ESID Newsletter

Contents

The ESID Newsletter is made for the members of ESID – the European Society for Immunodeficiencies.

It is published under the responsibility of the ESID Board, and at this moment it is edited by Esther de Vries.

Any ESID member who is interested in publishing his or her views, research, new ideas or other material in the ESID Newsletter is cordially invited to submit copy to the Editor. Suitability for publication is assessed by the Editor in consultation with the other members of the ESID Board.

Editor's address:

Dr. Esther de Vries, pediatric immunologist, Jeroen Bosch Hospital loc GZG, P.O. Box 90153, NL-5200 ME 's-Hertogenbosch, the Netherlands, tel. +31-73-6162965, fax +31-73-6162948, esther_de_vries_nl @ yahoo.co.uk .

Front page: Taste the French atmosphere in Versailles in October 2004!

Dear ESID members,

Already, we are starting the second year with a revised ESID Newsletter.

It contains the first invited review, from the group of prof. van Dongen, , as well as a report from our congress in Weimar in October.

In this issue, the 'Focus on a country' section shows you Hungary, with prof. László Maródi from Debrecen featuring as Established ESID member, and Lányi Arpád as Young Investigator. You will be able to get to know more about immunology in Hungary, the country where our congress will be held in 2006.

But before that, we will all meet again in Versailles, where the congress will be organised by prof. Alain Fischer and his team in 2004.

Of course you will also find the regular contributions from the ESID Board, ESID Information, News & Views, announcements about the ESID Summer School, as well as Working Party Reports.

If you feel like suggesting a country or person for the 'Focus on a country' section, want to attract attention to your symposium, express your opinion, write a review, or anything else of interest to the ESID community, **please don't hesitate to contact me at esther_de_vries_nl @ yahoo.co.uk.**

Best wishes to all of you!

Esther DE VRIES, Editor

= **ESID Information** =

ESID is the European Society for Immunodeficiencies. It was formed in 1994. The forerunner of ESID, the informal European Group for Immunodeficiencies (EGID) was established in 1983. Anyone who is interested in primary immunodeficiency diseases can become a member of ESID. You can find the necessary information to contact the treasurer Esther de Vries at www . esid . org.

Within ESID, six Working Parties are actively engaged in coordinating the member's joined efforts in patient care and research in primary immunodeficiency diseases: Bone marrow transplantation (chair: Andrew Cant), Pathology (chair Fabio Facchetti), Patient registries (chair: Bodo Grimbacher), Clinical (chair: Jean-Laurent Casanova), Genetics (chair: Anna Villa), and Education (chair: Anders Fasth). Anyone who is interested in participating in one or more of these Working Parties is invited to do so. Please contact the chairman of the relevant Working Party (contact information is available at www . esid . org).

In 1994, a main registry of patients with various forms of immunodeficiency in Europe was established. Altogether, data from some 10,000 patients from 26 countries were received until now. In 1995, the first locus-specific immunodeficiency mutation database accessible through the internet was established (BTKbase for X-linked agammaglobulinemia – curators Mauno Vihinen and C.I. Edvard Smith). Since then, several additional locus-specific data bases have been established: ADAbase (adenosine deaminase deficiency – curators Mauno Vihinen and Michael Hershfield), BLMbase (Blooms syndrome – curator Mauno Vihinen), CYBAbase (autosomal recessive p22 phox deficiency – curators Dirk Roos and Mauno Vihinen), CYBBbase (X-linked chronic granulomatous disease (XCGD) – curators Dirk Roos and Mauno Vihinen), CD3Ebase (autosomal recessive CD3 epsilondeficiency – curators Mauno Vihinen and Jose R. Regueiro), CD3Gbase (autosomal recessive CD3 gamma deficiency – curators Mauno Vihinen and Jose R. Regueiro), CD40Lbase (X-linked hyper-IgM syndrome – curators Luigi D. Notarangelo and Mauno Vihinen), JAK3base (autosomal recessive severe combined JAK3 deficiency – curators Luigi D. Notarangelo and Mauno Vihinen), NCF1base (autosomal recessive p47 phox deficiency – curators Dirk Roos and Mauno Vihinen), NCF2base (autosomal recessive p67 phox deficiency – curators Dirk Roos and Mauno Vihinen), RAG1base (autosomal recessive severe combined RAG1 deficiency – curators Mauno Vihinen and Anna Villa), RAG2base (autosomal recessive severe combined RAG2 deficiency – curators Mauno Vihinen and Anna Villa), SH2D1Abase (X-linked lymphoproliferative syndrome (XLP) – curators Luigi D. Notarangelo and Mauno Vihinen), TCIRG1base (autosomal recessive osteopetrosis (arOP) – curators Mauno Vihinen and Anna Villa), ZAP70base (autosomal recessive severe combined ZAP70 deficiency – curator Mauno Vihinen), WASPbase (Wiskott-Aldrich syndrome – curators Mauno Vihinen and Luigi D. Notarangelo) (information is available at www . esid . org).

ESID organizes a biennial congress to facilitate international contact between primary immunodeficiency specialists. The last congress was organised in 2002 in Weimar, Germany; the next congress will be organized in Versailles, France in October 2004.

= **ESID Information** =

President's letter

Dear colleagues and friends,

It is a special honour and a great privilege for me to act as the new President of our Society. First of all, I wish to thank Edvard Smith, who has been a very active and successful leader during the last four years. Those of you (and you were many!) who attended the last ESID Meeting in Weimar, have had the opportunity to appreciate how much our Society has grown in terms of numbers, but also of culture.

We started not too long ago as a small group of enthusiastic friends, who thought it was time to move from a "Group" (EGID) to a real "Society" (ESID). Under a series of very efficient Presidencies, and with the help of the entire community of European clinical and laboratory immunologists, pediatricians, as well as of specialists in various fields, we have all together succeeded in making Europe a world leader in Primary Immune Deficiencies. We should be proud of the fact that colleagues from the other side of the ocean are replicating several of the initiatives that ESID has taken during the last years, from the organization of a network of scientists and clinicians, to the first Educational Courses.

But, what we have achieved thus far should not be our final goal. We need to move further. We need to define our new goals for the next years. While I will certainly do my best to contribute to the identification of such goals, I would like the Newsletter to become a forum for this. If there is something where we need to improve, it is in the ability to communicate among us. I urge all of you to do your part!

From my personal perspective, I would like to propose some considerations. With a great number of PID genes discovered (many by European groups directly linked to ESID), I believe we have to transfer this information into something useful to our patients. Detailed analysis of the data contained in locus-specific databases, evaluation of the efficacy of currently used treatments (and even before that: comparison of clinical practices within Europe!), and definition of standardized guidelines for diagnosis and treatment may represent important goals. Once again, this can only be achieved if we all contribute. In this ESID Newsletter, you will find an addition of some diagnostic guidelines to those that were produced a few years ago through a joint effort of ESID and PAGID. Please, have a look at them, and send your comments to the ESID Newsletter editor and directly to the curators. Similarly, in Weimar Jean-Laurent Casanova presented the current situation of treatment for Wiskott-Aldrich syndrome in Europe. Not surprisingly, almost each centre has its own practice. It is perhaps time to compare our policies, and come up with a shared one. European centres involved in bone marrow transplantation have offered a unique example of tight collaboration over the years; the relative papers include the largest series of patients worldwide and thus have the value that only numbers (not impressions!) can give. It is perhaps time to launch similar collaborations among clinical centers dealing with other forms of PID.

Education should also remain a primary goal, particularly for young trainees. Forming the next generation of clinical experts and clinical investigators should remain an important commitment for our Society for the years to come.

We also have a duty to explore new ways. It has always been so in the history of PIDs, from Bruton's discovery to the first successful experience with BMT, up to gene therapy. For this reason, the ESID Board thought it was appropriate and necessary to take a position with regard to the severe adverse events registered in the course of the gene therapy trial headed by Alain Fischer and Marina Cavazzana-Calvo. In particular, we agreed with their statement concerning premature release of the information to the press. Moreover, while the French group itself immediately called for halting the enrolment of patients following the two cases of leukaemia-like reaction, we should also acknowledge that they

have given us, for the first time ever, a proof of evidence of efficacy of gene therapy in humans.

We may also expect some difficulties in the near future. In spite of the hopes that were raised during the Weimar meeting, there is little room for a joint ESID application to the Sixth Framework Programme. It is likely that different groups will apply for various, more specific, actions. Yet, the hope is that we do not lose our attitude to collaborate.

In my job, I will need the help of everybody, from the Board members, to each single registered ESID member. I wish all of you a peaceful, fruitful and successful year.

Luigi NOTARANGELO

Secretary's report

During the last biennial ESID meeting in Weimar, the ESID Board met for the second time in 2002. On the agenda was the biennial meeting after Weimar. As you know, it was decided to hold this meeting in Versailles, France, 21-24 October, 2004. The option of a joint meeting was raised by the Latin-American Group for Immunodeficiencies (LAGID), but the ESID Board decided to pursue this possibility separately from the biennial meetings, because this would fundamentally change the character of the biennial meetings. To improve contacts with LAGID, one key ESID representative could join their meeting every year, if funding can be raised. It was emphasized that one of the major goals of ESID should remain to support young European scientists, in particular from Eastern Europe; however, Latin-American participants to the ESID Summer Schools could be encouraged to some extent. Regarding the ESID business plan, Edvard Smith pointed out that there were years when ESID had little funding while EU grants were pending, so an alternative source of funding should be raised; a two-step approach could be chosen in ESID´s efforts to proceed with support from industry: instead of wanting support for the entire range of activities as illustrated in the business plan, support for specific ESID activities, e.g. the disease-specific network incl. Eastern Europe, should be considered.

Immediately after the ESID Board meeting, the Board met with representatives of PPTA companies invited by Edward Hutt from PPTA. The idea of this meeting was to offer an opportunity to inform ESID about the topics the industry is most interested in. Among others, Clive Dash from EPFA pointed out that ESID can be very helpful in improving diagnostic and therapeutic guidelines, e.g. for immunoglobulin treatment via the subcutaneous route. In this respect, the existing good cooperation among ESID members with regard to reporting of patients with specific diseases or treatment, e.g. BMT cases, was very helpful and enabled to work on homogeneous and concerted diagnostic and treatment guidelines. It was felt that the positive ESID experience with BMT should be transfered to the treatment of humoral immunodeficiencies. Helen Chapel thanked the industry representatives for the huge amount of previous donations intended to support the ESID Summer School, a proven success story. It was concluded to work together on a list of ESID activities relevant for potential sponsoring by industry (activity/price tag/benefit), as this would enable comparison and differentiation between the various activities. The possibilities but also the open questions of a CRO-like activity were finally considered.

During the Weimar meeting, the General Assembly of ESID took place. As always, only relatively few members attended, which was interpreted by Edvard Smith as a sign that the ESID members are generally satisfied with the Board.

It was once again pointed out by the president that funding of ESID projects has so far been achieved to a substantial part through EU grants donated to individual ESID members involved in the grant activities. Alternative sources of funding for the various ESID activities are actively pursued by the ESID Board, and without a contra votum the Assembly entrusted the Board to continue with these activities. In the Treasurer's Report, which was fully acknowledged by the Assembly, Esther de Vries showed details about the ESID Summer School account (174594,51 Euro) and the regular ESID account (54256,60 Euro); only 244 members have paid their membership fee due for 2002/2003, the remaining members were encouraged to follow as soon as possible. Furthermore, possibilities for new EU grant application(s) within the 6th framework were considered and should be pursued by the Board within the near future. The General Assembly ended with the Reports of the Working Parties, the presentation of Versailles as the next biennial meeting point and the election of Hungary as the next meeting place in 2006, organised by László Maródi.

Hermann WOLF

Treasurer's report

There are many people still who have not paid their ESID membership fee for the years 2002-2003. The Board has decided to send them a last reminder. If payment is not received after that, these people will be deleted from the list of members on the ESID website, and will cease to receive the ESID Newsletter. Of course, all this will be reversed as soon as they pay their membership fee after all. Although this may seem a bit strict, we have no other option. Sending the ESID Newsletter, updating the ESID website, organizing Working Party meetings, and all the other ESID activities: we can't do it for nothing!

Esther DE VRIES

News & Views

Information to ESID members about the second serious adverse event in a clinical trial of gene therapy for X-linked SCID

The ESID Board wishes to inform the ESID members about the different opinions that have been raised following the publication in the media in January 2003 of a second serious adverse event in a clinical trial of gene therapy for X-linked SCID. Information regarding this issue is available on the website of the European Society of Gene Therapy (ESGT) (www . esgt. org) with statements made by the ESGT, by the group of scientists performing the trial at the Hôpital Necker - Enfants Malades in Paris, France, as well as by the FDA, the Paul-Ehrlich-Institut and the German Medical Association (Bundesärztekammer), the

American Society of Gene Therapy and the French Medicine Agency (AFSSAPS).

Hermann WOLF
on behalf of the ESID Board

ESID Summer School, September 25 - 29, 2003, Portugal

This year, ESID will again organise the ESID Summer School, which has been a great success until now. We hope this year's School will be just as much of a success. The faculty will consist of Andrew Cant, Jacques van Dongen, Teresa Español, Anders Fasth, Georg Holländer, Susanna Müller, Gavin Spickett, and Esther de Vries. We cordially invite all young clinicians and investigators who are active in immunodeficiency diseases to consider participating in this event. (More) senior ESID members: please tell your younger colleagues about the School, to enable them to participate!

For further information and application form, please mail to Anders Fasth at anders.fasth @ pediat.gu.se; the deadline for application is May 30, 2003.

Anders Fasth

FOCIS Meeting

The Federation of Clinical Immunology Societies (FOCIS) will meet in Paris next May (15- 19, 2003), for information the web site is: www . focisnet . org/focismeeting.

Invitation to the XIth meeting of ESID in Versailles, France

Dear colleagues and friends,

The XIth meeting of the European Society for Immunology (ESID) together with the VIIIth meeting of the International Patient Organisation of Primary Immunodeficiencies (IPOPI) and the VIth meeting of the International Nursing Group for Immunodeficiencies (INGID) will be held in Versailles from October 21 to 24, 2004.

This will be a unique occasion to present and discuss innovations in the management of immunodeficiencies. Confrontation with the advances in immunology should open new tracks to further develop ideas and projects.

Take the opportunity to live a very unique moment where issues raised by the case of patients with primary immunodeficiencies will be challenged by the utmost scientific discoveries at a time where new ideas and results are blossoming. Come to take profit of the European contribution to this endeavour!

Versailles is a special place, marked by the beauty of classicism art at its top expression. The conference hall is neighbouring the famous Versailles castle and its garden, a place of inspiration you will enjoy to make this meeting a special event!

Welcome to Versailles
in October 2004!

Alain FISCHER

Working Party reports

Report from the BMT Working Party

The Joint ESID / EBMT Inborn Errors Working Party met in Zurich in September 2002. This was hosted by Reinhard Seger, and on the day before the Working Party there was a state-of-the-art symposium on BMT in children. The Working Party was held at the Seehotel Vitznauerhof and was followed by a workshop on the treatment of Hurlers disease by bone marrow transplantation.

Robert Bredius from Leiden spoke of his experience using Busulfex (I.V. Busulphan). In the discussion afterwards, it was mentioned that one should look for chimerism post-BMT and not just engraftment, and others weren't sure that the I.V. drug was any better (or any worse) than oral Busulphan.

Dr Wachowiak spoke of the experience of using Treosulfan in 8 patients transplanted with a genoidentical donor, 6 of whom had a bone marrow transplant for malignancy, 2 for inborn errors (WAS and ALD). All patients engrafted and this early experience was encouraging.

Paul Veys spoke on Great Ormond Street's use of the low intensity regimen using Fludarabine, Melphalan and Campath or ATG in 28 non-SCID BMT's and 5 SCID BMT's; 23 were fully matched, 9 had a one-antigen-mismatch and 1 was a two-antigen-mismatch. The regimen was Fludarabine 30 mg per m^2 per day for 5 days from -7 to –3, Melphalan 90-140 mg per m^2 on day –2, and either ATG 2.5 mg per day from –2 to +2 (19 patients), or Campath-1H 0.2 mg per kg for 5 days from –8 to –5 in 13 patients. The half-life of Campath was about 1 month. It is likely to act also by killing the antigen presenting cells of the host, improving the prevention of GVHD. 31 of the 33 patients survived, 29 of the 31 had T-cell engraftment, and 28 of the 31 had more than 5 % donor myeloid cells (3 patients rejected). They stopped Cyclosporin early, particularly as chimerism was « slipping ». It is important to note the occurrence of frequent infectious among them: 4 EBV-reactivations without LPD, 6 CMV diseases, 9 adenovirus and 1 fatal RSV infection, 1 BK and 5 cryptosporidioses for CD40L deficiency BMT, all in relationship with the administration of CsA. Susannah Matthes-Martin also briefly described their experience of low intensity conditioning in Vienna, where they found that Fludarabine, Melphalan, TLI and ATG was superior to Fludarabine, Busulphan and ATG. It was concluded that this regimen should be considered for MUD transplantation when a good donor (i.e. 10/10 antigen identical) is available.

Isabelle Andre updated the Working Party on allo-depletion studies, mentioned the use of IL-7 as a thymopoetic agent, and her work on the common lymphoid progenitors.

Reinhard Seger and Terry Flood presented very good results following BMT for CGD. 23 out of 27 patients survived, and all the deaths occurred in high risk patients with active fungal disease. Patients with severe active infection or inflammation seemed to have a higher risk of GvHD. Reinhard and Terry have drafted protocols for different groups of CGD patients; low risk with an HLA-id donor received Bu/Cyclo whilst high risk are given Campath as well. For patients receiving an URD BMT Bu/Cyclo and Campath is proposed for low risk patients, but Fludarabine / Melphalan and Campath for high risk patients. Please contact Terry Flood or Reinhard Seger for further details.

Juan Ortega described the 47 patients from 25 centres who had received cord transplants for immunodeficiency diseases. Encouragingly, 41 engrafted, although neutrophil and platelet recovery was slow. 30 % had severe GVHD which was mainly associated with mismatched cords being given. Survival was 70 % overall for those who received matched or one-antigen-mismatched cords, but only 55 % for those who received two-antigen-mismatched cords. The event-free survival was 79 % for SCID, 60 % for Wiskott Aldrich and 45 % for other immunodeficiency diseases.

Fulvio Porta reported the outcome in 4

patients affected by SCID who received an in-utero haploidentical transplantation with CD34+ positively selected cells. One patient with a T-B+ SCID form was grafted at five weeks of gestation, and at birth presented a good T-cell reconstitution but neither B- nor NK-cells. The second one with T-B- NK+ SCID was born with only 10 % donor cells; this patient was re-transplanted twice with a conditioning regimen and he died from GVHD. The third T-B+ NK+ SCID patient presented a T-cell reconstitution without any B-cells, and the last patient affected by an Omenn syndrome presented a poor T-cell reconstitution. In conclusion, a lot of problems remain unsolved and need further deep investigations.

Andy Gennery presented data on the CD40 Ligand bone marrow transplant survey, and Bobby Gaspar on long term follow up of ADA-deficient patients who had received a bone marrow transplant.

Robert Bredius reported their long-term follow-up study of 19 immunodeficiency patients more than 5 years after transplantation; it seems that conditioning was associated with a better quality of immune reconstitution.

Alain Fischer produced data on Chediak Higashi patients who had bone marrow transplants up to 20 years ago for the accelerated phase, and were now developing classical neurological complications as seen in surviving untreated Chediak patients.

He also reported the development of lymphoproliferation / leukaemia in one of the 11 X-SCID patients treated in Paris by gene therapy. As a consequence, the gene therapy programme has been suspended whilst this is further investigated. (By now, a second case has been described, see News & Views; editor)

Four patients affected by X-SCID have been treated by gene therapy by Adrian Thrasher's group in London. Claudio Bordigoni described the results reported in two ADA-deficient children treated by gene therapy following mild conditioning with Busulphan 2 x 2 mg/kg. Some pre-clinical results have also been reported by M. Cavazzana-Calvo and C. von Kalle on the gene therapy of RAG2-deficiency: use of lentivirus and study of the integration sites, respectively.

Future Studies and Protocols of the Working Party include:

- IV Busulphan or oral Busulphan will be used according to centre choice.
- Campath should be used instead of ATG for unrelated donor transplants, but ATG should be retained for haploidentical transplants. In replacing ATG by Campath it will need to be given earlier, but there is still ongoing discussion about the dose and timing.
- There is an opportunity for centres to take part in Isabelle Andre's allo-depletion study. Centres would however have to do their own laboratory work.
- Reinhard Seger and Terry Flood agreed to circulate protocols and to continue collecting data on CGD transplants.
- Andy Gennery and Graham Davies will continue to collect data on CD40 Ligand transplants.
- Paris and Ulm will collaborate on their long term follow up of Artemis and RAG SCID transplants.
- Bobby Gaspar will co-ordinate a study on long-term follow-up of ADA transplants.
- Hülya Özsahin is interested in studying the quality of immune reconstitution and complications following transplantation for Wiskott Aldrich Syndrome.
- Working Party members were asked if anybody had used the Busulphan / Fludarabine / Cyclosphosphamide conditioning regimen advocated for non-identical transplants in osteopetrosis and MHCII-deficiency. It appeared that this regimen had been used for 5 patients without serious toxicity, so it was agreed to continue to use it.
- Robert Bredius agreed to co-ordinate a study on immune reconstitution in SCID patients more than 10 years after transplantation.
- Selim Corbacioglu from Ulm then proposed a study of prophylactic Defibrotide for veno-occlusive disease, suggesting that 25 mg per kg per day was started as prophylaxis from the beginning of conditioning. Technical problems prevented a full discussion of this proposal, but members are invited to contact him if they wish to participate.

The Working Party will meet again at the EBMT meeting in Istanbul (March 2003) and on its own in Paris in September 2003.

The joint effort of this Working Party is still 'working well'! Several Collaborative Studies by Working Group Members were published:

- Antoine C, Müller S, Cant AJ, et al. Long term survival and hematopoietic stem-cell transplantation for immunodeficiencies: a survey of the European experience (1968-1999) The Lancet - in press.
- Seger RA, Gungor T, Belohradsky BH, Blanche S, Bordigoni P, Di Bartolomeo P, Flood T, Landais P, Müller S, Ozsahin H, Passwell JH, Porta F, Slavin S, Wulffraat N, Zintl F, Nagler A, Cant A, Fischer A. Treatment of chronic granulomatous disease with myeloablative conditioning and an unmodified hemopoietic allograft: a survey of the European experience, 1985-2000. Blood 2002;100(13):4344-50.

Andrew J. CANT, Chairman

Report from the Education Working Party

At the ESID meeting in Weimar the Education Working Party was formally formed, even if it has been highly active for the last four years. The driving force has been Helen Chapel, and she has been instrumental by starting the ESID Summer School on Primary Immunodeficiency. The Summer School has been a huge success. The School was held three years on row to meet an accumulated demand.

The concept of a Summer School on PID has also been adopted by the Clinical Immunology Society, and the Americans held their first course in August last year. The present chairman of the Education Working Party served as a bridge between the continents.

At the ESID biennial meeting, Helen Chapel was thanked by warm and long applauds for her outstanding work.

Also, at the meeting in Weimar the Educational Working Party organised a one-day pre-congress Educational Day, which started on Wednesday afternoon and continued up to lunchtime next day. The expected number of participants was around 60. Three times more turned up (!) and not only the young doctors and scientists the day was intended for, but many took the chance to listen to excellent contributions and discussions updating our knowledge on T- and B-cell deficiencies. The presentations during the first half-day concentrated on an update on lymphocyte development and function plus SCID, while the second half-day centered around B-cell development and hypogammaglobulinemia. Both half-days ended with superb demonstrations of illustrative cases.

For this year, the Educational Working Party plans its fourth Summer School, as you can see in a separate advertisement in this ESID Newsletter. The School will be held close to Faro at the Algarve coast of Portugal. As in previous years, the course is intended for young colleagues specialising in primary immunodeficiency diseases: pediatricians, or those involved in any adult medicine speciality, or basic scientists involved in laboratory work-up of PID patients or research in PID. Young is loosely defined as below 35 years of age, and as an ESID activity it is mainly intended for Europeans.

Anders FASTH, Chairman.

Report of the Genetic Working Party

I want to thank the members and the Board of ESID for having given me the opportunity to chair the Genetic Working Party after Mauno Vihinen. It is not easy to succeed Mauno since he did a wonderful job, and, as you may know, he has set up a large database of primary immunodeficiencies (so far there are 36 immunodeficiency mutation databases available at http: // bioinf.uta.fi. Mauno will continue to work on the mutation databases. The update of these data will be crucial for the genetic and clinical analysis of the primary immunodeficiency diseases. At this point, it would be interesting to correlate the molecular results to the clinical phenotype and to the different prognosis.

The patient database system is going to change in the near future. To this end, there is a proposal by Dr. Grimbacher to create an internet database for the ESID main registry. The project is to create a new data bank containing the follow-up data on the already registered patients. In this way, physicians will be able to access the protected website by using a personal password. It will be interesting to link the main registry to different existing registries, which are currently maintained and developed in order to facilitate the registration of the patients.

If you have any suggestions for the Genetics Working Party, please contact me at villa @ itba.mi.cnr.it!

Anna VILLA

Report from the ESID Registry of Primary Immunodeficiencies

1. A letter of introduction from the new Chairman:

Dear Colleagues,

In Weimar, October 2002, I have been elected as successor of Lennart Hammarström for hosting the main ESID patient registry.

My name is Bodo Grimbacher. I am receiving my clinical education at the University of Freiburg, Germany, where I am training in the Department of Clinical Immunology and Rheumatology under the supervision of H.-H. Peter. In 1997, I joined Jennifer M. Puck's laboratory at the National Human Genome Research Institute, NIH, USA, for my postdoc. My project was to conduct genetic linkage analysis in primary immunodeficiencies (PID). Since then, I am involved in the gene-hunt for various PID's, leading to the localisation of the genetic loci for diseases such as the hyper-IgE syndrome, chronic mucocutaneous candidiasis, congenital neutropenia, and, most recently, common variable immunodeficiency (CVID). Recently, I was involved in the identification of ICOS deficiency as a genetic defect causing CVID.

Lennart Hammarström has accomplished an immeasurable work for the registry, and I would like to take this opportunity to thank him in the name of all ESID members and patients. He will continue to be my mentor in the field of databases for PID. Lennart Hammarström accomplished to collect almost 10.000 patients with PID from 26 different European countries for the registry!

I really appreciate your vote, and I will do my best to serve ESID and its patient registry.

Regards, Bodo GRIMBACHER

2. Today's Topic: Shall we put the ESID pa-

tient registry online?

The next task will be to obtain updates on the clinical course, laboratory values, and treatment-outcome on all those collected patients. To reach this goal, the idea was born to put the ESID patient registry online as an internet-based database system. This would facilitate two major achievements: data could be entered any time online, and there would be no further need to mail paperwork, and data could be accessed and analysed any time by any ESID member; data maintenance and accessibility for the community would be greatly improved.

Therefore, we would like to make the following proposal to the ESID community.

Our general purpose is to develop a clinical data management system that fulfills a number of central ideas, all of which are relevant for a modern approach of a multi-centre clinical research database system. The aim is to set up a central data storage which holds data-subsets of every participating centre and presents these data to authenticated users (User-Password authentication - passwords are stored in encrypted form on the server). The system will be accessible via standard internet browsers. The data-presentation depends on the role a specific user occupies on the system and allows or restricts the access (views and rights to write or modify) to the data. Access rights are precisely defined by the head administrator of the system and the administrators of individual centres.

The current ESID patient registry has until now been maintained by Dr. Lennart Hammarström at Huddinge. This database is an intranet solution and runs on a dedicated computer which is not connected to any external network. Therefore, data need to be sent by hardcopy mail and entered in Huddinge.

At the moment, there is no possibility to access the data online. Furthermore, the current database (FileMakerPro) does not implement necessary features a common enterprise database system provides (transaction integrity, data integrity after system breakdown, online backup, online optimisation, scalability, standard SQL data querying, etc.). These would be necessary in a European-wide clinical data management and research system.

Therefore, we propose to develop a system based on an enterprise database system (SAP™DB), which satisfies the particular needs of an internet-based clinical data management and research system able to cope with huge amounts of data.

Data access will be provided through secure (SSL encrypted) internet sessions via standard internet browsers. The server handles these internet requests through a J2EE™ (Java™ 2 Enterprise Edition) server, the web application will be set up under the J2EE™ framework.

The general concept will be as follows. The ESID patient and research registry/database with clinical, laboratory, treatment and research data/information will be capable of assigning any follow-up data to a specific case. The system will be accessible via standard internet browsers. The internet sessions will be encrypted by 128 Bit (1024 Bit keys) SSL encryption. The data will be centrally stored, and can be used for clinical data management, research and even for sponsorship. If allowed by the administrators, data may be provided for external institutions, companies, researchers, etc. The application will be easy to set up. The implementation and integration of new modules will be possible and existing modules and data structures will be modifiable. The system must of course show good performance with huge amounts of data.

Data input can be done manually via encrypted internet sessions or through automated import functionalities from other data storage systems (i.e. local or national patient databases). For manual data entry, the authorised users will get a password to a protected and secure web application and can then enter the new data with 128bit encryption online. For automated import, there will be import interfaces from local and national registries and databases, which are currently maintained and developed (e.g. English, Spanish, Italian, etc.) to this European database to facilitate the registration and documentation of the patients

(eliminating the need to enter patients twice).

With passwords, each user will not only be able to maintain and update the data, but also to view and analyse accessible data. Access is restricted by a user-role concept. A user occupies a specific role on the system. The administrators can precisely define access rules to the existing roles.

If any user likes to analyse additional patients, he/she needs to contact the database administrator with an inquiry to obtain a corresponding user-role. The database administrator will contact the respective centre to get the approval. It now is at the centres discretion if it allows the access of its data by the inquiring user. However, a subset of the data (like the diagnosis, the gender, the age at diagnosis, the immunoglobulin levels at diagnosis, and the information if this is a sporadic or a familial case) will be available for all registered users at all times.

There are special concerns according to clinical data accessible through the internet. We therefore need to guarantee data security by defining a data-set through which direct identification of individual cases (patients) is impossible. Each documenting centre needs to get approval for this data-set by its own data protection officer. There needs to be a written consent form signed by each patient or their legal representatives. For this purpose, the centre in Freiburg, Germany, can provide a patient consent form which has already been approved by the data protection authorities of the state Baden-Württemberg.

The purpose is to build a standardised system that shall be developed and validated by the standards set by FDA CFR 21 Part 11 for Good Clinical Practice and ISO 9001 for Quality Control.

The system will be set up as a client-server system. The clients will be thin-clients (i.e. no further installations and configuration are necessary except the existence of a standard internet browser able to handle encrypted internet sessions). The server consists of a database system (SAP™DB) and a web-application server. The database system must fulfill specific needs, data-holding in the context of clinical data management requests (transaction integrity, data integrity even after break-down, online backup, etc.). The web application server will be J2EE™ (Java™ 2 Enterprise Edition) conform. The application itself will fulfill the Model-View-Controller (MVC) principle and thus handles different stages during data request and view processes through different modules. Standard components are defined by XML-Documents that determine layout, data requests and data modification tasks. Furthermore, the web application server handles user-role authentication, session management, database connections and encrypted internet sessions. The server shall be physically situated in a secure server net that restricts the access to the server through a validated firewall.

The function of the system can be differentiated into four points:

Clinical data management:

- Manual data input
- Viewing data (patient- and population-specific)
- Generating clinical reports
- Exporting reports and views into standardised output formats (RTF, CSV, SQL)
- Automatic import and export of data from and to other database systems and networks

2. Set-up-Installation-Configuration:

- Clear and easy Set-up- and Build-Process through XML- and Java™-Technology
- New (complex) components can be integrated through JavaServlets™ and JavaServerPages™ Technologies

3. Research:

- Central storage of data from different centres
- Viewing and analysis of huge populations not restricted to one centre (if allowed by administrators)
- Connecting University research and pharmaceutical industry:

providing valuable data for mercantile purpose

To make this also a financial success, we need to be prepared to answer queries of the pharmaceutical companies. Our database

will allow to search the data for specific questions. However, if the datafield the companies are interested in has not been documented, there is no use of such a database. Therefore, we need to know from the companies (i.e. the IG producing companies) what their questions to such a database will be, so that we can design fields for those items to be documented. Then we'll be able to sell the data to the companies and make money for ESID with the registry. This money may also be used to give the documenting centres an incentive to document their patients, and thereby increase the quality of the data.

We would like to receive your input on

Report of the Clinical Working Party

The Clinical Working Party has been active with three projects during the past months: extension of the diagnostic guidelines, drawing up of treatment guidelines (Wiskott-Aldrich syndrome questionnaire), and establishing a European diagnostic protocol for the suspicion of immunodeficiency. We would like to thank all of you who reacted until now, and those of you who didn't: feel free to do so still!

We are still working on the treatment guidelines and the diagnostic protocol for the suspicion of immunodeficiency. On the next pages you will find our proposals for diagnostic guidelines of several primary immunodeficiency diseases. Please send us your comments before March 31st at casanova @ necker.fr. !!

Jean-Laurent CASANOVA

Hyper-IgM due to mutation of the CD40 gene

Definitive

Male or female patients with an immunological phenotype compatible with an hyper IgM syndrome (IgG and IgA concentration at least 2 SD below normal for age, and normal or elevated levels of IgM) and one of the following:

- absent expression of CD40 molecule on lymphocyte surface by flowcytometric analysis.
- absent expression of CD40 protein in lymphocytes by western blot analysis.
- mutation in CD40 gene.

Probable

Male or female patients with an immunological phenotype compatible with an hyper IgM syndrome and all of the following:

- normal number of circulating T-cells and normal or elevated number of circulating B-cells.

parental consanguinity

- normal lymphocyte proliferative response to mitogens.
- defective IgG antibody response to vaccines.
- clinical history with one or more of the following infections or complications:

* recurrent bacterial infections in the first five years of life.
* opportunistic infections (i.e. Pneumocystis carinii) usually occurring in the first few years of life
* neutropenia
* cryptosporidium-related diarrhea.

Possible

Male or female patients with serum IgG and IgA levels at least 2 SD below normal for age, normal T- and B-cell counts and normal lymphocyte proliferative responses to mitogens, and one or more of the following:

normal/elevated IgM serum levels.

opportunistic infections early in infancy.

bacterial infections in the first five years of age.

growth delay.

Spectrum of disease

Very few patients with hyper IgM syndrome due to mutation of the CD40 gene have been reported so far. The clinical spectrum is characterized by recurrent bacterial infections in the first five years of life, and by opportunistic infections early in infancy (Pneumocystis carinii pneumonia and Cryptosporidium-related diarrhea). Neutropenia and remarkable eosinophilia in the absence of overt infections have been also reported.

Exclusion criteria

Multiple affected males in multiple generations suggest X-linked hyper-IgM syndrome or NEMO deficiency. The latter is also associated with clinical features of hypohydrotric ectodermal dysplasia. Defects in T-cell activation (i.e. defective expression of CD69 or CD25 after in vitro T cell stimulation) and proliferation are suggestive of a T-cell immunodeficiency. Secondary immunodeficiencies (HIV, congenital infections) should also be excluded.

Alessandro PLEBANI

Inherited disorders of the IL-12 - IFNγ axis

Definitive

Male or female patients with impaired production of, or response to, IL-12 or IFNγ and one of the following:
- mutations in *IL12B* (AR, loss of function).
- mutations in *IL12RB1* (AR, loss of function).
- mutation(s) in *IFNGR1* (AR, loss of function or hypomorphic; AD, loss of function and dominant-negative).
- mutations in *IFNGR2* (AR, loss of function or hypomorphic).
- mutation(s) in STAT1 (AR, loss of function; AD, loss of function and dominant-negative).

Probable

Male or female patients with impaired production of, or response to, IL-12 or IFNγ and one of the following:
- clinical disease caused by BCG vaccine.
- clinical disease caused by an environmental *Mycobacterium*.
- clinical disease caused by *Mycobacterium tuberculosis*.
- clinical disease caused by *Salmonella*.
- clinical disease caused by other intra-cellular pathogens (bacteria, fungi, parasites, viruses).

Possible

Male or female patients with disseminated and/or recurrent infection caused by one of the following micro-organisms:
- BCG.
- environmental Mycobacteria.
- non-typhi Salmonella.

Spectrum of disease

Infections caused by poorly virulent Mycobacteria and Salmonellae are the hallmarks of inherited disorders of the IL-12 - IFNγ axis. Infections caused by other intra-cellular agents are much less frequent. A high level of allelic and non-allelic genetic heterogeneity accounts for the considerable phenotypic variability in terms of age of onset of infection (infancy, adulthood), severity of infection (local, disseminated; with or without recurrence), granuloma structure (tuberculoid, lepromatous-like), and clinical outcome (benign, lethal).

Jean-Laurent CASANOVA

Diagnostic Criteria for LAD II

Definite

A male or female patient with decreased intensity of expression of CD15 (SleX) or other fucosylated glycoproteins on leukocytes (less than 5% of normal) and:
- mutation in tne FUCT1 gene.

Probable

A male or female patient with defective expression of CD15 on leukocytes and all of the following:
- persistent leukocytosis (neutrophil count above 20,000).
- severe growth retardation.
- severe mental retardation.

Possible

Infant with marked leukocytosis (neutrophil count above 10,000) and one of the following:
- recurrent infections.
- growth retardation.
- mental retardation.
- specific facial features.

Spectrum of Disease

Marked leukocytosis and severe growth and mental retardation exist in all patients. Facial features include: puffy eyelids, depressed and wide nasal bridge and prominent mandible. The severity of the infections tends to decrease with the years. The syndrome is a general defect in fucose metabolism due to a specific mutation in the fucose transporter to the Golgi apparatus. In a few cases supplementation of oral fucose may be benificial.

Amos ETZIONI

Omenn Syndrome

Definitive

Male or female infant patient with severe erythrodermia, hepatosplenomegaly, and lymphadenopathy who has one of the following:

- high levels of IgE, absence of B-cells, markedly reduced proliferative response to mitogens, and presence of oligoclonal, activated autologous T-cells
- mutations in Rag1 or Rag2 (Recombination activating gene) allowing a partial V(D)J recombination activity

Probable

Male or female patient with severe eczema, hepatosplenomegaly, and lymphadenopathy, and with at least one of the following:

- recurrent or severe infections
- absence of B-cells

Possible

Male or female patient with erythrodermia or severe eczema, high serum levels of IgE and normal response to mitogens, who has one of the following:

- hepatosplenomegaly
- lymphadenopathy
- failure to thrive

Spectrum of Disease

The presence of immunodeficiency and a severe skin rash, due to the infiltration of activated oligoclonal T-cells, is the diagnostic hallmark of Omenn Syndrome. Patients show the symptoms of the disease very early in life, the median age at onset is 4 –6 weeks. Most patients develop hepatosplenomegaly, chronic diarrhea, and lymphadenopathy, often accompanied by recurrent infections, alopecia and failure to thrive.

Serum concentrations of IgE are usually elevated, whereas the levels of IgM and IgG can be low. White blood cell counts are normal or, in half the cases, increased through the presence of eosinophilia. B-cell counts are significantly decreased (less than 2%), while T-cell counts are elevated. T-cells show an activated phenotype, with low or absent responses to mitogens. The analysis of the T-cell repertoire demonstrates oligoclonality. The molecular analysis of Rag-genes shows the presence of a hypomorphic mutation on at least one allele, which allows a very low level of V(D)J recombination activity.

Differential Diagnoses

- severe atopic dermatitis
- SCID with T-cell maternal engraftment
- histiocytosis X
- other syndromes with immune disregulation (IPEX syndrome)
- DiGeorge syndrome

Anna VILLA

Following soon:

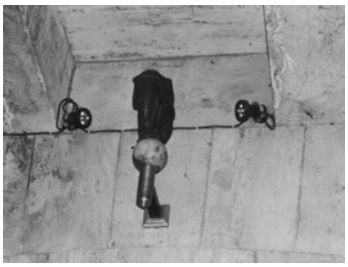
- hyper-IgM syndrome type 2
- autosomal recessive agammaglobulinemia
- IgG-subclass deficiency (+/- IgA deficiency)

Please let us know your comments and suggestions, by sending an email to the editor and/or the author of the Guideline in question before March 31st! (emailaddress see www . esid . org)

Report of the ESID Meeting in Weimar

The Xth Meeting of the ESID took place in Weimar, Germany from 16th to 20th October 2002. The Meeting was attended by more then 600 participants from 40 different countries from all 5 continents!

A total of 250 abstracts were submitted. The meeting started off with an Educational Symposium, providing state-of-the-art information in the field of T-cell and B-cell deficiencies. In several Plenary Sessions, presentations on normal lymphocyte development, homeostasis and regulation provided an excellent forum to present and discuss corresponding immunodeficiency disease states. A number of workshops gave opportunities to deal with several important and partially controversial topics. It was unfortunate that the proportion of oral presentations had to be limited in order to prevent parallel sessions. All abstracts were published in the ESID Newsletter (2002 – Supplement), and can be found on the ESID website as well (www . esid . org). The list of participants can be found on the website as well, thus facilitating communication and exchange of ideas. The city of Weimar proved to be a superb Meeting place, and the many attractive cultural and historical aspects of the city were highly appreciated by all.



Wilhelm FRIEDRICH

Invited review

FLOWCYTOMETRIC ANALYSIS OF THE PRECURSOR B-CELL COMPARTMENT IN BONE MARROW FOR GUIDING MOLECULAR DIAGNOSTICS IN IMMUNODEFICIENT CHILDREN

Mirjam van der Burg,[1] Jeroen G. Noordzij,[1] Nico G. Hartwig,[2] Ronald de Groot [2] and Jacques J.M. van Dongen.[1] Depts. of Immunology[1] and Pediatrics,[2] Erasmus MC, University Medical Center Rotterdam, The Netherlands

Introduction

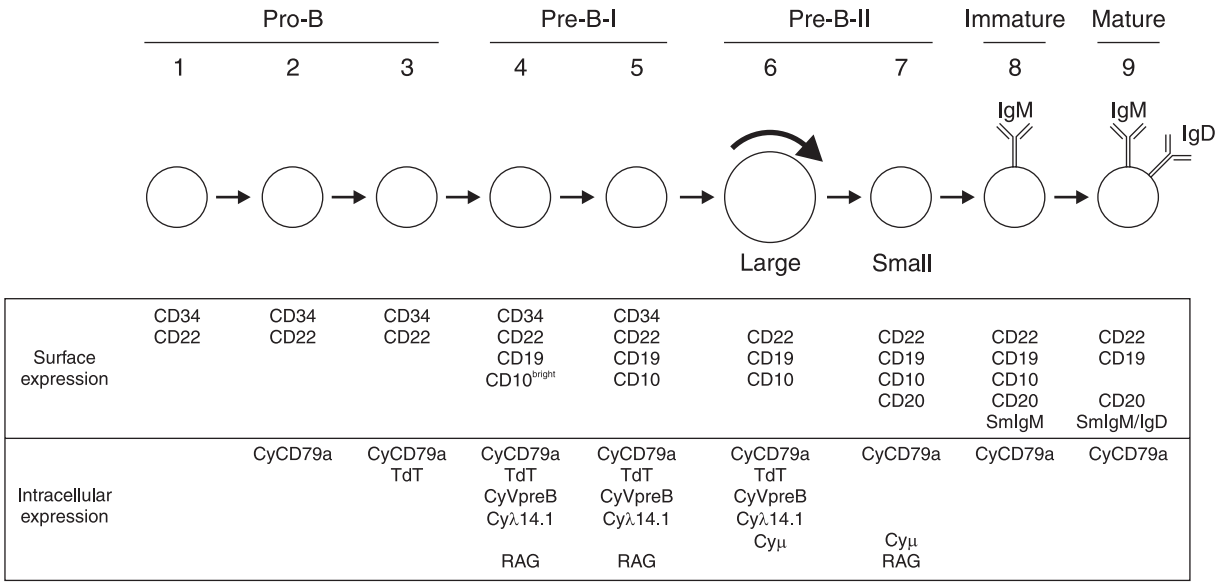
During recent years, several mutated genes have been identified, which are involved in the pathogenesis of primary immunodeficiency diseases (PID).[1] This has resulted in the possibility of making a molecular diagnosis in many PID patients. However, the frequency of most gene defects is low. In addition, PID are characterized by heterogeneous clinical pictures in patients with identical gene defects, but also by identical clinical pictures caused by different gene defects. Therefore, it is important to apply the relatively expensive molecular techniques in a selective way. The diagnostic process in PID patients can generally be subdivided in three steps: (1) careful description of the clinical picture of the patient and classification based on type and location of infections and on laboratory results, (2) flowcytometric analysis of peripheral blood cells, and (3) molecular analysis of a candidate gene.[2]

During the last few years, we developed a new protocol for flowcytometric immunophenotyping of the precursor B-cell compartment in bone marrow, which can contribute to the diagnostic process of PID cases with a B-cell defect, especially agammaglobulinemia and B-cell negative severe combined immunodeficiency (SCID). Firstly, we analyzed a number of bone marrow samples of healthy donors to get insight in the composition of the normal precursor B-cell compartment and the relative size of the various precursor B-cell subpopulations.

Flowcytometric analysis of the precursor B-cell compartment in bone marrow of healthy controls

B-cell differentiation in the bone marrow occurs in sequential steps, starting with early precursor B cells eventually leading to the generation of mature B lymphocytes.[3-6] Using cell sorting and single-cell PCR, Ghia *et al.* have characterized several precursor B-cell differentiation stages (pre-B-I cells, cycling pre-B-II cells, resting pre-B-II cells, immature and mature B cells), based on cytoplasmic (Cy)VpreB, CyIgm and RAG expression.[3] Because cell sorting and single cell PCR are complex and time-consuming techniques, which cannot be implemented routinely, we have adapted this differentiation scheme using additional markers. These additional markers can be subdivided into lineage-specific pan-B-cell markers (CD22, CyCD79a, and CD19), and stage-specific markers (CD34, CD10, CD20, and TdT). This approach allowed us to discriminate nine differentiation stages (Figure 1).[7,8]

The pro-B-cell fraction was defined as $CD19^-$ and this fraction could be further divided based on CyCD79a and TdT expression (stage 1-3, Figure 1). Pre-B-I cells were defined as $CD19^+$, $CD10^+$, TdT^+, $CyVpreB^+$ and $CyIgm^-$ and were further subdivided on basis of the level of CD10 expression (stage 4 and 5). Pre-B-II cells were defined as $CD19^+$, $CD10^+$, TdT^-, $CD34^-$, $CyIgm^+$ in combination with presence or absence of CyVpreB expression, respectively (stage 6 and 7). Pre-B-II cells in stage 7, which are $CyIgm^+$, but $CyVpreB^-$, were also recognized by Schiff *et al.* and probably represent non-cycling small pre-B-cells which have upregulated RAG expression, allowing rearrangement of the Ig light chain genes.[4] However, TdT expression was



Surface expression	CD34 CD22	CD34 CD22	CD34 CD22	CD34 CD22 CD19 CD10^{bright}	CD34 CD22 CD19 CD10	CD22 CD19 CD10	CD22 CD19 CD10 CD20	CD22 CD19 CD10 CD20 SmIgM	CD22 CD19 CD20 SmIgM/IgD
Intracellular expression		CyCD79a	CyCD79a TdT	CyCD79a TdT CyVpreB Cyλ14.1 RAG	CyCD79a TdT CyVpreB Cyλ14.1 RAG	CyCD79a TdT CyVpreB Cyλ14.1 Cyμ	CyCD79a Cyμ RAG	CyCD79a	CyCD79a

Figure 1. Hypothetical scheme of precursor B-cell differentiation stages in bone marrow from healthy children under age of 16. The distinction between large and small pre-B-II cells and expression of RAG proteins were deduced from Ghia *et al.*,[3] Schiff *et al.*,[4] and Rolink *et al.*,[5]. The expression patterns of the other markers were determined in studies by Noordzij *et al.*[7,8]

beyond detection during this stage.[4] Immature B-cells were defined as CD19$^+$, CD10$^+$, surface membrane bound (Sm)IgM$^+$, and SmIgD$^-$ (stage 8), whereas mature B-cells are CD19$^+$, CD10$^-$, SmIgM$^+$ and SmIgD$^+$ (stage 9).

At this moment, we use twelve quadruple labelings to analyze the composition of the precursor-B-cell compartment.[8] The analyses are performed within CD22$^+$, CyCD79a$^+$ and CD19$^+$ B-cell gates. To ensure that the B-cell gates include B-lineage cells only, we added a mix of CD3, CD16 and CD33 monoclonal antibodies to exclude T cells, NK cells, granulocytes and other myeloid cells. In addition, an antibody against CD36, which is expressed on platelets, mature monocytes and macrophages, and some macrophage-derived dendritic cells, is added to some essential labelings, to further improve the purity of the B-cell gates.

Figure 2 shows two examples of the flowcytometric labelings, which illustrate the composition of the precursor B-cell compartment.[7] The expression patterns of two molecules are analyzed within a CD19$^+$ B-cell gate. Within this CD19$^+$ gate, regions were defined representing different subpopulations (from immature to mature as indicated with an arrow). In the CD10/CD20 dot plot, five subpopulations could be discriminated: CD10^{++}/CD20$^-$; CD10$^+$/CD20$^-$; CD10$^+$/CD20$^{+/-}$; CD10$^+$/CD20$^+$; and CD10$^-$/CD20$^+$ (Figure 2A). In the CyIgm/SmIgM dot plot, three subpopulations could be discriminated: CyIgM$^-$/SmIgM$^-$, CyIgM$^+$/SmIgM$^-$; en CyIgM$^+$/SmIgM$^+$ (Figure 2B).

The relative distribution of the different subpopulations can be summarized in a bar diagram (Figure 3A). The size of the mature B-cell population in the bone marrow varies and is dependent on the amount of peripheral blood contamination with CD10$^-$/SmIgM$^+$/SmIgD$^+$ B-lymphocytes. For this purpose we choose to omit stage 9 from our further calculations and focused on stage 1 to stage 8, which were set together at 100%.

Flowcytometric analysis of the precursor B-cell compartment in bone marrow of patients with agammaglobulinemia

Bone marrow samples of nine X-linked agammaglobulinemia (XLA) patients, with proven

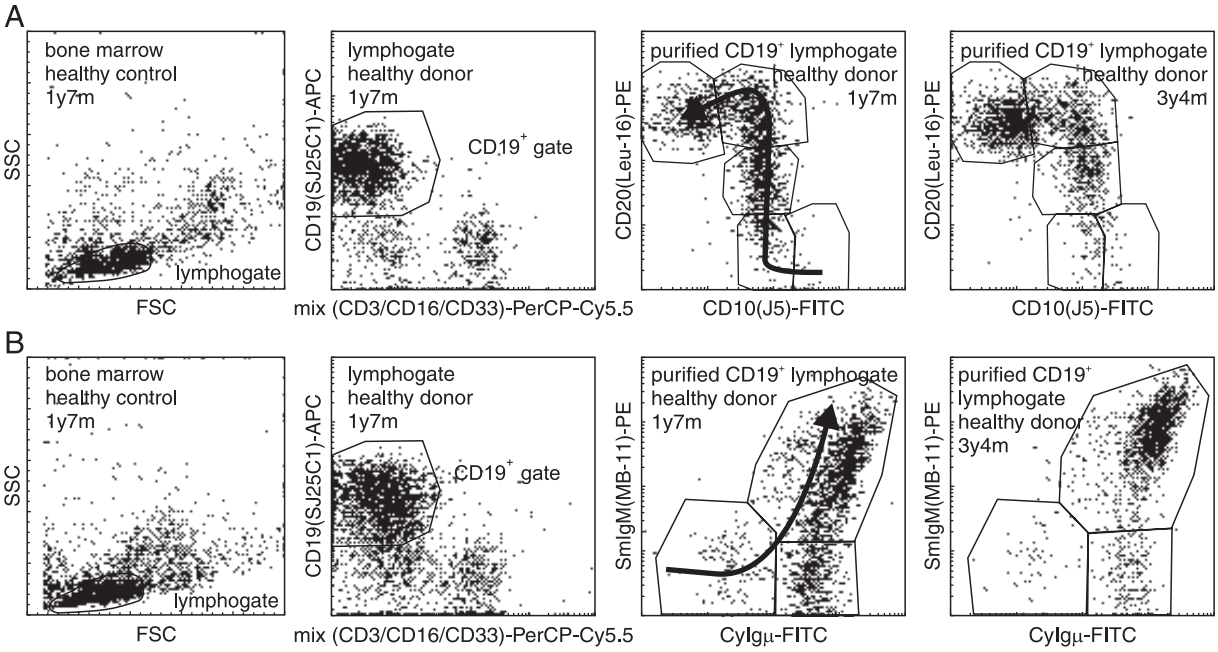


Figure 2. Flowcytometric immunophenotyping of bone marrow of a healthy donor. The composition of the precursor B-cell compartment was analyzed within a lymphogate and a $CD19^+$ B-cell gate with exclusion of cells positive for CD3, CD16 and/or CD33. The cells in the purified $CD19^+$ lymphogate were subsequently analysed for expression of CyIgm and SmIgM **(A)**, and CD10 and CD20 **(B)**. The order of the B-cell differentiation stages is indicated with an arrow.

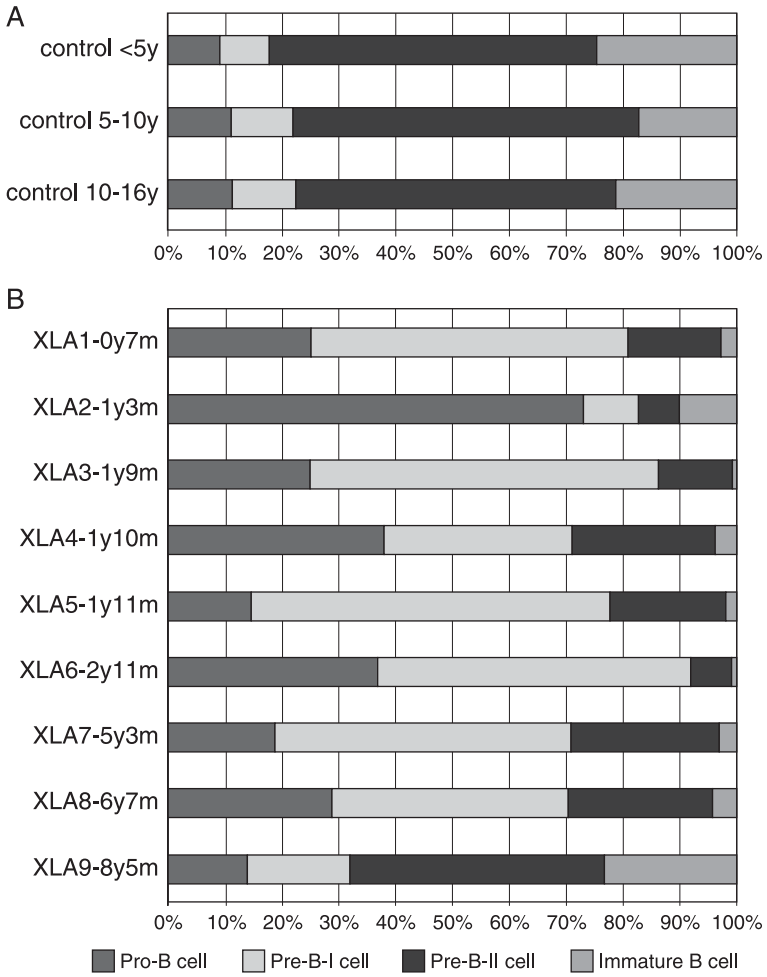


Figure 3. Composition of the precursor B-cell compartment in healthy children (A) compared to XLA patients (B). The precursor B-cell compartment (stage 1-8) were set at 100%.[7]

BTK mutations, were analyzed according to the above-described protocol. *BTK* mutations in man result in a 'leaky' block before the pre-B-II stage, which implies that some cells pass this block and can appear (in low numbers) in the peripheral blood (Figure 3B).[7] The degree of leakiness appeared to vary between the different patients (Figure 4). Especially XLA patient 9, with a splice-site mutation in the *BTK* gene, showed a high degree of leakiness. In a next study, we analyzed whether the type of mutation correlated with the degree of leakiness (Figure 3B and Figure 4). This study focused on XLA patients with *BTK* splice-site mutations and showed that low levels of wild type *BTK* transcripts can be present in such cases.[9] However, in the eight studied patients, the presence of low levels of wild-type *BTK* transcripts did not show a clear correlation with the percentage, absolute number, or immunophenotype of B-lymphocytes nor with age or serum immunoglobulin levels at diagnosis.[9]

Analysis of the precursor-B-cell compartment in XLA patients showed a heterogeneous pattern of the distribution between the different subsets, which is largely due to different levels of leakiness. Based on these studies and on the fact that 90-95% of boys with agammaglobulinemia have a *BTK* mutation, we recommend that these patients are directly subjected to molecular analysis of the *BTK* gene.

However, in cases of autosomal recessive agammaglobulinemia, mutations might be found in several genes, including *IGHCM, CD79A, l14.1,* and *BLNK*.[10] The protein expression of most of these genes can be assessed by immunophenotyping of the bone marrow precursor B-cell compartment. The type of differentiation block and the absence of protein expression of certain genes may direct further molecular studies. Via this approach we could identify patients with a defective *IGHCM* gene (Figure 5) or a defective *CD79A* gene (manuscript in preparation).

Flowcytometric immunophenotyping in the diagnostics of B-cell negative SCID

Detailed flowcytometric immunophenotyping of the bone marrow precursor B-cell compartment might also be informative in the diagnosis of B-cell negative SCID patients. $T^-/B^-/NK^+$ SCID patients generally have a defect in the recombinase enzyme system leading to the inability to rearrange the immunoglobulin (Ig) and T-cell receptor (TCR) genes, which is essential for the generation of antigen-specific B- and T-cell receptors. Such defects result in a complete block *before* the pre-B-II cell stage ($CyIgM^+$) (Figure 5). Mutations in the recombinase activating genes (*RAG1* and *RAG2*) are the most frequent defects found in $T^-B^-NK^+$ SCID. However, not all $T^-B^-NK^+$ SCID patients suffer from mutations in the *RAG* genes. It has been shown that a subgroup of these patients are sensitive to ionizing radiation.[11] A part of the radiosensitive $T^-B^-NK^+$ SCID patients was proven to suffer from defects in DNA double strand break repair caused by mutations in the recently discovered *Artemis* gene.[12] Analysis of the precursor B-cell compartment in *Artemis*-negative patients also showed a differentiation arrest before the pre-B-II cell stage ($CyIgM^+$).[13] Immunogenotyping, i.e. PCR analysis of the Ig gene rearrangements in bone marrow cells, could further determine residual recombinase activity in *RAG*-negative and *Artemis*-negative SCID patients, as assessed by the presence of certain Ig gene rearrangements.[8,14]

$T^-/B^-/NK^-$ SCID patients can show a completely different B-cell differentiation pattern with the presence of all differentiation stages, but with a prominent decrease of the more mature B-cell stages (Figure 5). $T^-/B^-/NK^-$ SCID is generally caused by a mutation in the adenosine deaminase (*ADA*) gene, resulting in the accumulation of toxic metabolites of nucleic acids, which are particularly toxic for mature lymphocytes.

Conclusion

Flowcytometric analysis of the precursor B-cell compartment in the bone marrow can play a crucial role in guiding molecular diagnostics of primary immunodeficiencies. Based on our

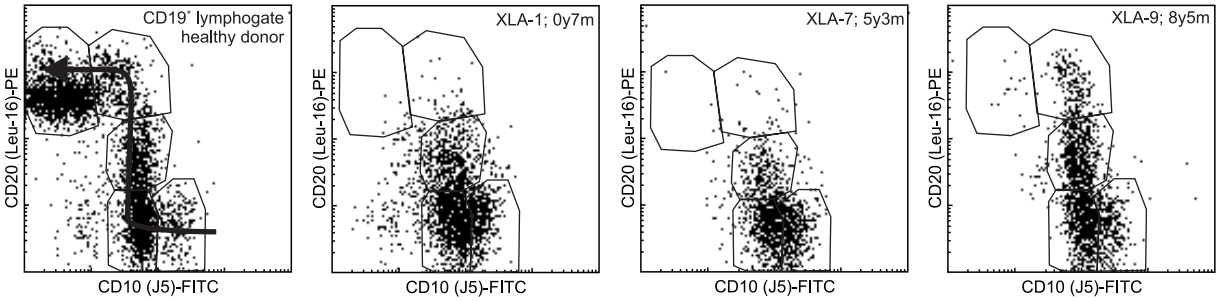


Figure 4. Flowcytometric analysis of the precursor B-cell compartment in bone marrow of a healthy donor and three XLA patients. The precursor B-cells were evaluated for their CD10 and CD20 expression profile within a CD19$^+$ lymphogate (see Figure 2 for details). Patients XLA1 and XLA7 showed a severe reduction of the more mature B-cell differentiation stages (CD10$^+$/CD20^{bright} and CD10$^-$/CD20^{bright}), whereas patient XLA 9 (with a splice-site mutation) contained a prominent CD10$^+$/CD20^{bright} B-cell subset in his bone marrow.[7,9]

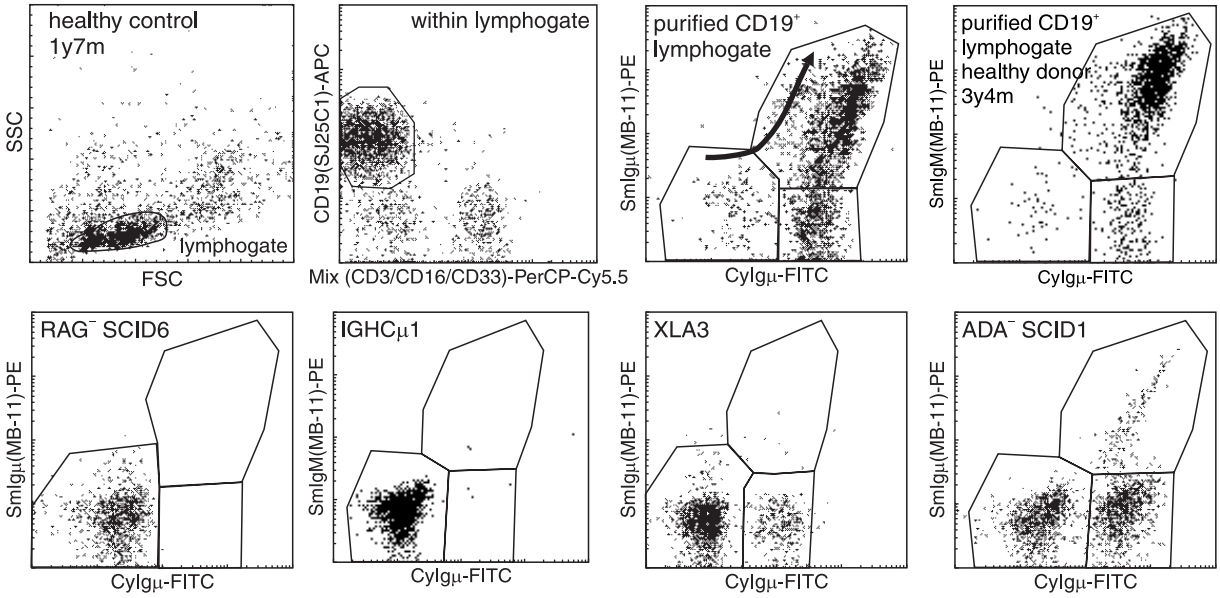


Figure 5. Flowcytometric analysis of bone marrow of two healthy donors as compared to patients with a *RAG*, a *IGHCMU*, *BTK* and a *ADA* gene defect. In healthy control the vast majority of the precursor B-cells were SmIgM$^-$/CyIgm$^+$ or SmIgM$^+$/CyIgm$^+$, which was in full contrast to the four immunodeficient patients in the lower panels, which showed different types of differentiation blockades, dependent on the type of mutated gene.

combined flowcytometric and molecular studies, we recommend to start with analysis of the *BTK* gene in boys with agammaglobulinemia. However, if no *BTK* mutation is found or if an autosomal recessive agammaglobulinemia might be present, flowcytometric immunophenotyping of the precursor B-cell compartment in the bone marrow can be very informative.

In SCID patients, both immunophenotyping and immunogenotyping are important tools to define and to understand the molecular defects, possibly resulting in new insights in the recombination process.

Acknowledgements

We are grateful to our colleagues in many European institutes for providing cell samples of agammaglobu-

linemia and SCID patients: C.M.R. Weemaes, Nijmegen, NL; A. Lankester, J.M.J.J. Vossen, M. Z. Zdzienicka and W. Wiegand, Leiden, NL; T.W. Kuijpers, Amsterdam, NL; E. de Vries, 's Hertogenbosch, NL; L.A.M. Sanders, N. Wulfraat and T. Revesz, Utrecht, NL; B. Gerritsen, Rotterdam, NL; S. van Lierde, Leuven, BE; E. Bernatowska, M. Pac, and B. Wolska, Warsaw, PL; D. Kowalczyk, Cracow, PL; L. Marodi, Debrecen, HU; C. Heilmann, Kopenhagen, DK; W. Friedrich, Ulm, DE; D. Wolf-Eichbaum, Minden, DE; J. Litzman, Brno, CZ; I. Tezcan, Ankara, TR; E. Pachanov, Moscow, RUS; A. Sediva, Praag, CZ; E. Savilahti, Helsinki, SF; I. Reisli, Konya, TR; and P. Klemetti, Helsinki, FI. We would like to thank mrs. W.M. Comans-Bitter for making the figures.

Contact information

Dr. M. van der Burg PhD, mirjam.vanderburg @ erasmusmc.nl
Prof. dr. J.J.M. van Dongen, M.D., PhD, j.j.m.vandongen @ erasmusmc.nl

References

1. Fischer A. Primary immunodeficiency diseases: an experimental model for molecular medicine. Lancet. 2001;357:1863-9.
2. De Vries E, Noordzij JG, Kuijpers TW, van Dongen JJM. Flow cytometric immunophenotyping in the diagnosis and follow-up of immunodeficient children. Eur J Pediatr. 2001 ;160 :583-91.
3. Ghia P, ten Boekel E, Sanz E, de la Hera A, Rolink A, Melchers F. Ordering of human bone marrow B lymphocyte precursors by single-cell polymerase chain reaction analyses of the rearrangement status of the immunoglobulin H and L chain gene loci. J Exp Med. 1996;184:2217-29.
4. Schiff C, Lemmers B, Deville A, Fougereau M, Meffre E. Autosomal primary immunodeficiencies affecting human bone marrow B-cell differentiation. Immunol Rev. 2000;178:91-8.
5. Rolink AG, Schaniel C, Andersson J, Melchers F. Selection events operating at various stages in B cell development. Curr Opin Immunol. 2001;13:202-7.
6. Nomura K, Kanegane H, Karasuyama H, Tsukada S, Agematsu K, Murakami G, Sakazume S, Sako M, Tanaka R, Kuniya Y, Komeno T, Ishihara S, Hayashi K, Kishimoto T, Miyawaki T. Genetic defect in human X-linked agammaglobulinemia impedes a maturational evalution of pro-B cells into a later stage of pre-B cells in the B-cell differentiation pathway. Blood. 2000;96:610-617.
7. Noordzij JG, De Bruin-Versteeg S, Comans-Bitter WM, Hartwig NG, Hendriks RW, De Groot R, Van Dongen JJM. Composition of the precursor B-cell compartment in bone marrow from patients with X-linked agammaglobulinemia compared to healthy children. Pediatr Res. 2002;51:159-68.
8. Noordzij JG, De Bruin-Versteeg S, Verkaik NS, Vossen JMJJ, De Groot R, Bernatowska E, Langerak AW, Van Gent DC, Van Dongen JJM. The immunophenotypic and immunogenotypic B-cell differentiation arrest in bone marrow of RAG deficient SCID patients corresponds to residual recombination activities of mutated RAG proteins. Blood. 2002;100:2145-52.
9. Noordzij JG, De Bruin-Versteeg S, Hartwig NG, Weemaes CM, Gerritsen EJA, Bernatowska E, Van Lierde S, De Groot R, Van Dongen JJM. XLA patients with *BTK* splice-site mutations produce low levels of wild-type *BTK* transcripts. J. Clin. Immunol. 2002;22:306-18.
10. Gaspar HB, Conley ME. Early B cell defects. Clin Exp Immunol. 2000;119:383-9.
11. Nicolas N, Moshous D, Cavazzana-Calvo M, Papadopoulo D, de Chasseval R, Le Deist F, Fischer A, de Villartay JP. A human severe combined immunodeficiency (SCID) condition with increased sensitivity to ionizing radiations and impaired V(D)J rearrangements defines a new DNA recombination/repair deficiency. J Exp Med. 1998;188:627-34.
12. Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, Le Deist F, Tezcan I, Sanal O, Bertrand Y, Philippe N, Fischer A, de Villartay JP. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. Cell. 2001;105:177-86.
13. Noordzij JG, Verkaik NS, Van der Burg M, Van Veelen LR, De Bruin-Versteeg S, Wiegant W, Vossen JMJJ, Weemaes CMR, De Groot R, Zdzienicka MZ, Van Gent DC, Van Dongen JJM. Radiosensitive SCID patients with Artemis gene mutations show a complete B-cell differentiation arrest at the pre-B-cell receptor checkpoint in bone marrow. Blood. 2003;In press.
14. Noordzij JG, Verkaik NS, Hartwig NG, De Groot R, Van Gent DC, Van Dongen JJM. N-terminal truncated human RAG1 proteins can direct T-cell receptor but not immunoglobulin gene rearrangements. Blood. 2000;96:203-9.

Focus on a country:

Established member Q & A
László MARÓDI
University of Debrecen,
Debrecen, Hungary

Can you give me some information about your background ?

I was born in Bököny, an East-Hungarian and catholic village, and lived there until I was 14. I went to grammar school in Nagykálló in 1964, and to medical school to Debrecen in 1968. I received my doctor of medicine degree from the University School of Medicine in Debrecen in 1974, and in 2003 I am still working there, currently as the Head of the Department of Infectiology and Pediatric Immunology. Klára, my wife, is a dermatologist; we have two great children, Klári and Laci. I like mountains and poetry.

Can you tell me something about your career history, and how you became interested in immunodeficiency?

Debrecen is not only a city open to the world, but also a center for clinical immunology. After my training in pediatric immunology in Debrecen, I spent a year in Leiden, in Ralph van Furth's group, and studied functional and biochemical characteristics of cord blood phagocytic cells. This work resulted in a 'Leiden' Ph.D. thesis in 1984. This stay in Holland determined my later carreer! Firstly, because I fell in love with phagocytes, and my research interest is still concentrated on these fascinating cells that orchestrate natural immunity. Secondly, because Ralph introduced me to Jaak Vossen who was interested in the pathogenesis of primary immunodeficiency diseases and saving lives of patients without adaptive immunity by BMT. Importantly, both Ralph and Jaak were physicians as well as scientists.

When I came back from Leiden, I established a clinical immunology group, and set up a research lab in Debrecen. When I was ready with the work, I organized a Workshop on primary phagocytic cell deficiencies and invited EGID to Debrecen, in 1988.

Postdoctoral studies followed in the lab of Richard B. Johnston, Jr, in Philadelphia, Steven M. Holland at NIH, and Siamon Gordon in Oxford. All these were very important to further develop the level of pediatric immunology in patient care and research in Debrecen.

What have been your achievements in research and patient care in the field of immunodeficiencies?

My approach and scope to immunodeficiency is much wider than just primary immunodeficiencies. There are so many patients with deficient immune function! Immunodeficiency doesn't only mean large groups of patients, but it is also a way of thinking when we deal with patients. Every individual passes through developmental immaturity of the immune system. Consequently, all of us were or are severely immunodeficient in early life. Our colleagues from neonatology and general pediatrics do not always pay attention to this developmental immaturity of the immune system. They should, however, because neglecting this immunodeficiency can be as harmful to the baby as inappropriate ventilation.

I did a lot of research to better understand the pathophysiology of neonatal host defense against bacteria and fungi. The immaturity of mononuclear phagocytes, in contrast to granulocytes, was already defined in my PhD work. In collaboration with the groups of Dick Johnston and Gábor Szabó I was able to define the molecular basis more precisely and concluded that neonatal macrophages cannot be fully activated with interferon (IFN)-γ, the most potent macrophage-activating agent in vivo. This deficiency can be explained by impaired signaling through the IFN-γ receptor as indicated by decreased STAT-1 phosphorylation. The hyporesponsiveness to activation by IFN-γ may have broad implication and it explains, at least in part, the lack of appropriate Th1-type responses in early infancy.

Another line of research we did with Dick Johnston relates to functional and molecular mechanisms of host defense against candida species, formidable pathogens in patients with various immunodeficiency disorders. This research revealed that macrophages use the mannose receptor to recognise candida, and that resistance to phagocyte-derived toxic compounds may explain differential pathogenicity of various candida species. Our data on modulation of macrophage candidacidal function in newborns and adults by proinflammatory cytokines may provide a basis for clinical trials in patients with invasive candidiasis. A continuation of these studies with Rita Káposzta, Rosangela da Silva, and Siamon Gordon revealed morphologic characteristics of C. albicans-related pathogenicity.

Our research on the mode of action of IVIG concentrates, the life-saving medication for patients with B-cell deficiency, revealed that IgG molecules initiate respiratory burst activation in resting granulocytes. We found with Imre Szabó and Ágnes Kalmár that this effect can be inhibited by monoclonal antibodies to FcγRII and FcγRIII receptors. We believe that Fc receptor binding may be involved in the immunomodulatory effect of IVIG in patients with inflammatory and autoimmune diseases.

The macrophage mannose receptor is the link between our research interest in macrophages and Gaucher disease, a lysosomal storage disorder (the recombinant enzyme used to treat Gaucher patients is taken up by macrophage mannose receptors). A research project on biochemical characteristics of macrophages in type 1 Gaucher patients has been running for 10 years now in my lab. We described that glucocerebroside inhibits NADPH oxidase activity in a specific fashion, which may explain the deficient superoxide release and microbicidal capacity of macrophages from Gaucher patients.

We have been running a pediatric immunology clinic and inpatient Division in Debrecen for twenty years, which is now part of our Department. This is not only a PID clinic but includes patients with various autoimmune and inflammatory disorders. I am among those European pediatricians who consider immunology as one of the most important areas of pediatrics, which is overlapping and intervening with infectious diseases. As such, I am an advocate for the ESID/ESPHI/ESPID Training Program in Pediatric Infectious Diseases and Immunology at CESP. It is unbelievable that in 2003 we need to argue with pediatricians in CESP that immunology is not just a lab discipline but a very important clinical area of pediatrics. Our Department, and a couple of others in Europe, are living examples of this.

Together with Kálmán Nagy and Pál Megyeri, I have been running a Pediatric Immunology working group in Hungary since 1985. This is the most important professional group in the country to concert diagnostic and treatment activities for the benefit of immunodeficient children. We achieved that all patients with immunoglobulin deficiency in the country receive IVIG free of charge, and there are two pediatric centers for BMT, one in Miskolc, run by Kálmán Nagy, and one in Budapest, run by Gergely Kriván and previously by László Timár. There is no limitation of C1 esterase supply for Hungarian patients with this complement defect.

What kind of developments in immunodeficiency do you expect in the near future?

The last decades have been a remarkable era of progression in pathogenesis and treatment of immunodeficiencies. In genetic defects, I expect new vectors/carriers to target genes to stem cell DNA. Viral vectors may be substituted by chemical carriers, which will open new ways for gene therapy. More sofisticated therapeutic regimens should replace the robust immunoreconstitution by BMT. In developmental and secondary immunodeficiencies, targeted immuno-augmentation and immunomodulation can be expected to occur. Cytokine therapy is one example.

As a pediatric immunologist in Debrecen, I have local expectations and responsibilities. Hungary is a small country in East-Central Europe and it is difficult to limit our profes-

sional responsibilities inside geographic borders. Therefore, we worked out a program to establish an East-Central European PID center with molecular diagnostic facilities. The program was highlighted in Debrecen, last year at the International Symposium on PID attended by internationally recognized speakers from all over the world and 60 doctors from East-European countries. Important part of the program is the ESID-related EURO-PID-NAS project coordinated by Edvard Smith and supported by the EU in the coming two years. I anticipate significant progress in terms of recognition and management of patients in this large area of Europe. Additional support from other societies, e.g. ESPHI, and additional European and USA sources would add special strength to our East-European initiative.

What is your advice for young people who want to launch their career in immunodeficiency?

For PhD's I advice to work together with clinicians on their immunodeficiency research project. Research by PhD's and MD's are not interchangable but complimentary. The "so what" has to do with the questions that MD's ask which are different from those that the PhD's ask.

For MD's, I advice to work together with PhD's. In addition, I recommend for them to become clinician-scientists. Simply because our patients with immunodeficiencies need "bench-to-bedsite" doctors who understand the whole spectrum of the particular disease they have. There is no other way you can manage PID patients. You cannot stay in the clinical dimension of the disease because you will loose your way and will not be able to fulfill the expectations of the patients and their families. This holds true for tertiary care pediatricians in general, and for pediatric immunologists, in particular. Your professional scope of knowledge should cover the whole spectrum of pathology and molecular biology from disease manifestation to the gene defect.

It is very important for immunologist MD's to do full-time biological research early in their career and keep an eye, or preferably a hand, on what is going on in the lab throughout their career. PhD's are enormously important but they are educated differently from that of MD's. That is why clinician-scientists are needed.

To lead a clinician-scientist life is not an easy thing, and you may develop a kind of professional split personality what "pure clinicians" do not have. It requires a kind of person who likes a bridge, who likes going back and forth between the bench and bedside in both directions.

And — last but not least — what does ESID mean to you?

ESID is an intellectually stimulating and friendly company of bright people from all over the world. For me, it is like a family to which it is assuring to belong to. ESID is a predictable background for professional activity and research in the field of PID; it is a balanced combination of clinical responsibility and research progression on a society dimension.

Young investigator Q & A

Árpád LÁNYI
University of Debrecen
Debrecen, Hungary

Can you give me some information about yourself and your background?

I was born in Budapest, Hungary. I am the father of two "energetic" boys of three and six. The family spent nine years in the US. We moved back home about a year ago. At present, I live and work in Debrecen and it feels like home by now.

Can you tell me something about your career history?

I had my first degree in Biology and Chemistry at the Eotvos University in Budapest. In 1991, I got a scholarship sponsored by the European Community and studied Biotechnology at the University of Newcastle upon Tyne, Newcastle (UK). I received my PhD at the University of Nebraska Medical Center in Omaha, Nebraska, USA in Prof. Janos Sumegi's laboratory, where I worked on the positional cloning of the X-linked lymphoproliferative disease (XLP) gene. At that time, it was already clear that to solve some of the mysteries of the disease the defective gene had to be identified. After a rather long quest, in 1998, the XLP gene was cloned by three groups independently. Since then, I have been working on the characterization of the function of the protein (SH2D1A/SAP) that is defective or missing in XLP boys. An active collaboration has developed between Janos Sumegi's lab and prof. Cox Terhorst's lab who found the gene by a functional approach. In 2000 I was fortunate to spend four months in the prof. Terhorst's lab as a visiting researcher, working on the characterization of protein interactions of SAP and kinases of the src family protein tyrosine kinases. In 2001, I was offered an Assistant Professor position at the Institute of Immunology of the University of Debrecen, Health Science Center headed by Prof. Eva Rajnanolgyi. Soon, my natural interest in primary immunodeficiencies brought me together with prof. László Maródi, whose vision of creating a regional immunodeficiency center in Debrecen with molecular diagnostics for rare immunodeficiencies is appealing to me, as it will be to the benefit of basic scientists, as well as clinicians, and patients of course.

How did you become interested in immunodeficiencies?

It was inevitable, working in a department lead by such a devoted person as the late David Purtilo, but I could continue the list with many other physicians and scientists working in Omaha. In addition, XLP, like other immunodeficiencies (or perhaps even more so) holds clues to better understand basic questions in immunology, cell biology or even in microbiology.

For example, signaling by SLAM (Signaling Lymphocyte Activation Molecule), a co-receptor found on activated T-cells and antigen presenting cells is defective in XLP patients. Unexpectedly, SLAM was found to be the "true" receptor for the measles virus.

What have been your achievements in patient care and/or immunodeficiency research?

The Sumegi lab was part of the International XLP Consortium that identified the XLP gene. Later, still in the Sumegi lab I found that SAP can bind directly to the T-cell specific form of the src kinase fyn, which raised the possibility that SAP works as an adaptor protein in SLAM signaling. During my visit in prof. Terhorst's laboratory the adaptor concept was further developed, and in collaboration with the laboratory of Dr. Michael Eck at the Dana Faber Cancer Institute in Boston, SAP was shown to be the first single SH2-domain protein simultaneously interacting with a receptor (SLAM) and a src-kinase (Fyn).

What do you hope to achieve in the future?

As to basic research, I would like to study the role of SAP and the SAP homologue EAT-2 in the regulation of T-helper cell polarization, primarily by investigating the role of these proteins in dendritic cell function. Through this I hope to better understand the delicate balance between the immune system and the Epstein-Barr virus in healthy individuals. In addition, I would like to contribute to the development of the regional center for immunodeficiency research and diagnostics here in Debrecen.

How are you planning to reach this goal?

Everyone does it by regular hard work. Good planning and decision making is essential too. One needs to exchange ideas with the right people. So I will try to maintain active collaboration with my mentors, co-workers and other scientists in my general research area. I also work with enthusiastic, talented people. This is the plan, basically.

And - last but not least - what does ESID mean to you?

Just as I said, exchange of information is vital to good research. ESID is a great forum for this. In addition, as a new member I can already see that ESID has developed to be a real community.

What would you want to change if you were president of ESID?

I need a lot more time to see how it (ESID) works, perhaps I should keep the readers in suspense until then!