentioned the conver[t]ion between file formats, namely from columnar data to UNIFORMAT, and from this one back and forth to Arlequin.

The system presented above is new because it generalises existing ones, in the sense of not having the same limitations of other programs currently available. As one would expect, there is no restriction in the number of cases to include in the reactivity data file, the length or number of probes detected per case, as well as in the number of probes to include in the kit description file. Furthermore, the use of kit description files gives you complete freedom to use your own probes and build your own kits.

A very brief bibliographic account must mention that the gene-counting method was conceived by Smith, [CSS55] and [Smi57] in the late 50's in the context of allele frequency estimation. The method was later shown to be a particular case of the Expectation–Maximisation algorithm, [DLR77]. The extension of gene–counting to haplotype frequency estimation appears to date to the 1980's, [BD80], but as of 1991, 11th HLA workshop, the connection with the EM algorithm is still absent, cf. [IAK+92]. This connection, in the context of haplotype estimation, dates to 1995, [LWU95] and [HPK94], and has been accompanied by computer implementations, one of which is the well known Arlequin, [SRE00]. Although at least one implementation of the EM gene–counting method is able to deal with ambiguous phenotypes, there seems to be no interest in such prior to the 12th HLA workshop, [CLM+97]. Further details about the programs presented here, their algorithms, and a formal description of UNIFORMAT, are given in [Nun03].

Acknowledgement

The author wishes to express his gratitude to Berta Martins da Silva and Alicia Sánchez-Mazas.

References


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1For some people a convenient way to produce such files is the use of a spreadsheet where each column stands for a locus or the initial identifier. Each locus column contains the list of allele pairs corresponding to the locus: two pairs are separated by a space, and the two elements of the pair are separated by a comma. To use such a file it suffices to export it as text, selecting tab or tabulation as the text delimiter.

2This situation is the ultimate degree of ambiguity: an untyped locus corresponds to a list with all distinct pairs of alleles existing at the locus. While conceptually simple, this is a little tricky to program, but just tedious to compute.

3This form has been conceived to represent phenotypes as lists of pairs of alleles. Hence, a HLA data file is in UNIFORMAT if each line is a case or a comment—a line starting with the # sign or a blank line—and each case starts with an identifier followed by a tab—or tabulation mark—and then by a list of allele pairs. If several loci are to be included, followed by a tab—or tabulation mark—and then by a blank line—and, each case starts with an identifier.
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