Sanquin

Spring Seminars
19 & 20 April 2007

Antibodies in Disease, Diagnosis, and Treatment

Sanquin Spring Seminars

Antibodies in disease, diagnosis and treatment

Chairman: Prof. Lucien Aarden PhD, Amsterdam, The Netherlands

19 & 20 April 2007

Royal Tropical Institute, Amsterdam, The Netherlands

Special support is given by Fresenius Kabi, Transfusion Technology Division, Emmer Compascuum, The Netherlands; Roche Diagnostics Nederland B.V., Almere, The Netherlands.

Genmab, Utrecht, The Netherlands; Haemonetics B.V., Breda, The Netherlands; Hemocue, Waalre, The Netherlands; Tecan Benelux B.V.B.A, Giessen, The Netherlands; Sanquin Blood Supply Foundation, Amsterdam, The Netherlands.

www.sss.sanquin.nl

Scientific Committee

Lucien Aarden PhD (chairman)
Ernest Briët MD PhD
Dirk Roos PhD
C. Ellen van der Schoot MD PhD
Marinus van Oers MD PhD

Organizing Committee

Ernest Briët, Chairman
Ingeborg van der Heijden
Fatima Muntar
Jan Willem Smeenk

Seminar Secretariat

Eurocongres Conference Management
J. van Goyenkade 11
1075 HP Amsterdam, The Netherlands
Tel: +31-20-679.3411, Fax: +31-20-673.7306
sanquin@eurocongres.com
http://www.sss.sanquin.nl

Venue

Royal Tropical Institute
Mauritskade 63
1092 AD Amsterdam
The Netherlands
Tel: + 31-20-568.8698, Fax: + 31-20-668.4579

Scientific programme, Thursday 19 April 2007

09.00 hrs: Registration, coffee & tea

10.30 hrs: Welcome & opening by Ernest Briët, Amsterdam, The Netherlands

Session I: Clinical Aspects of Immunoglobulins

Chair: Jos van der Meer, Nijmegen, The Netherlands

10.35 – 11.10 hrs: Critical steps in B-cell Differentiation and Antibody Production: What can we learn from Antibody Deficiencies?
Jacques van Dongen, Rotterdam, The Netherlands

11.10 – 11.25 hrs: Intravenous Immunoglobulins Reduce Allogeneic T-cell Activation after Liver Transplantation by Modulating the Interaction between Dendritic Cells and NK-CELLS
Thanyalah Tha-In, Rotterdam, The Netherlands

11.25 – 11.40 hrs: Apoptosis: A Target for Potentiation of UV-Induced Interleukin Receptor Antagonist (il1-ra) Synthesis by Intravenous Immunoglobulins in the Perspective of New Treatment
Etienne Dupont, Brussels, Belgium

11.40 – 12.15 hrs: Natural Antibodies, Auto Antibodies and Complement Activation in Tissue Injury
George Tsokos, Boston, USA

12.15 – 14.00 hrs: Lunch break / Visit to the exhibition and posters

Session II: Antibody Response in Man

Chair: Cees van Kooten, Leiden, The Netherlands

14.00 – 14.35 hrs: Humoral Immuneresponse to RhD
C. Ellen van der Schoot, Amsterdam, The Netherlands

14.35 – 14.50 hrs: Differential Regulation of Humoral Autoimmunity Versus Alloimmunity in Rheumatoid Arthritis
Onno Teng, Leiden, The Netherlands

14.50 – 15.25 hrs: Title: to be announced
Antonio Lanzavecchia, Bellinzona, Switzerland

15.25 – 16.00 hrs: Coffee & tea break / Visit to the exhibition and posters

Scientific programme, Friday 20 April 2007

08.45 – 09.30 hrs: Registration, coffee & tea

Session IV: Monoclonal antibodies, a new class of drugs

Chair: Lou de Leij, Groningen, The Netherlands

09.30 – 10.05 hrs: Mechanisms of Antibody Therapeutics for Cancer
Paul Parren, Utrecht, The Netherlands

10.05 – 10.20 hrs: Extreme Variability in Clearance of RH D-Positive Red Blood Cells by Monoclonal and Recombinant RH D Antibodies
Belinda Kumpel, Bristol Institute of Transfusion Science, Bristol, United Kingdom

10.20 – 10.55 hrs: Nanobodies™ as New Therapeutic Entities
Hans de Haard, Gent, Belgium

10.55 – 11.30 hrs: Coffee & tea break /
Visit to the exhibition and posters

Session III: Structural Aspects of Immunoglobulins

Chair: Paul Parren, Utrecht, The Netherlands

16.00 – 16.35 hrs: The physiological generation of bispecific IgG4 antibodies
Rob Aalberse, Amsterdam, The Netherlands

16.35 – 16.50 hrs: Expression of Recombinant Human IGM Anti-K in the Presence or Absence of J Chain and the Effect on Serological function
Jacqueline Gilmour, Bristol Institute for Transfusion Science, Bristol, United Kingdom

16.50 – 17.25 hrs: Functional Properties of Antibodies for Diverse Applications
Sherie Morrison, Los Angeles, USA

17.30 – 21.00 hrs: Visit to the posters & drinks and conference buffet

20.00 hrs: Poster award ceremony

Session V: Antibodies against pharmaceuticals

Chair: Jan Voorberg, Amsterdam, The Netherlands

11.30 – 12.05 hrs: The Antibody Response to Therapeutic Monoclonal Antibodies
Lucien Aarden, Amsterdam, The Netherlands

12.05 – 12.20 hrs: Identification of Humira-specific T Cell Epitopes
Josine van Beek, Amsterdam, The Netherlands

12.20 – 12.55 hrs: The Antibody Response to Blood Coagulation Factor VIII
Pete Lollar, Atlanta, USA

12.55 – 14.30 hrs: Lunch break / visit to the exhibition and posters

Session VI: Antibodies and inflammation

Chair: Erik Hack, Leiden, The Netherlands

14.30 – 15.05 hrs: Intravenous Immunoglobulins: more then a mere transfer of Antibodies
Srini Kaveri, Paris, France

15.05 – 15.20 hrs: FCRN: An Igg Receptor on Phagocytes with a Novel Role in Phagocytosis
Gestur Vidarsson, Amsterdam, The Netherlands

15.20 – 15.55 hrs: The Influence of Antibody Glycosylation on IVIG Activity
Falk Nimmerjahn, Erlangen, Germany & New York, USA

15.55 hrs: Closing remarks & farewell reception

Abstracts of Sessions I – VI

Session I Thursday 19 April, 10.35

J. van Dongen, MD PhD, Erasmus Medical Center, Rotterdam, The Netherlands

Critical steps in B-cell Differentiation and Antibody Production: What can we learn from Antibody Deficiencies?

T. Tha-In, H.J. Metselaar, H.W. Tilanus, Z.M.A. Groothuismink, P.M. van Hagen, G. Geert, E.J. Kuipers, R.A. de Man, J. Kwekkeboom, Erasmus MC, Rotterdam, The Netherlands

Intravenous Immunoglobulins Reduce Allogeneic T-cell Activation after Liver Transplantation by Modulating the Interaction between Dendritic Cells and NK-cells

We have shown that intravenous immunoglobulins (IVIg) reduce the incidence of acute rejection after liver transplantation from 31% to 13% and suppress the allogeneic T-cell priming by dendritic cells (DC). Here, we investigated the mechanism by which IVIg prevent immune activation after liver transplantation.

Human DC, NK-cells and T-cells were isolated from blood of healthy individuals. DC were stimulated with TNFα/IL1 in absence or presence of IVIg. IVIg were then removed and allogeneic NK-cells were added. NK-cell phenotype and apoptosis of DC were determined by flowcytometry. T-cell priming capacity of DC was assessed by culturing DC with allogeneic T-cells with or without NK-cells using 3H-thymidine incorporation and CFSE-dilution techniques. *Ex vivo* changes in peripheral blood leukocyte populations were monitored in patients treated with IVIg (N=11).

DC matured in presence of IVIg (IVIg-DC) activated allogeneic NK-cells and increased their interferon-γ production, compared to control-DC. Subsequently, the activated NK-cells induced apoptosis of IVIg-DC, as shown by increased Caspase-3 expression and increased 7-AAD staining (IVIg-DC: 33 ± 9% 7-AAD positive, control-DC: 17 ± 8%, $p<0.01$). In presence of NK-cells, IVIg-DC were impaired in their allogeneic T-cell priming capacity by 81 ± 15% ($p<0.05$) compared to control-DC. This was due to NK-cell mediated Antibody Dependent Cytotoxicity (ADCC) to IVIg-DC, which can be abrogated by blockade of FcγRIII on NK-cells. This effect of IVIg could be mimicked by aggregates of a humanized monoclonal antibody, indicating that ADCC of DC is restricted to multimers in IVIg preparations. Furthermore, IVIg-DC promoted in vitro expansion of CD56brightCD16-CCR7+ lymph node type NK-cells, which correlated with a decrease in the numbers of circulating NK-cell after IVIg-treatment.

In conclusion, IVIg reduce the incidence of acute rejection after liver transplantation by promoting NK-cell mediated ADCC of DC, which subsequently reduces the allogeneic T-cell priming. By modulating the early control switch of antigen-presentation, IVIg can prevent T-cell activation, and may therefore be a promising candidate for future non toxic immunosuppressive regimen after liver transplantation.

R. Laub[1], L. Craciun[2], M. Di Giambattista[1], M. Goldman[2], E. Dupont[2]

[1.] CAF-DCF Red Cross, Brussels, Belgium

[2.] ULB Erasme, Department of Immunology, Brussels, Belgium

Apoptosis: A Target for Potentiation of UV-Induced Interleukin Receptor Antagonist (il1-ra) Synthesis by Intravenous Immunoglobulins in the Perspective of New Treatment

Background

Besides classical activators of IL-1Ra production, such as bacterial lipopolysaccharides and granulocyte-macrophage colony-stimulating factor, immunoglobulins G and anti-D immunoglobulins stimulate IL-1Ra secretion both in vitro and in vivo. Likewise, therapeutic intravenous immunoglobulin (IVIG) infusions have been shown to activate IL-1Ra production in patients. When administered as a drug in the recombinant form, IL-1Ra, which prevents IL-1-induced inflammatory signalling, displays a protective effect against graft rejection and graft-versus-host disease. This effect can also be achieved by pharmacological activation of endogenous IL-1Ra production. UV light and IVIG have been shown to increase monocyte/macrophage IL-1Ra secretion. The aim of this study was to determine optimal in vitro conditions for induction of IL-1Ra secretion, with a view to finding ways to improve the efficacy of photochemotherapy and IVIG treatment. As both agents induce lymphocyte apoptosis, we focused our analysis on the influence of IVIG on UV-induced IL-1Ra production on this mechanism.

Materials and Methods

After overnight pre-incubation at 37, UVC-irradiated peripheral blood lymphocytes (PBL) mixed with IVIG at two concentrations (1 and 25 mg/ml) (Multigam, CAF-DCF, Brussels) were cocultured with autologous peripheral blood mononuclear cells. Apoptosis was measured by annexin-V, necrosis by propidium iodide detection. IL-1Ra and IL-10 secretion were evaluated by the reverse-transcriptase polymerase chain reaction and the Luminex-microbead-array assay.

Results

A significant additive dose-dependent influence of IVIG (+85%; p=0.0005) on UV-induced IL-1Ra secretion involved both Fc-receptor activation at low dosage (1 mg/ml) and a potent apoptotic action on PBL at high concentration (25 mg/ml), reinforcing the UV effect.

Conclusion

Lymphocyte apoptosis represents an important pathway contributing to enhancement by IVIG of UV-induced monocyte/macrophage IL-1Ra production, mostly when high doses are used. Combining UV and IVIG therapy could be a powerful way to improve treatment efficacy in cases of immunological disorders, acquired or not, such as graft-versus-host disease or arthritis.

Session I Thursday 19 April, 11.40

G.C. Tsokos, MD, Beth Israel Deaconess Medical Center, Harvard Medical School

Natural Antibodies, Autoantibodies and Complement Activation in Tissue Injury

Activation of complement represents an effector mechanism of intestinal ischemia/reperfusion injury. Mice deficient in complement receptors 1 and 2 fail to produce a component of the natural antibody repertoire that binds to ischemia-conditioned tissues and activate complement. In contrast, mice prone to autoimmunity display accelerated and enhanced tissue injury that results from the binding of autoantibodies to injured tissues. Antibody avidity and concentration determine the magnitude of tissue damage. Our experiments demonstrate that naturally occurring antibodies and autoantibodies mediate tissue injury only after an organ has been subjected to a stressor such as ischemia.

Session II Thursday 19 April, 14.00

C. E. van der Schoot, MD PhD, Sanquin Research, Amsterdam, The Netherlands

Humoral Immuneresponse to RhD

Session II Thursday 19 April, 14.35

Y.K.O. Teng[1], R.J. Verburg[1], K.N. Verpoort[1], G.W.J. Diepenhorst[2], I. Bajema[1], M. van Tol[1], E. Jol[1], R.E.M. Toes[1], T.W.J. Huizinga[1], J.M. van Laar[1]

[1.] Leiden University Medical Center, Leiden, The Netherlands

[2.] Sanquin Research, Amsterdam, The Netherlands

Differential Regulation of Humoral Autoimmunity versus Alloimmunity in Rheumatoid Arthritis

Purpose

Circulating autoantibodies are a characteristic phenomenon of autoimmunity in rheumatoid arthritis (RA). The chronic mechanisms underlying the production of RA-specific autoantibodies are unknown. The present study investigated antibody responses towards exogenous and endogenous antigens in RA patients, who were treated with high dose chemotherapy followed by autologous stem cell transplantation (HDC+HSCT). HDC+HSCT is an experimental therapy for severe, refractory RA patients and is specifically targeted to eliminate proliferating cells.

Methods

Eight RA patients treated with HDC+HSCT were followed for up to 2 years after treatment. The effects of HDC+HSCT on circulating B-cell and T-cell counts were measured by flowcytometry. Serum titers of total immunoglobulins, exogenous antibodies (IgM-phosphoryl-choline (IgM-PC) and IgG-rubella (IgG-RL)) and autoantibodies (IgM-rheumatoid factor (IgM-RF) and IgG-cyclic citrullinated peptide (IgG-CCP)) were measured before treatment and during follow-up. Additionally, avidity of IgG-tetanus toxoid (IgG-TT) and IgG-CCP were measured to analyze neo- and memory responses in normal immune responses compared to autoimmune responses.

Results

In 3 out of 6 patients titers of ACPA-IgG were nearly completely eradicated after HDC+HSCT (before median 215 AU/mL to nadir median 34 AU/mL ; p=0,05). One patient had persistent seroconversion of ACPA-status. This contrasted with the stable titers of the Rubella-IgG. HDC+HSCT also significantly reduced IgM titers of both RF-IgM autoantibodies (p=0,043) as well as PC-IgM (p=0,043). Importantly, serum titers of total immunoglobulin were also affected by treatment, notably IgM. The reduction in RF-IgM paralleled the reduction of total IgM ($r = 0,74$; $p< 0,001$), whereas the effects on ACPA-IgG correlated only weakly with total IgG titers ($r = 0,21$; $p=0,04$), indicative of non-selective depletion of RF-IgM.

To further unfold the pathologic mechanism of ACPA-IgG responses, we measured avidity of serum ACPA-IgG during follow-up and of serum tetanus toxoid IgG (TT-IgG). Before immunoablation, a wide range of ACPA-IgG avidity was measured (mean SEM: 1,11 ± 0,46). After immunoablation, in 3 of 3 patients the ACPA-IgG avidity had decreased (mean SEM: 0,65 ± 0,28), but more importantly, in all (6 out of 6) patients rises in ACPA-IgG titers were dominated by the emergence of low avidity autoantibodies (mean SEM: 0,80 ± 0,44). These drops in avidity of ACPA-IgG indicated that reactivation of ACPA autoimmunity was derived from an immature humoral immune . This observation was substantiated by the avidity of TT-IgG alloantibodies in the same patients which remained stable after immunoablative therapy (pre-treatment mean SEM: 3,04 ± 0,17 and post-treatment: 2,93 ± 0,17). Following repeated immunizations, serum titers of TT-IgG increased as expected, yet the avidity of TT-IgG remained stable (mean SEM after 1st boost: 2,56 ± 0,33; after 2nd boost: 2,53 ± 0,38; after 3rd boost: 2,63 ± 0,22), indicative of an intact, matured memory response despite HDC+HSCT

Conclusion

The present study provides evidence for the differential regulation of long-term humoral immunity to alloantigens versus autoantigens in RA. Whereas long-lived humoral alloimmunity depends upon memory responses, humoral autoimmunity is a continuous process with recruitment of autoreactive B cells that can be selectively targeted with immunoablative treatment.

Session II Thursday 19 April, 14.50

A. Lanzavecchia, Institute for Research in Biomedicine, Bellinzona, Switzerland

Title: to be announced

Session III Thursday 19 April, 16.00

R.C. Aalberse, PhD, Sanquin Research, Amsterdam, The Netherlands

The physiological generation of bispecific IgG4 antibodies

Immunoglobulin G4 (IgG4) antibodies have been known for some time to be functionally monovalent. We proposed a structural basis for this monovalency: the in vivo exchange of IgG half-molecules (one H-plus one L-chain) among IgG4. Such a process results in bispecific antibodies that in most situations will behave as functionally monovalent antibodies. We assumed that this abnormal behaviour of IgG4 was largely the result of a single amino acid change relative to human IgG1: the change of a proline in core hinge of IgG1 to serine. This results in a marked shift in the equilibrium between interchain disulphide bridges and intrachain disulphide bridges, which for IgG4 results in 25-75% absence of a covalent interaction between the H-chains. Because of strong non-covalent interactions between the CH3 domains IgG4 is a stable four-chain molecule and does not easily exchange half-molecules under standard physiological conditions *in vitro*. We postulated that the exchange is catalysed *in vivo* by protein disulphide isomerase (PDI) and/or FcRn (the major histocompatibility complex (MHC)-related Fc receptor) during transit of IgG4 in the endosomal pathway in endothelial cells. Because IgG4 is predominantly expressed under conditions of chronic antigen exposure, the biological relevance of this exchange of half-molecules is that it generates antibodies that are unable to form large immune complexes and therefore have a low potential for inducing immune inflammation. In contrast to monovalent immunoglobulin fragments, these scrambled immunoglobulins have a normal half-life. The significance of the ensuing bispecificity needs further evaluation, because this will be relevant only in situations where high IgG4 responses are found to two unrelated antigens that happen to be present in the body at the same time and place. In this context the significance of IgG4 autoreactivity might have to be re-evaluated. The main function of IgG4, however, is presumably to interfere with immune inflammation induced by complement-fixing antibodies, or, in the case of helminth infection or allergy, by IgE antibodies.

Session III Thursday 19 April, 16.35

J.E.M. Gilmour, S.J. Pittman, R.J. Nesbitt, M.L. Scott,
Bristol Institute for Transfusion Science, Bristol, United Kingdom

Expression of Recombinant Human IgM Anti-K in the Presence or Absence of J Chain and the Effect on Serological function

The ability to directly agglutinate red blood cells is a desirable property in a blood-grouping antibody as it simplifies the diagnostic assay. However, many of the blood-group specific monoclonal antibodies that have been produced are IgG isotype, which cannot cross-link the RBCs directly. The polymeric immunoglobulin IgM has the ability to cross-link but the isotype is not often isolated during hybridoma production. It would therefore be useful to convert IgG with the desired specificity and affinity into an IgM isotype for use as a diagnostic reagent. This work describes the construction of a human recombinant IgM anti-K by grafting the variable regions of an IgG anti-K heavy chain onto an IgM heavy chain constant region and co-expressing with the appropriate light chain. The construction was simplified by not co-expressing a third polypeptide normally associated with IgM, the J chain. The J chain is not required for the polymerisation of IgM, but it has a role in stabilising the pentameric form. The human IgM construct was transfected into two rodent cell lines: NS0, which expresses mouse J chain, and CHO, in which J chain is absent. Recombinant IgM was expressed from both lines and it was shown that both produced multimeric IgM that could directly agglutinate Kell positive RBC, demonstrating that the specificity of the parent IgG antibody was retained.

Mouse J chain was shown to interact with the recombinant human IgM produced in NS0. It has been previously shown by others (e.g. Randall et al. 1992 J.Biol. Chem. 267 18002-7) that, in the absence of J chain, IgM can form structures larger than the normal pentamer. This could have an effect on the serological properties of the antibody; for example, larger structures such as hexamers could interfere with antigen binding but, alternatively, higher valency could result in higher functional affinity and better cross-linking. Comparison of the two rIgM showed that there was no significant difference in their serological ability whether formed in the presence or absence of J chain. The work demonstrates that, for diagnostic purposes, recombinant human IgM can be successfully produced from either cell line without the need to isolate and co-express human J chain.

Session III Thursday 19 April, 16.50

S.L. Morrison, University of California, Los Angeles, USA

Functional Properties of Antibodies for Diverse Applications

Antibodies have properties that make them uniquely appropriate for numerous applications. They can be used to treat malignancy and infectious disease and can be used as vehicles to specifically deliver agents containing targets recognized by their binding sites. The functional properties of an antibody are determined both by the amino acid sequence of its heavy and light chains and by the structure of its attached glycans. The presentation will discuss the contributions of different amino acid sequences and carbohydrate structures to antibody function and will address the role of the properties of an antibody in determining its functional efficacy.

Session IV Friday 20 April, 9.30

P.W.H.I. Parren, Genmab, Utrecht, The Netherlands

Mechanisms of Therapeutic Antibodies for Cancer

Epidermal growth factor receptor (EGFr) over-expression is common in a large number of solid tumors and represents a negative prognostic indicator. Over-expression of EGFr is strongly tumor-associated, and this receptor tyrosine kinase is considered an attractive target for antibody therapy. Zalutumumab - a human IgG1 EGFr antibody - blocks EGF binding, interferes with cellular signaling and efficiently recruits effector cells for antibody-dependent cell-mediated cytotoxicity (ADCC). Mechanisms of action for Zalutumumab were further delineated at the molecular level and in animal models. Our studies indicate that HuMax-EGFr has two therapeutic mechanisms. First, it blocks tumor growth by arresting EGFr in an inactive conformation which results in an efficient inhibition of signaling. Second, induction of ADCC represents an additional effector mechanism, which is effective at relatively low dose and is likely to be important for preventing metastases.

Session IV Friday 20 April, 10.05

B.M. Kumpel, Bristol Institute of Transfusion Science, Bristol, United Kingdom

Extreme Variability in Clearance of Rh D-Positive Red Blood Cells by Monoclonal and Recombinant Rh D Antibodies

The administration of anti-D Ig (Rh Ig) to Rh D-negative women during and after pregnancy has reduced the incidence of Rh D immunisation to fetal D+ red blood cells (RBC) and subsequent haemolytic disease of the fetus and newborn by over 90% since anti-D prophylaxis was introduced nearly 40 years ago. Prevention of anti-D responses depends on rapid clearance of D+ RBC from the circulation to the spleen. Anti-D Ig is manufactured from plasma of deliberately immunised donors. Monoclonal and, more recently, recombinant anti-Ds have been developed as replacement therapies and several from different institutions now tested in clinical trials. Varying protocols were used in 14 studies to measure clearance of 0.5-15 ml D+ autologous (presensitised) or allogeneic RBC. One anti-D produced by a human EBV-B cell line cleared almost as effectively as anti-D Ig (1) whereas other human monoclonal anti-Ds have not. No monoclonal antibodies produced by human-mouse heterohybridoma cells have efficiently mediated clearance (2). A recombinant anti-D derived from rat myeloma YB2/0 cells cleared RBC extremely rapidly but with some removal to the liver and with signs of a pro-inflammatory response (3). In contrast, clearance mediated by a recombinant anti-D produced by a Chinese hamster ovary (CHO) cell line was variable and generally very slow with rapid loss of antibody from plasma (4). Surprisingly, the in vivo performance of anti-Ds did not always reflect their IgG Fc receptor-mediated functional activity assessed by *in vitro* bioassays. A hypothesis of why these unexpected results occurred is that additional (as yet uncharacterised) cellular or molecular interactions of monoclonal and recombinant anti-D occur *in vivo*.

(1) Kumpel BM et al, Blood 1995;86:1701-9. (2) Thomson A et al, Lancet 1990;336:1147-50. (3) Armour KL et al, Blood 2006;107: 2619-26. (4) Miescher et al, Blood 2004;103:4028-35.

Session IV Friday 20 April, 10.20

H.J. de Haard, Ablynx N.V., Gent, Belgium

Nanobodies™ as New Therapeutic Entities

Nanobodies are antibody-derived therapeutic proteins with the structural and functional properties of naturally occurring single-chain antibodies derived from camelids. These proteins combine the affinity and selectivity of conventional antibodies with unparalleled biophysical stability, small size, easy tailoring of half-life, low immunogenic potential and microbial manufacturing. Ablynx has established the therapeutic potential of Nanobodies in multiple *in vivo* models, including animal models for rheumatoid arthritis, cancer and thrombosis and for inflammatory bowel disease. In this presentation I will review the progress made in the technologies to isolate and engineer Nanobodies, and as an example will review one of Ablynx's therapeutic lead programs that builds on the many advantages provided by this new platform.

Session V Friday 20 April, 11.30

L.A. Aarden, Sanquin Research, Amsterdam, The Netherlands

The Antibody Response to Therapeutic Monoclonal Antibodies

Patients, chronically treated with therapeutic monoclonal antibodies, develop HACA's or HAHA's in quite high frequencies. Such antibodies lead to increased turnover of the drug, hence to loss of efficacy. I will present a detailed analysis of the antibody response to various monoclonal antibodies and its consequences. Our data suggest that monitoring of the immune response to monoclonal antibodies will lead to more economical, safer and efficient dosing schemes.

J. van Beek[1], E.C. de Jong[2], M.L. Kapsenberg[2], P.W.H.I. Parren[3], G.J. Wolbink[1], S.M. van Ham[1]

1. Sanquin Research, Amsterdam, The Netherlands
2. Cell Biology and Histology, Academic Medical Center, Amsterdam, The Netherlands
3. Genmab, Utrecht, The Netherlands

Identification of Humira-specific T Cell Epitopes

Tumor Necrosis Factor-alpha (TNF-α) is one of the key inflammatory mediators in the maintenance of the chronic inflammatory process in Rheumatoid Arthritis (RA). In addition, it acts as a driver for the inflammation that damages cartilage and bone tissues. Inhibition of TNF-α, for example by monoclonal antibodies, leads to significant clinical improvement and reduction of this damage.

Humir® (Adalimumab) is a fully human anti-TNF-α monoclonal antibody that binds with high affinity to TNF-α. However, in a subset of patients anti-Humir® antibody formation occurs, which results in a loss of therapy efficacy. The anti-Humir® antibodies are of the IgG1 and 4 subclasses, pointing to a T-cell-dependent mechanism.

At present, we are investigating the Humir® specific CD4+ T-cell response. Therefore, we predicted a set of immunogenic peptides in the Humir® sequence using 2 distinct HLA class II prediction methods. In addition, we selected several control peptides. The immunogenicity of the peptides is analyzed in proliferation assays using peptide-loaded antigen presenting cells of HLA-typed RA patients with and without anti-Humir® antibody formation, and healthy donors. CFSE-mediated FACS analysis is used as read-out. These studies are the first steps in the analysis of the immunogenicity of human therapeutic antibodies, which should result into more sensible application of biologicals.

Conclusion
Our results show that erythroid cells selectively express P2Y13. ADP inhibited ATP release by acting on this receptor. This negative feedback system could be important for controlling tissue circulation and of interest for efforts to preserve intracellular ATP in erythrocytes during storage.

Session V Friday 20 April, 12.20

J.S. Lollar, Emory Children's Center, Atlanta, USA

The Antibody Response to Blood Coagulation Factor VIII

Inhibitory antibodies (Abs) to factor VIII (fVIII inhibitors) are the most significant complication in the management of hemophilia A. Additionally, autoantibodies to fVIII can develop in nonhemophiliacs, producing a condition called acquired hemophilia A, which is the most common autoimmune bleeding disorder involving the coagulation system. The analysis of fVIII inhibitors is confounded by polyclonality and the size of fVIII. The A2 and C2 domains in the ~300-kDa A1-A2-B-ap-A3-C1-C2 fVIII molecule contain immunodominant inhibitor epitopes. These epitopes are also immunodominant in hemophilia A mice that receive human fVIII. We have characterized hybridoma Abs to dissect the polyclonal response to human fVIII in hemophilia A mice undergoing a dosage schedule that mimics human use. The florid humoral response to fVIII produces hundreds of hybridomas per mouse. To characterize the Abs, we developed a novel ELISA to assign domain specificity. In addition to prominent inhibitory anti-A2 and anti-C2 responses, these studies yielded the relative contribution of non-inhibitory antibodies to the overall immune response, identified significant domain/Ig isotype associations, discovered a novel inhibitory A2 epitope and an identified an anomalous class of antibodies with dual specificity for the A1 and A3 domain.

Session VI Friday 20 April, 14.30

S.V. Kaveri, INSERM Centre de Recherche des Cordeliers, Paris, France

Intravenous Immunoglobulin: Harnessing the Therapeutic Potential of Natural Antibodies

Intravenous immunoglobulin (IVIg) has increasingly been used for the treatment of autoimmune and systemic inflammatory diseases in addition to supportive therapy of immunodeficient patients. IVIg is beneficial in several diseases including acute and chronic/relapsing diseases, autoimmune diseases and inflammatory disorders. Therapeutic efficacy of IVIg has also been established in a number of dermatologic diseases. Although a considerable progress has been made in understanding the mechanisms by which IVIg exerts immunomodulatory functions in autoimmune diseases, they remain not fully elucidated. The mode of action of IVIg is complex, involving modulation of expression and function of Fc receptors, interference with activation of complement and the cytokine network, modulation of idiotype network, regulation of cell growth, alteration of cellular adhesion process, and effects on the activation differentiation and effector functions of T and B cells and of antigen-presenting cells. The therapeutic effects of IVIg most likely reflect the functions of natural antibodies in maintaining immune homeostasis in healthy people. The ability of IVIg to interact through V regions with complementary V regions of antibodies and antigen receptors as well as with relevant soluble and surface molecules provides the basis for inducing the selection of immune repertoires.

Session VI Friday 20 April, 15.05

G. Vidarsson[1], A.M. Stemerding[2], N.M. Stapelton[1], S.E. Spliethoff[1], H. Janssen[3], F.E. Rebers[1], M. de Haas[1], J.G.J. van de Winkel[2]

[1.] Sanquin Research, Amsterdam, The Netherlands

[2.] University Medical Center, Utrecht, The Netherlands

[3.] Netherlands Cancer institute, Amsterdam, The Netherlands

An IgG Receptor on Phagocytes with a Novel Role in Phagocytosis

Introduction

The neonatal FcγR (FcRn), is a 2M containing MHC-I homolog and is present various cell types and organs, where it has been implicated in transport of IgG across mucosal cells, from mother to fetus, and regulation of IgG half-life, respectively. FcRn only binds IgG in acidic environment (pH =6.5) when histidine residues in the Fc-tail of IgG become protonated, e.g. in endocytic vacuoles. Here we describe the expression and a novel function of FcRn on phagocytes.

Methods

The expression FcRn in human immune cells was analysed by FACS, real time quantitative RT-PCR, confocal and electron microscopy. To compare phagocyte functions we compared IgG recycling and phagocytosis using A) Recombinant human IgG1 directed against pneumococci with that of a mutated variant unable to bind FcRn; B) comparing WT mouse phagocytes with that of 2M knock-out or FcRn a-chain knock-out mice; and C) comparing phagocytes functions in the presence of proton pump blockers and TAT-peptides consisting of the intracellular tail of FcRn.

Results

We found FcRn to enhance phagocytosis in a pH dependent manner which was independent of IgG recycling. IgG-opsonized bacteria were inefficiently phagocytosed by neutrophils from 2M knock-out or FcRn a-chain knock-out mice, which both lack expression of FcRn. Similarly, low phagocytic activity was also observed with mutated IgG (H435A), which is incapable of binding to FcRn, while retaining normal binding to classical leukocyte Fcγ Receptors. Finally, a TAT peptide representing intracellular endocytosis- and transport motifs within FcRn, strongly inhibited IgG-mediated phagocytosis.

Conclusion

These findings support a novel concept in which FcRn fulfills a major role in IgG-mediated phagocytosis

Session VI Friday 20 April, 15.20

F. Nimmerjahn, Nikolaus Fiebiger Center for Molecular Medicine, Erlangen, Germany & Rockefeller University, New York, USA

The Role of Antibody Glycosylation in IVIG Activity

The intravenous administration of the pooled polyclonal IgG fraction of thousands of donors (IVIG-therapy) has gained wide use as an anti-inflammatory drug for the treatment of autoimmune diseases and is approved for this use in immunothrombocytopenia (ITP) and Kawasaki's Disease. The mechanisms by which IVIG, administered at high doses (1g/kg), provides anti-inflammatory activity have been the subject of much speculation, stemming from the fact that IgGs are promiscuous in their binding interactions, through their antigen binding domain as well as their Fc domain. However, evidence from both, clinical trials in the treatment of ITP and many animal models suggests that IVIG and its Fc fragments have equivalent anti-inflammatory activity. Thus, the talk will discuss recent models that have been proposed to explain the anti-inflammatory acitvity of the Fc-portion of IVIG, including the role of the neonatal (FcRn), activating and inhibitory Fc-receptors. One major focus will be on the importance of differentially glycosylated IVIG subfractions and the mechanism by which they mediate their enhanced anti-inflammatory activity.

Posters of selected abstracts for oral presentations

For abstracts, please refer to the speakers' abstract section.
Numbers refer to the poster boards.

5. Expression of Recombinant Human IGM Anti-K in the Presence or Absence of J Chain and the Effect on Serological function.
J.E.M. Gilmour, S.J. Pittman, R.J. Nesbitt, M.L. Scott

9. Extreme Variability in Clearance of RH D-positive Red Blood Cells by Monoclonal and Recombinant RH D Antibodies.
B.M. Kumpel

10. Apoptosis: A Target for Potentiation of UV-Induced Interleukin Receptor Antagonist (il1-ra) Synthesis by Intravenous Immunoglobulins in the Perspective of New Treatment.
R. Laub, L. Craciun, M. Di Giambattista, M. Goldman and E. Dupont

17. Differential Regulation of Humoral Autoimmunity Versus Alloimmunity in Rheumatoid Arthritis.
Y.K.O. Teng, R.J. Verburg, K.N. Verpoort, G.W.J. Diepenhorst, I. Bajema, M. van Tol, E. Jol, R.E.M. Toes, T.W.J. Huizinga and J.M. van Laar

18. Intravenous Immunoglobulins Reduce Allogeneic T-cell Activation after Liver Transplantation by Modulating the Interaction between Dendritic Cells and NK-cells.
T. Tha-In, H.J. Metselaar, H.W. Tilanus, Z.M.A. Groothuismink, P.M. van Hagen, G. Geert, E.J. Kuipers, R.A. de Man and J. Kwekkeboom

19. Identification of Humir®-specific T Cell Epitopes.
J. van Beek, E.C. de Jong, M.L. Kapsenberg, P.W.H.I. Parren, G.J. Wolbink, S.M. van Ham

22. FcRn: An IgG Receptor on Phagocytes with a Novel Role in Phagocytosis
G. Vidarsson, A.M. Stemerding, N.M. Stapelton, S.E. Spliethoff, H. Janssen, F.E. Rebers, M. de Haas, J.G.J. van de Winkel

Posters

Numbers refer to the poster boards

1. Filamin A stabilizes FcγRi Surface Expression and Prevents its Lysosomal Routing

J.M. Beekman, C.E. van der Poel, J. van der Linden, J. Griffiths, M. Kleijmeer, J.G.J. van de Winkel, and J.H.W. Leusen

Introduction

Filamin A, or actin-binding protein 280, is a ubiquitously expressed cytosolic protein that interacts with cytoplasmic domains of multiple receptors to control their subcellular distribution, and signaling capacity.

Methods

Here, we document interaction between FcγRI, a high affinity IgG receptor, and filamin A by yeast two-hybrid techniques. FcγRI and filamin localisation was studied using confocal microscopy. The filamin-deficient cell line M2, and a filamin-reconstituted M2 subclone (A7) were used to study filamin function after transfection with FcγRI cDNA.

Results

FcγRI co-immunoprecipitated with filamin A. Both proteins co-localized at the plasma membrane of monocytes and resided in intracellular structures upon FcγRI triggering. FcγRI transfection in M2 cells without filamin resulted in low surface expression of FcγRI, whereas A7 cells with filamin resulted in high plasma membrane protein levels. Co-expression of the FcRγ -chain could not reconstitute surface expression of FcγRI on M2 cells. In filamin-deficient cells, FcγRI routed by default towards multivesicular bodies and lysosomes as shown by confocal and electron microscopy. Bafilomycin treatment restored the lower total protein levels of FcγRI seen in M2 cells.

Conclusion

These data support a pivotal role for filamin in FcγRI surface expression via retention of FcγRI from a default lysosomal pathway.

2. Elucidation of the Working Mechanism of RhD Immunoprophylaxis

A.A. Chhatta, G. Vidarsson, and C.E. van der Schoot

Rhesus D (Rh or RhD) is a multispanning membrane protein that is expressed on Red blood cells. It is highly polymorphic, and deletion mutations are frequent. RhD is highly immunogenic in RhD negative individuals, which is of a major concern for blood transfusion and pregnancy. Prevention of RhD immunization by the administration of anti-D immunoglobulin is one of the most widespread and successful applications of immunotherapy. Although RhD immunoprophylaxis was introduced in the 60s, the suppressive mechanism is still poorly understood. Passive antibodymediated immune suppression has been studied in mice immunized with sheep red blood cells (SRBC) with or without anti-SRBC IgG. These studies led to the concept that the humoral immune response is inhibited as a result of co-crosslinking of antigen receptors and inhibitory FcγRIIb of RhD-specific B cells by an RBC coated with the passively administered IgG anti RhD. However, in contrast to the postulated role of FcγRIIb, it has recently been shown that the same degree of immune suppression was found in FcγRIIb-deficient mice immunized with SRBC. The suppressive effect of Ig in this model was thought to be mediated by masking of antigenic epitopes, thereby preventing B cells from binding and responding to this antigen. However, this mechanism cannot be the dominant mechanism in human RhD prophylaxis, since most (>90%) of the RhD antigens remain unoccupied with IgG at effective doses of RhD immunoglobulin. This lack of insight in the working mechanism hampers the introduction of monoclonal IgG for hD immunoprophylaxis, and makes it difficult to develop strategies to increase effectiveness of the current treatment. The mouse-SRBC model is not a good model to study human RhD prophylaxis as the xenotypic SRBCs are cleared from the murine circulation within minutes, irrespective of presence of administered anti-SRBC IgG. The aim of this project is to unravel the mechanism of RhD prophylaxis by generating a new model using RhD transgenic mice.

3. Th 1 Cytokine Profiles in Patients with Hepatitis C Virus Infection: Relationship together and to Serologic Inflammation Parameters

N. Farhadi, M. Najafizadeh, and Z. Karayer

Background

Th1 cytokines are required for host antiviral immune responses. However little is known about the production and progression of cytokines in hepatitis C virus (HCV) infected patients. The aim of this study was to assess the serum levels of Th1 cytokines and also their association with inflammatory indicators in HCV-infected and normal individuals.

Methods

Fifty four HCV-infected patients along with thirty one healthy controls were selected using the sequential sampling method. Serum levels of interleukine-2 (IL-2), interferon-gamma (IFN-γ) and tumor necrosis factor-alfa (TNF-α) was determined in all the precipitants by enzymelinked immunosorbent assay (ELISA). Moreover serum levels of alanine aminotransferase (ALT), aspartat aminotransferase (AST), alkaline phosphatase (ALP), C-reactive protein (CRP) and rheumatoid factor (RF) were also determined in both patient and control groups.

Results

The results showed that serum levels of IFN-γ, TNF-α and IL-2 were higher in HCV infected patients than controls group but the difference was significant only for TNF-α ($p<0.05$). A positive correlation was found between the serum level of TNF-α and IL-2 in patient group ($p<0.05$).

Conclusion

TNF-α is the main mediator of the acute inflammatory responses to microbial infections and in our study serum level of TNF-α in HCV-infected patients was higher than healthy subjects. Positive correlation of serum TNF-α and IL-2 levels in HCV-infected patients may contribute to the role of innate immunity in stimulating the adaptive immune responses, thus suggests role of TNF-α in antibody production.

4. Liver Echogenicity and Inflammatory Indicators Relationship with HCV Antibody Production in Hepatitis C Virus Infected Patients

N. Farhadi, M. Najafizadeh, and Z. Karayer

Background/Objective

Immune-mediated mechanisms are believed to play an important role in pathogenesis of HCV infection. Our aim was to investigate whether liver echogenicity and serum inflammatory indicators level have relation with HCV antibody production.

Methods

Serum HCV antibody titer determined by ELISA in referred people and thereby fifty-four patients with Hepatitis C virus (HCV) infection and thirty-one healthy controls selected by sequential sampling method. Liver echogenicity determined by ultrasonography method in all of participants. Serum levels of inflammatory indicators contain alanin aminotransferase (ALT), aspartat aminotransferase (ALT) and alkaline phosphates (ALP) detected by spectrophotometer. Data analyzed by Chi-square and T-independent tests.

Results: Liver echogenicity was increased in 61.5% of positive HCV antibody group but only in 23.3% of negative one. This difference was significant ($p < 0.05$). Elevated serum ALT level was observed in 47.1% of patient but in 6.5% of control groups. In 74.5% of positive and in 38.7% of negative HCV anti body groups, serum ALP level was elevated. Differences between groups in both serum ALT and ALP level were significant ($p < 0.05$). Data analysis also showed significant difference between means of serum AST level according to liver echogenicity result in patient group (41.47± 8.11 in increased echogenicity and 36.82± 4.49 in normal echogenicity groups, $p < 0.05$).

Conclusion

ALP more than ALT and liver echogenicity associated with HCV antibody production.

Relationship between AST and liver echogenicity may be to introduce AST as biochemical marker for prediction of liver density or steatosis.

6. Comparison of Structure, Functional Activity and Glycosylation of Recombinant Human IgM Expressed From NS0 and CHO Cell Lines.

J.E.M. Gilmour, S.J. Pittman, R.J. Nesbitt, A.J. Rowe, and M.L. Scott

IgM consists of 3 polypeptides: heavy, light and J chains. The polymeric structure of IgM can form in the absence of J chain, as the tail of the heavy chain allows disulphide links to be formed between the immunoglobulin monomers. However, J chain has a role in stabilising the pentameric form and if it is missing, higher polymeric structures may be formed. A human IgG was converted into human IgM. The construct was expressed from 2 cell lines, NS0 and CHO, resulting in recombinant human IgM either containing mouse J chain or lacking J chain respectively. In this work, the structure and functionality of the two forms of IgM were studied.

Analytical ultracentrifugation demonstrated that both cell lines produced pentamers. However, both also gave a high molecular weight peak running at approximately 33S which equates to a dipentameric structure. There was no evidence of hexamers which have often been associated with IgM lacking J chain (e.g. Randall et al. 1992 JBC 267 18002) C1q binding was measured as an indication of complement fixation. Both were able to bind C1q *in vitro* demonstrating that the constant regions were functional. However, rIgM lacking J chain bound less C1q compared to the form containing mouse J chain. IgM can interact with polymeric immunoglobulin receptor (pIgR) and be transported across epithelial cells into mucosal secretions. It was demonstrated that both rIgM could interact with a portion of pIgR, secretory component (SC), in an ELISA. Again, lack of J chain resulted in lower binding. To test interaction with intact pIgR, transport assays were carried out in which the ability of rIgM to cross an epithelial cell layer was determined. The results demonstrated that the mouse J chain was sufficient to allow a human IgM to be transported by human pIgR at levels similar to serum IgM. IgM lacking J chain was not transported.

In addition to being a polymeric immunoglobulin, IgM differs from IgG in the level and type of glycosylation. As glycosylation state can also influence function, it is of interest to know the glycosylation of rIgM produced from different cell lines. IgM has a high mannose structures which are absent from IgG. Although the protein must contain the correct motif to allow N-glycosylation, it is the cell line and growth conditions that determine the glycosylation state of the glycoprotein. It was therefore of interest to examine the effect of expressing the same IgM in two commonly used cell lines, CHO and NS0, on the glycans of the rIgM.

Examination using HPAE-PAD demonstrated that both cell lines attached high mannose structures to the rIgM. It was also found that the mouse line (NS0) and the hamster line (CHO) produced similar, but not identical, patterns with CHO cells attaching more high mannose structures than the mouse line.

7. IgG Dimers in Monoclonal and Polyclonal IgG Preparations

T. Guhr, R.L. Aalberse, A.H.L. Koenderman, and H.G.J. ter Hart

Intravenous IgG preparations are widely used for substitution therapy in antibody deficiency or as an immunomodulating agent in autoimmune diseases. Neutralization of auto antibodies by antiidiotype antibodies, blocking Fc receptors and scavenging of activated complement are postulated mechanisms of the effects on the immune system.

Considering the different binding affinities of Fc receptors for IgG, dimeric IgG seemed to be the relevant component of the intravenous IgG preparation. Three types of IgG dimers can be discriminated; tail-tail (Fc-Fc) dimers, tail-head (Fc-Fab) dimers and head-head (Fab-Fab) dimers, also known as idiotype anti-idiotype dimers. According to Tankersley (1988) the IgG dimers in IVIG are idiotytpe anti-idiotype dimers. His hypothesis is supported by the finding that IgG dimerisation occurs between F(ab)2 fragments and that the IgG dimer content in IVIG is correlated with the numbers of donors contributing to the plasma pool.

In this study we investigate the formation and stability of dimeric IgG in 1) monoclonal Ab preparations, 2) polyclonal single plasma preparations and 3) plasma derived immunoglobulin preparations. The preparations were stored at 4°C and 37°C and analysed at different time points by size exclusion chromatography.

Based on our results we conclude that IgG dimer concentration is an equilibrium, which is temperature and concentration dependent. The rate of IgG dimer dissociation is high. Within 24 hours equilibrium between monomeric and dimeric IgG is reached.

Conclusion

Our preliminary results support a significant contribution of idiotype anti- idiotype dimers to the dimer pool in plasma derived immunoglobulin preparations. However, the dimeric fraction is more heterogeneous then previously reported.

8. A Single Gift of Antenatal Anti-D-Prophylaxis of 1000 IU in week 30 reduces the Prevalence of Rhesus-D-Immunization and Haemolytic Disease of the Fetus and Newborn (HDFN) in the next Pregnancy

J.M. Koelewijn, M. de Haas, C.E. van der Schoot, T.G.M. Vrijkotte, and G. Bonsel

Background

Rhesus-D(RhD)-immunization can cause severe haemolytic disease of the fetus and newborn (HDFN). In the Netherlands, since 1969, 1000 IU of anti-D-Ig is administered to RhDnegative women after delivery of an RhD-positive child, resulting in 80% reduction of RhDimmunizations. To achieve further reduction of RhD-immunization resulting from ongoing fetomaternal haemorrhage in pregnancy, since July 1998, a single gift of 1000 IU of anti-D-Ig is administered in week 30 to RhD-negative women in first pregnancies. Evidence on effectiveness of antenatal anti-D-Ig prophylaxis is limited, moreover dosage and timing of anti-D-Ig gifts in published studies differ from the Dutch regimen.

Objective

To determine the effect of antenatal anti-D-prophylaxis (1000 IU in week 30), on the prevalence of new RhD-immunizations, detected by routine irregular erythrocyte antibody (IEA-)screening in (1) week 12 or (2) week 30 of the next pregnancy, and (3) on the resulting prevalence of severe HDFN (i.e. perinatal mortality, need for intra-uterine or exchange transfusion).

Methods

Population study with post hoc observational analysis. Nation-wide all pregnant primiparae (one foregoing delivery) with RhD-immunization newly detected by IEA-screening in 1999, 2002 and 2004 were identified. Patients history and outcome data were collected from obstetric caregivers. The total number of RhD-negative primiparae (15.3% of pregnants) with a first RhD positive child before (control group) and after (intervention group) introduction of antenatal anti-D-prophylaxis, was calculated from data of the Office of Vital Statistics. The coverage of antenatal anti-D-prophylaxis was estimated to be 100 percent, judged from the number of provided ampoules of anti-D (1000 IU) by Sanquin (single provider in the study period).

Results

New RhD-antibodies were detected in 198 primiparae. Excluded from further analysis were 46 women: 26 because of first delivery outside the Netherlands, 20 received no postnatal anti-D-prophylaxis (8 after incorrect RhD-typing of the mother, 9 after negative RhD-typing of the child, 3 for unknown reasons). In 152 included women, 99 RhD-immunizations were detected in week 12 and 53 in week 30, of which 39 respectively 29 received antenatal prophylaxis. Severe HDFN occurred in 18 pregnancies in the control group and 15 in the intervention group. The total number of RhD negative primiparae with a first RhD positive child in the control group was estimated as 8,645 in week 12, and 8,811 in week 30; in the intervention group these numbers were estimated as 12,576 in week 12 and 11,519 in week 30. The prevalence of new RhD-immunizations upon 12th week screening, was 0.69% (95%-CI:0.52-0.87) in the control group versus 0.31% ((95%-CI: 0.21-0.41) in the intervention group.
The prevalence of new RhDimmunizations in week 30 was similar for the control and the intervention group (0.255%, resp. 0.252%).
The prevalence of severe HDFN by new RhD-antibodies in the control group was 0.23% (95%- CI:0.13%-0.33%) and in the intervention group 0.10% (95%-CI:0.05%-0.16%).

Conclusion

Antenatal anti-D-Ig prophylaxis with a single gift of 1000 IU reduces the prevalence of RhD-immunizations, newly detected upon 12th week screening in the next pregnancy, with 50 - 60%. This is in concordance with published studies. There was no effect on immunizations detected later in pregnancy. Antenatal anti-D-Ig reduced the prevalence of severe HDFN in the next pregnancy by new RhD-immunizations with 50-60%.

11. Monitoring Anti-Pneumococcal Antibodies in Healthy Children and in Paediatric Bone Marrow Transplant Patients Treated With Intravenous Immunoglobulin using a New Universal Standard.

R. Laub, M. di Giambattista, J. Duchateau, T. Branckaert, and A. Ferster

Introduction

Hematopoetic bone marrow transplantation, originally developed for the treatment of aplasic anemia and haematological malignancies, is also used to treat solid tumors, congenital immunodeficiencies and autoimmune disorders. These procedures include severe temporay alterations in the host defence mechanism, generally caused by a combination of the underlying disease, preparative treatment for transplantation (immunosuppressive drugs in allogeneic transplantation) and GVHD. Regular replacement therapy with intravenous immunoglobulin (IVIG) has proven effective against infection. In such patients, *S. pneumoniae* is a major cause of morbidity and mortality worldwide. Monitoring pneumococcus-specific antibodies (APAb) provides an assessment of immunity, although it is rendered complex by the diversity of serotypes, the lack of standardised data, and the fact that the minimum concentration required is still largely unknown.

Aim

To develop a universal standard representative of the natural immunity against pneumococcal serotypes. To monitor IVIG treatment by measuring total anti-pneumococcal antibodies using a new standard.

Materials, Methods and Patients

Total anti-pneumococcal antibodies were determined by ELISA (Elizen, Zentech, Liège). The quantification test was carefully validated according the ICH guidelines. Total IgG and IgG2 were measured by nephelometry. Plasma pools were produced with donations from healthy, unpaid volunteers. Specific protective anti-pneumococcal antibodies were determined according WHO recommendations for vaccines. Plasma samples from 15 healthy children (6 to 17 years old) were collected with the informed consent of their parents. Seventeen patients between 1 and 22 years of age, after allogeneic or autologous hematopoietic stem cell transplantation, were infused with IVIG (400mg/kg), Immunoglobulins I.V. (CAF-DCF, Brussels), and with Sandoglobuline (ZLB, Zwitserland). The pathologies transplanted were severe Sickle Cell Disease, Acute Myeloid Leukemia, Myelodysplasia and severe Juvenile Idiopathic Arthritis. The allo-transplanted patients were still on immunosuppressive drugs.

Results

To establish a standard for measuring total anti-pneumococcal antibodies, replicate plasma pools were made from 24 to 5,000 donations. The results show that the APAb concentration in the pools of >5,000 donations remained constant, independently of the year of donation collection, as well as the concentrations determined for 13 specific individual pneumococcal serotypes. The large plasma pool was used successfully as a standard for total APb or for specific neutralizing APb upon incubation in the presence of the cell wall polysaccharide C. The effect of 22F was studied. To check the relevance of using the large plasma pool as a standard (PPS), 131 donations were analysed individually. The results show that the concentrations ranged from 149 to 7,536 mU/ml, the mean value being 1,042 mU/ml. No significant age- or sex-related difference was observed. Similarly, the mean concentration of APb in 15 healthy children (ranging in age from 6 to 17 years) was 1,042 mAU.
Before any IVIG infusion, total APb concentrations were rather low in all patients. After IVIG infusion, the APb concentration rose (up to 4-fold), reaching levels comparable to those measured in healthy children.

Discussion and conclusion

APb concentration measurement and monitoring was easily assessed in healthy individuals (children and adults) and IVIG-treated paediatric patients. Quantification of the APb level could successful be performed in patients and compared to the general population using a pool of more than 5,000 donations as a standard, highly reproducible in time and space and available in large amount.

12. The Fc Receptor Gamma-Chain ITAM is Required for Antibody Immunotherapy of Melanoma

J.H.W. Leusen, S. de Haij, J.H.M. Jansen, J.E. Bakema, J.S. Verbeek, and J.G.J. van de Winkel

Background

In vitro studies have indicated antibody-dependent cellular cytotoxicity (ADCC) as the primary mechanism for tumor-kill by therapeutic antibodies. *In vivo* studies with FcRγ-chain deficient mice have demonstrated the importance of FcR for antibody therapy. However, as these mice lack expression of activating FcR, it is not possible to discriminate between ADCC and apoptosis induced by FcR cross-linking. Therefore, the mechanism of FcR-mediated tumor kill *in vivo* is still unknown and aim of the present study.

Materials and methods

We have generated a novel transgenic mouse expressing a mutated human FcRγ-chain in which the ITAM-associated tyrosines at positions 65 and 76 have been replaced by phenylalanines. These so-called NOTAM transgenic mice were crossed with FcRγ-chain deficient mice. Expression of the transgene was determined by western blot and FcR expression by FACS analysis. The ADCC capacity of isolated neutrophils was determined by chromium release assays. Tumor therapy *in vivo* was determined in the protective B16F10 melanoma model in mice as described (Van Spriel, Blood 2003).

Results

Strong expression of the NOTAM γ-chain was observed in isolated neutrophils, macrophages and dendritic cells. Furthermore, NOTAM transgenic mice showed reconstitution of FcγRI, III and IV to wild-type levels. Neutrophils from NOTAM mice were unable to kill several tumor cell-lines *ex vivo*. *In vivo*, antibody treatment resulted in protection of wild-type mice but not of NOTAM transgenic mice.

Conclusions

We have developed a novel mouse model expressing a signalling defective FcRγ-chain with intact FcR expression levels. With this transgenic mouse, we have established for the first time that FcRγ-chain signalling via the ITAM-associated tyrosines is required for tumor therapy of mouse melanoma *in vivo*.

13. The Most Frequent Diseases and Health Disorders in a Student Population in Medical University-Pleven

P.O. Pudovka

Introduction

To work normal human organism has to be in dynamic equilibrium with environment. Stress is a central theme of scientific literature considering many different aspects through which stress causes diseases. The daily student life is full with stress and tense moments when all organ systems are involved. It is accepted commonly that medicine is one of difficult studied sciences connected with stress.

Aims

To access the prevalence of diseases, health disturbances in students on medicine.

Materials and Methodology

A total of 60 students aged between 18 and 26 years from Medical University-Pleven (47% girls and 53% boys) were inquired by an original self-completed questionnaire. The study was done in December, 2006.

Results

The response rate is 99 %. The most frequent health problems in students were optical and connected with nervous system. The relationship between study results and body mass index, study results and blood pressure were accessed too. (Despite on our opinion there is frequent discrepancy between knowledge and marks. It is relative connection).

Conclusions

The health diseases of students on medicine are a problem which deserves special attention. In combination with some addictive habits as an alcohol and drug abuse it can embarrass young peoples realization.

14. The Significance and Supply of Opiates for the Medical and Scientific Purposes in University Hospital 'G. Stranski'-Pleven

O.A. Pudovka, S. Simeonev, and G. Baichev

Aims

To investigate the use of opiates and to access the ways, frequency of their use on practice of Bulgarian doctors in University hospital- Pleven

Introduction

The activation of the different classes of opioid receptor produces a wide array of cellular responses as analgesia, suppression of protein synthesis, schizophrenia, and immune response regulation. It ensures the medical and scientific benefits of opiates.
Narcotic drugs, including opiates, have a variety of medical uses. They are used as an anaesthetic or analgesic, and to treat diarrhoea, cough or narcotic addiction, as well as for veterinary, dental and laboratory purposes. It has to be an adequate supply of narcotic drugs for medical and scientific purposes and limit of their production and use only to such purposes in order to prevent illicit narcotic drug production, trafficking and use. Not least among these are the opiates which are widely used for the treatment of severe pain, particularly in cancer patients.

Materials and Methods

Most relevant articles on the subject from 1995 to 2005 were reviewed. We studied the documentation of patients with opiate therapy in different departments of University hospital- Pleven and especially in cabinet of chronic pain.

Results

The most frequent use of opiate therapy is on the patients treated in Oncological Center, Surgery and Neurology Departments.
Conclusions: While focusing on the abuse of the opiates, it should not be forgotten that they have important medical uses.

15. Detection of Conformational Changes in IGg using Isothermal Titration Calorimetry with Low Molecular-Weight Probes

T. Rispens, C. Lakemont, and R.C. Aalberse

The aim of this project is to develop procedures to measure trace amounts of “damaged” IgG in therapeutic immunoglobulin preparations. As a model system we compared native IgG with IgG heated at 63 ºC. Conformational changes in IgG were monitored by measuring the enthalpies of binding of the fluorescent probe 8-anilino-1-naphthalene sulfonate (ANS) using isothermal titration calorimetry (ITC) in combination with optical measurements (fluorescence and CD). Analysis reveals that at pH 7.4 approximately two molecules of ANS bind to IgG with a dissociation constant of ca. 0.2 mM, and 50-100 molecules of ANS bind weakly to IgG. Heating at 63 ºC induces partial denaturation and aggregation of IgG, causing the enthalpy of binding to increase more than fifteen-fold. Therefore, heat-aggregated IgG can be detected already at levels below 5%. At pH 4.5, heating resulted in partially denatured IgG that hardly aggregates. Nevertheless, significant differences in the enthalpies of binding are observed between heated and non-heated IgG using the non-fluorescent probes 4-amino-1-naphthalene sulfonate and 1-naphthalene sulfonate. Despite the significant differences in enthalpy of binding, the relatively strong binding sites are still present in the aggregated IgG, and the association constant is hardly affected. It suggests that the structure of aggregated IgG resembles the native structure. This was confirmed by fluorescence and CD measurements.

16. Transplacental IGg Transport is FcRn Mediated and Favors IGg1 over IGg3

N.M. Stapleton, A.M. Stemerding, R. Verheul, A.Y. Zhao, M. de Haas, C.E. van der Schoot, and G. Vidarsson

FcRn, the neonatal MHC class I related Fc receptor, transports IgG across various cell layers and is probably responsible for IgG transport across the placenta. It also prolongs the lifespan of IgGs by rescuing them from degradation. FcRn binding and release is dependent on a histidine-rich region in the Fc-part of IgG that is either positively charged (endo-lysosomes) or neutral (plasma) dependent on the pH of the surroundings. Of the four IgG subclasses, only IgG3 has a half-life comparable with that of non-recycled serum proteins, suggesting FcRn-mediated rescuing to be deficient. This may be linked to the presence of an arginine in position 435 in IgG3, whereas all other subclasses have a histidine in this position. However, the defective rescue function contrasts with the observation that IgG3 is transported across the placenta to neonates, albeit less than IgG1.

We used an experimental setup whereby we could study IgG transport across a monolayer of cultured cells (Transwell).

In this system we studied a placenta derived cell line (JAR, a syncytiotrophoblast cell line) and an FcRn negative cell line (A375), comparing it with its FcRn transfected counterpart (A375FcRn).

In this system we specifically measure FcRn dependant transport, as we observed that if FcRn is not present or blocked by the minimal binding domain of protein A (Z domain) no active transport can be detected.

When only one subclass is present, FcRn-mediated IgG3 transport is equal to IgG1 transport. In agreement with this, exchanging the 435 His normally found in IgG1 with the 435 Arg normally found in IgG3 doesnt affect transport rates. However we do find that mutating the 435 residue to an alanine severely inhibits transport of both IgG1 and IgG3. Unexpectedly, when IVIg (a polyclonal mixture of all subclasses) is tested in this system, IgG1 transport rates are higher that those of IgG3. This can be explained by our observation that IgG3 transport is inhibited by the presence of IgG1. We provide evidence that this inhibition is due to the difference between IgG subclasses in amino acid composition in position 435 as His435-engrafted IgG3 is not inhibited by IgG1. This His435-containing IgG3 furthermore inhibits Arg435-engrafted IgG1 to a similar extent as WT-IgG1 inhibits WT-IgG3. These results indicate that the affinity of FcRn for an Ig-molecule with a His at postion 435 is higher than for Ig molecules with an Arg. This may explain the shorter half-life of IgG3 compared to IgG1 and the relatively lower transplacental IgG3 transport.

20. The Value of Antibodies to Mutated Citrullinated Vimentin in early Arthritis

A.R. van de Horst, J. Ursum, D. van Schaardenburg, M.M.J. Nielen, R.J. van der Stadt, B.A.L. Dijkmans, and D. Hamann

Objective

To investigate the diagnostic and prognostic value of antibodies to mutated citrullinated vimentin (anti-MCV) in early arthritis in comparison with the second generation antibodies to cyclic citrullinated peptides (anti-CCP2) test and to study the relationship between disease activity and anti-MCV.

Methods

Anti-MCV (ORGENTEC Diagnostica) was measured in 162 patients with early arthritis (70% females, mean age 55,7 years, maximum symptom duration 3 years) at baseline and one and two year follow-up. Antibodies against CCP (second generation test, Euro-Diagnostica) were measured only at baseline. After one year follow up patients were diagnosed as rheumatoid arthritis (RA) or undifferentiated arthritis (UA) after chart review by an experienced rheumatologist (BD), who was blinded for the results of antibody tests. Disease activity was measured by the DAS28 score and serum C-reactive protein (CRP). Radiographic progression between 0 and 2 years was measured by the Sharp-van der Heijde score of hands and feet. Multivariate logistic regression analyses were used to assess the diagnostic value (clinical diagnosis of RA at one year follow-up) and the prognostic value (radiographic progression score after two years) of the anti- MCV test in these patients, expressed as odds ratio (OR) with 95% confidence interval (CI). Linear regression was used to assess the relation between anti-MCV concentration and disease activity.

Results

123 patients were diagnosed as RA and 39 as UA. The specificity for the diagnosis RA was comparable for anti-CCP2 and anti-MCV with 92.1% and 92.3%, respectively. The sensitivity of anti-MCV was 59.3%, slightly higher than that of anti-CCP2 (55.3%). Anti-MCV was the best predictor for the diagnosis RA (OR = 24.8, 95%CI 6.1-101.3). The best predictive variable for radiographic progression was anti-CCP2 (OR 10.7, 95%CI 4.0-29.0) followed by anti-MCV (OR 9.6 95%CI 3.6-25.9). At all time points, the group of anti-MCV positive patients had significantly higher median ESR and CRP levels and Sharp-van der Heijde score than the group of anti-MCV negative patients ($p<0.005$). Disease activity (DAS28) was higher in anti-MCV positive patients compared to anti-MCV negative patients at two years follow-up ($p<0.05$) (Figure 1). However, a direct relation between anti-MCV levels and disease activity was not found ($r = 0.17$, $p<0.01$) The correlation between anti-MCV level and CRP ($r=0.23$, $p<0.01$) and between anti-CV level and disease activity (DAS28, $r=017$, $p<0.05$) was very low.

Conclusion

Anti-MCV is a good marker for predicting the diagnosis and prognosis of early RA. The higher level of inflammation and radiographic damage in the group of anti-MCV positive patients versus the group of anti-MCV negative patients already at baseline and even at two years suggest that anti-MCV positive is RA is an aggressive form of RA.

21. Activating FcGr2c Predisposes to Idiopathic Thrombocytopenia

E. van Mirre, W.B. Breunis, M. Bruin, J. Geissler, M. de Boer, M. Peters, D. Roos, M. de Haas, H.R. de Koene, and T.W. Kuijpers

Gene copy number polymorphisms (CNPs) and single nucleotide polymorphisms (SNPs) count as important sources for inter-individual differences, including differential responsiveness to infection or predisposition to autoimmune disease as a result of unbalanced immunity.

By a novel multiplex ligation-dependent probe amplification (MLPA) assay, we demonstrate extensive variation in the FCGR2 and FCGR3 gene clusters, including previously unrecognized CNPs as well as FCGR2C gene translation into an activating IgG receptor that exerts antibody-mediated cellular cytotoxicity (ADCC) by immune cells.

As indicated by the prevalence of an FCGR2C open reading frame (ORF) of the gene, this receptor is expressed in less than 20% of healthy individuals but strongly associated with the hematological autoimmune disease ITP (present in 35% of ITP patients.

23. Factor VIII-Specific Memory B-Cells in Patients with Hemophilia A

J. Voorberg, P.M.W. van Helden, P.H.P. Kaijen, S.E. Dohmen, S.D. van Haren, F.P.J. Mul, K. Fijnvandraat, and H.M. van den Berg

Memory B cells rapidly differentiate into antibody secreting cells (ASC) following antigenic stimulation. Persisting levels of memory B cells have been found after exposure to viruses and following vaccination. Less is known with respect to the frequency of memory B cells in the context of immune responses to therapeutic proteins like blood coagulation FVIII.

We have developed a protocol that allows for quantification of circulating FVIII-specific memory B cells in small amounts of peripheral blood. CD19+ B lymphocytes were sorted on a layer of irradiated EL4B% thymoma cells expressing CD40L in the presence of supernatant of mitogenstimulated T cells. These experimental conditions induce proliferation of memory B cells and allow them to differentiate into ASC. After 9-10 days of culture, total IgG and FVIII specific IgG was determined by ELISA and number of ASC was determined by ELISpot.

Blood samples of five hemophilia A patients with inhibitors, five multi-transfused non-inhibitor patients and six patients successfully treated by ITI were analyzed. For all five inhibitor patients FVIII-specific ASC could be detected. The number of circulating FVIII-specific memory B cells ranged from 10-244 per 106 peripheral B cells. These values are similar to the number of antigenspecific memory B cells that are found following vaccination. Inspection of a panel of hemophilia A patients without inhibitors revealed that low levels of FVIII specific memory B cells were present in 2 out of 5 patients. Similarly, low levels of circulating memory B cells were observed in 2 out of 6 patients who were successfully treated by immune tolerance induction (ITI). Our findings reveal that the frequencies of circulating FVIII-specific memory B cells in hemophilia patients with inhibitors are similar to that observed following vaccination. Reduced levels of circulating FVIII specific memory B cells are present in hemophilia A patients without inhibitors as well as in patients who have been successfully treated by ITI. These results suggest that FVIII specific memory B cells are successfully eradicated during ITI.

24. Human VH1-69 Germ Line Gene Encoded Anti-Spacer Domain Antibodies that develop in Patients with Acquired TTP interfere with the Binding of ADAMTS13 to VWF

J. Voorberg, W. Pos, B.M. Luken, E.A.M. Turenhout, J.A. Kremer Hovinga, J.F. Dong, and R. Fijnheer

Acquired thrombotic thrombocytopenic purpura (TTP) is characterized by the presence of antibodies directed towards ADAMTS13. In all presently characterized patients with acquired TTP antibodies directed towards the spacer domain have been found. In order to study anti- ADAMTS13 antibodies at the molecular level, we have isolated a panel of human antibodies from two patients with acquired TTP using phage display. The characteristics and biochemical properties of two of the selected human antibodies were studied. Sequence analysis revealed that both human antibodies, designated I-9 and II-1, were encoded by gene segment VH1-69 that is incorporated into only 3% of IgG-expressing peripheral B cells. Epitope-mapping studies employing a hybrid ADAMTS13 variant containing the spacer domain of ADAMTS1 showed that both antibody I-9 and II-1 were directed towards the spacer domain. Competition experiments revealed that antibodies with similar epitope specificity as I-9 were present in plasma of patients with acquired TTP. Both antibody I-9 and antibody II-1 inhibited ADAMTS13 activity as measured by cleavage of VWF-FRETS73 substrate. Antibody II-1 was 100 times more efficient than antibody I-9. Biosensor analysis revealed that antibody I-9 dissociates more rapidly from immobilized ADAMTS13 when compared to antibody II-1. Antibody I-9 also effectively inhibited the cleavage of VWF multimers both under static conditions and under flow on the surface of endothelial cells. Both antibody I-9 and antibody II-1 interfere with the binding of ADAMTS13 to immobilized VWF. Together our results suggest that VH1-69-encoded anti-ADAMTS13 antibodies are commonly observed in the immunoglobulin repertoire of patients with acquired TTP. Biochemical characterization revealed that VH1-69 encoded human antibodies inhibit ADAMTS13 by interfering with spacer domain-mediated binding to VWF.

List of participating companies – medical technical exhibition

1. Fresenius Kabi, Transfusion Technology Division
   Runde ZZ 41
   7881 HM Emmer Compascuum
   The Netherlands

2. Roche Diagnostics Nederland B.V.
   Transitorstraat 41
   1322 CK Almere
   The Netherlands

3. Genmab
   Postbus 85199
   3508 AD Utrecht
   The Netherlands

4. Haemonetics BV
   Tinstraat 107
   4823 AA Breda
   The Netherlands

5. Hemocue
   Gestelsestraat 15h
   5582 HH Waalre
   The Netherlands

6. Tecan Benelux B.V.B.A
   Industrieweg 30
   4283 GZ Giessen
   The Netherlands

7. Sanquin Blood Supply Foundation / Sanquin Reagents
   P.O. Box 9892
   1006 AN Amsterdam
   The Netherlands

Social programme

Poster visits, drinks and congress buffet - Thursday April 19th

The drinks and congress buffet will be held in the main hall of the Royal Tropical Institute. The Poster Award Ceremony will be held at 20.00 hours.

Farewell drinks - Friday April 20th

On Friday after the last session a farewell reception will be organised in the main hall of the Royal Tropical Institute. You are most welcome to join this reception.

General information

(in alphabetical order)

Accreditation and certificate of attendance

The following societies have rewarded accreditation points. γ
Please, ask at the registration desk for certificates of attendance.
You may be asked to sign a list of attendance for the society in question.

- Dutch Society for Immunology: 12 points for 2 days γ
- Dutch Society for Rheumatology: 4 hours each day
- Dutch Association of Hospital Pharmacists: 9 hours for 2 days
- Dutch Pediatric Society: 9 points for 2 days
- Dutch Society for Internal Medicine (allergy/clinical immunology; blood transfusion medicine; haematology): 9 points for 2 days
- European Accreditation Council for Continuing Medical Education (EACCME): 12 European credits for 2 days
- American Medical Association AMA PRA category 1 credits will be awarded on the basis of the EACCME credit points. Please refer to the conference website or the AMA website for further information.

Badges

All participants will receive a personal badge upon registration.
You are kindly requested to wear your name badge when attending any meeting or social gathering.

Banking facilities

The official currency in The Netherlands is the Euro. It is recommended that foreign currencies will be converted to Euros at Dutch chartered banks, which are usually open from Monday through Friday from 09.00 - 16.00 hours. Exchange of foreign money and travellers' cheques is also possible in most hotels.

Cloakroom and luggage

At the Royal Tropical Institute a cloakroom is located near the registration area.

Electricity

In the Netherlands, electricity is supplied at 220 V - 50 Hz AC.

Insurance

In registering for the Sanquin Spring Seminars, participants agree that neither the Organizing Committee nor the Seminar Secretariat assume any liability whatsoever. Participants are requested to make their own arrangements for health and travel insurance.

Language

The official language of the Sanquin Spring Seminars is English.

Registration desk

The registration desk will be open at the following times:

Thursday, April 19, 2007	07:30 - 18:00 hours
Friday, April 20, 2007	07:30 - 16:00 hours

Shops

Most shops in Amsterdam are open from 09.00 to 18.00 hours.
On Thursdays, shops are open till 21.00 hours.

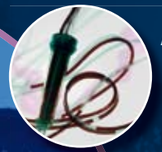
Taxis

Numerous taxi stands are located throughout Amsterdam.
The telephone number of the central taxi service is 020-7777777

Weather

While April may offer lovely spring weather, it can be quite unpredictable and might be chilly in the evening. Temperatures range from 7 to 14°C. As showers might occur, we advise you to bring a raincoat or an umbrella.

Blood Processing
Disposables



Apheresis Systems

Blood Processing
Equipment

Your partner for blood management and transfusion technology

Fresenius HemoCare

The Blood Care Company

Fresenius
Kabi

Caring for Life

Fresenius HemoCare Netherlands B.V.
A company of the Fresenius Kabi group
Runde ZZ 41 · 7881 HM Emmer-Compascuum · Netherlands
Phone: +31 (0) 591-35 57 75 · Fax: +31 (0) 591-35 10 55
www.fresenius-kabi.com

Sanquin

Spring Seminars
16 & 17 April 2009

The next Sanquin Spring Seminar will again be held in Amsterdam, in the spring of 2009, Thursday 16 and Friday 17 April 2009, Amsterdam, The Netherlands

Cellular therapies: insights and new horizons

Themes:

Hematopoietic stem cell transplantation

Mesenchymal stem cell therapy

Tissue engineering

Dendritic cell immunotherapy

Adoptive T cell transfer

Sanquin
Plesmanlaan 125
P.O. Box 9892
1006 AN Amsterdam
The Netherlands
Telephone +31 20 512 30 00
www.sanquin.nl