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SECOND EFIS/EJI BELGRADE SYMPOSIUM

Inflammation at the Interface of Innate and Acquired Immunity

7 – 10, SEPTEMBER 2008

EJI European Journal of Immunology
efis European Federation of Immunological Societies

PROGRAMME AND ABSTRACTS PROGRAMME AND ABSTRACTS PROGRAMME AND ABSTRACTS

Serbian Journal of Experimental and Clinical Research

2008

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2ND EFIS/EJI BELGRADE IMMUNOLOGY SYMPOSIUM

INFLAMMATION AT THE INTERFACE OF INNATE AND ACQUIRED IMMUNITY

PROGRAM AND ABSTRACTS
September 7th – 10th, 2008
Belgrade, Serbia

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2nd EFIS/EJI BELGRADE

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European Journal of Immunology (EJI)

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Immunological Society of Serbia

Faculty of Medicine, University of Kragujevac

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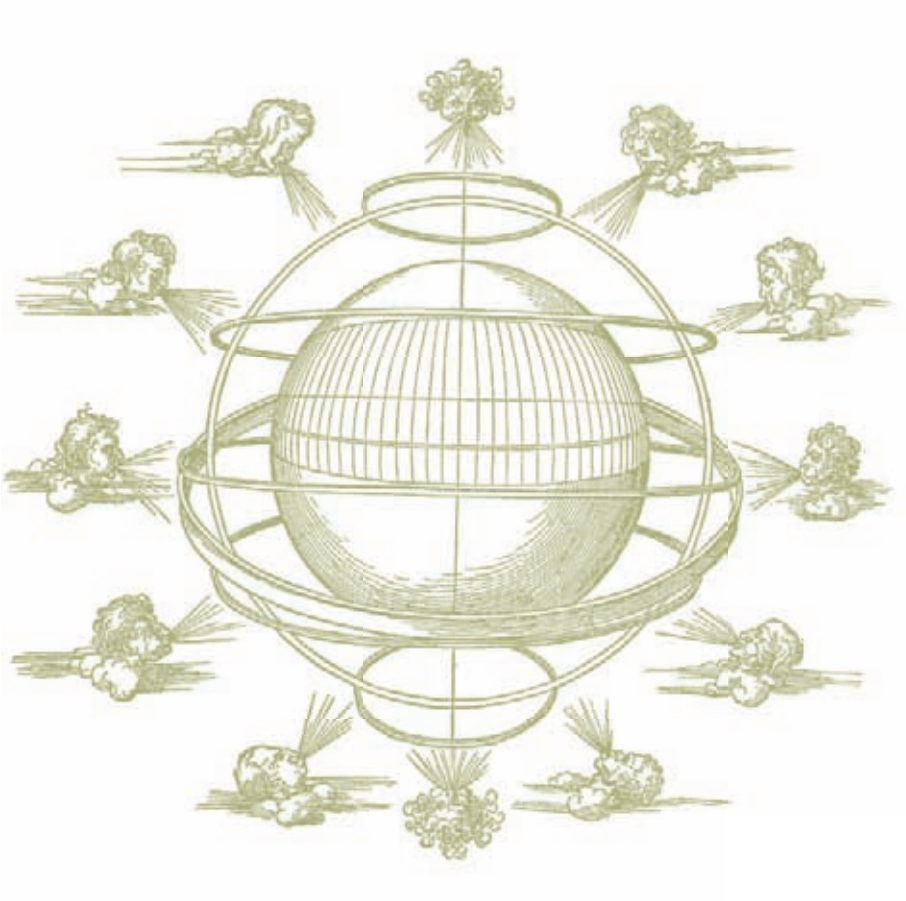
Minister of Science and Technological Development



Welcome,

It is my pleasure to invite you to participate in the 2nd Belgrade Meeting on Immunoregulation entitled: *Inflammation at the interface of Innate and Acquired Immunity*.

As the previous one, the meeting is organized under the auspices and with support of European Federation of Immunological Societies and European Journal of Immunology. The meeting will offer the lectures and discussions related to several major topics on contemporary immunology. The list of speakers includes outstanding scientists from Europe, USA and Australia (see list of invited speakers) and presentations selected from the participants. There will be also time for poster presentations and informal discussions with invited speakers. With support of EFIS/EJI and Government of Serbia, we are able to secure low registration fees and an attractive program

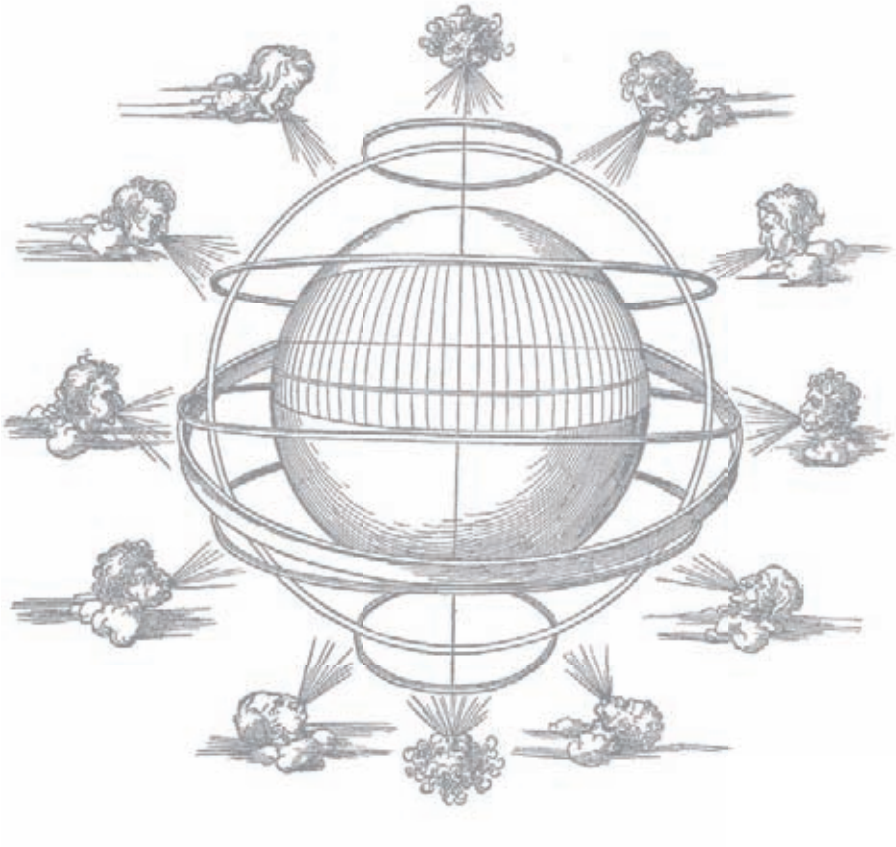
We hope that we are putting together an exciting scientific program and social events. A highlight of the conference will be the participation of a international invited speakers that have made milestone contributions to the field.

We look forward to a great meeting with lots of excellent immunology and translational research of high clinical relevance

Belgrade is a hospitable city at the confluence of the two major European rivers (Danube and Sava). It is one of the oldest cities in Europe with tumultuous history and vibrant cultural life and entertainment at present.

I wholeheartedly welcome you in Belgrade.

Miodrag L. Lukic
Meeting Chairman



PROGRAMME

DAY 1: SUNDAY, SEPTEMBER 7th, 2008

17:00 – 17:30
National Assembly Building (Main Hall, Nikola Pasic Square 13)
• Welcome and Opening Remarks

17:30 – 17:45
Break

Opening Lectures
Session Chairs – M Lukic, N Arsenijevic

17:45 – 18:25
F Y Liew (Glasgow)
• Novel cytokines in infectious and inflammatory disease

18:25 – 19:05
Sergio Romagnani (Florence)
• Properties and origin of human Th17 cells

19:05 – 19:45
Olivera Finn (Pittsburgh)
• Cancer immunosurveillance and immunotherapy

20:15 – 22:00
Welcoming Reception

DAY 2, MONDAY, SEPTEMBER 8th, 2008

THEME I: INNATE AND ACQUIRED IMMUNITY IN INFECTION

Hotel Hyatt Regency (Belgrade/Budva Halls)
Registration from 08:00

Session Chairs: F Y Liew and M Mostarica-Stojkovic

08:30 – 09:00
Stipan Jonjic (Rijeka)
• Activation and inhibition of NK cells by murine cytomegalovirus

09:00 – 09:20
Vladimir Badovinac (Iowa City)
• CD8 T cell memory

Keynote Lecture

09:20 – 10:00

Peter Doherty (Melbourne/Memphis)

• Cell-mediated immunity in influenza

10:00 – 10:30

Coffee break

10:30 – 10:50

Janko Nikolich-Zugich (Tucson)

• T Cell response to viral infections in the old age: homeostatic and functional aspects.

10:50 – 11:10

Ljiljana Sofronic-Milosavljevic (Belgrade)

• Trichinella spiralis – helmint that holds back autoimmunity

11:10 – 11:30

Desa Lilic (Newcastle)

• Defects of dendritic cell cytokine and Th 17 immunity in human chronic candidiasis

11:30 – 12:00

Sergei Nedospasov (Moscow/ Berlin)

• Tumor necrosis factor as mediator of innate immunity and inflammation: importance of mouse models

12:00 – 12:20

Miodrag Colic (Belgrade)

• Immunology of periapical lesions

12:20 – 14:00

Break

THEME II – IMMUNOPATHOLOGY OF INFLAMMATORY DISEASES

Session Chairs: S Romagnani, A Djukic

14:00 – 14:30

Georg Wick (Insbruck)

• Classical risk factors as inducers of anti-endothelial cell immune reactions in atherosclerosis

14:30 – 14:50

Vera Pravica (Los Angeles)

• Short arm of chromosome 6 : Genetic contribution to innate and acquired immunity

14:50 – 15:10

Ratko Djukanovic (Southemphton)

• Mechanisms of T cell activation and recruitment in asthma

15:10 – 15:30

Stanislav Vukmanovic (Washington)

• Alloimmunization in patients with sickle cell diseases: an experimental model to study "immune response genes" in humans

15:30 – 15:50
Nada Pejnovic (London/Belgrade)
• Increased atherosclerotic lesions in interleukin-18 deficient apolipoprotein e-knockout mice fed high-cholesterol diet reveal a role for Th17 cells in atherosclerosis

15:50 – 16:10
Nikola Vujanovic (Pittsburgh)
• Tumor necrosis factor alpha: the masterkey of inflammation and immune reactions

17:00 – 22:00
Free evening

DAY 3, TUESDAY, SEPTEMBER 9th, 2008

THEME III – IMMUNOREGULATION
Session Chairs: S Nedospasov, M Colic

09:00 – 09:30
Hannes Stockinger (Vienna)
• Regulatory pathways in T cells analyzed under nanometer precision and microsecond time resolution using single molecule imaging

09:30 – 09:50
Miodrag Lukic (Kragujevac/Al Ain)
• Galectin-3 in autoimmunity

09:50 – 10:20
Dragana Jankovic (Bethesda)
• Self control by Th-1 lymphocytes

10:20 – 10:50
Coffee break

10:50 - 11:20
Anna Erdei (Budapest)
• The role of complement system in the pathogenesis of experimental allergic encephalomyelitis

11:20 – 11:35
Lazar Vujanovic (Pittsburgh)
• Activation of human NK cells by adenoviraly-engineered dendritic cells

11:35 – 11:55
Milan Basta (Bethesda)
• Immunoglobulins and inflammation: switching between stimulation and suppression

11:55 – 12:25
Mathias von Herrath (La Jolla)
• Viruses and Tregs–two sides of a coin

12:25 – 14:00
Break

THEME IV: AUTOIMMUNITY
Session Chairs: M von Herrath, G Leposavic

14:00 – 14:30
Alexander Gabibov (Moscow)
• Catalytic antibodies and autoimmunity

14:30 – 15:00
Hartmut Wekerle (Munich)
• Autoimmune T cell migration in experimental autoimmune encephalomyelitis: A journey through multiple milieus

15:00 – 15:20
Ivana Stojanovic (Belgrade)
• MIF – A cytokine at the top of the inflammatory cascade

15:20 – 15:40
Marija Mostarica-Stojkovic (Belgrade)
• Th1 and Th17 cells - partners or foes in CNS autoimmunity?

15:40 – 15:55
Alexey Belogurov (Moscow)
• Myelin basic protein epitope library: Path to diagnostics and treatment of multiple sclerosis

15:55 – 16:10
Oliver Burton (Cambridge)
• Schistosoma mansoni egg antigens directly and indirectly induce regulatory T cells that prevent diabetes in NOD mice

16:10 – 16:25
Milica Vukmanovic-Stejic (London)
• The kinetics of CD4+ Foxp3+ regulatory T cell accumulation and proliferation during an antigen- specific memory response in humans

16:25 – 17:30
Poster discussion

19:00
Boat trip and conference dinner

DAY 4, WEDNESDAY, SEPTEMBER 10th, 2008

THEME V: IMMUNOTHERAPEUTIC PERSPECTIVES IN CHRONIC INFLAMMATORY DISEASES
Session Chairs: A Erdei, S Stosic-Grujicic

08:30 – 08:50
Miodrag Stojkovic (Kragujevac/Valencia)
• Adult and embrional stem cells for treatment of human diseases

08:50 – 09:20
Mario Abinun (Newcastle)
• Haematopoietic stem cell transplantation for severe rheumatic disorders in children

09:20 – 09:50
Steffen Gay (Zurich)
• Innate immunity, epigenetics and autoimmunity in rheumatoid arthritis

09:50 – 10:20
Tchavdar Vassilev (Sofia)
• Selective silencing of disease-associated autoreactive B lymphocytes by chimeric antibodies targeting their inhibitory FcgammaIIB and CD22 receptors

10:20 – 10:50
Coffee break

Closing Lecture
Session Chairs: H Stockinger, LJ Sofronic-Milosavljevic

10:50 – 11:30
Charles Dinarello (Denver)
• The Role of the IL-1 Family in Inflammation

11:30 – 11:50
N.A. Mitchison (London)
• Concluding comments

11:50 – 12:10
Closing of the Meeting

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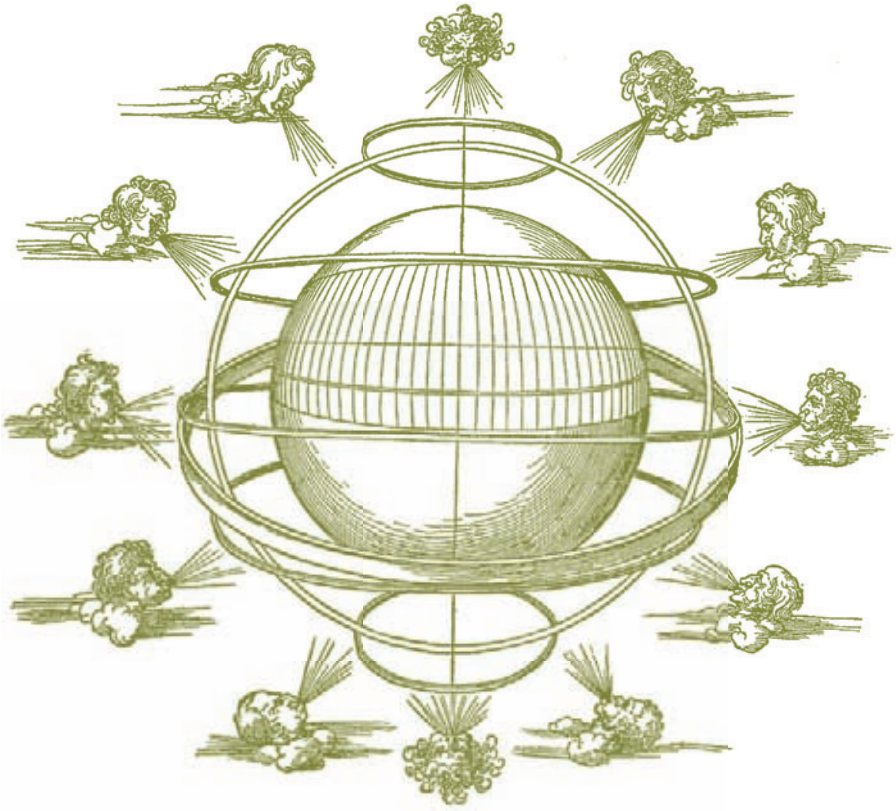


SPEAKERS AND SESSION CHAIRS

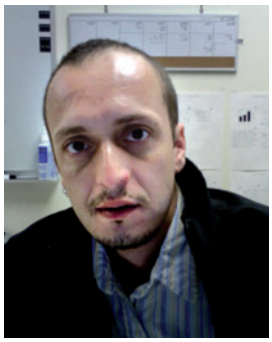
Having in mind heterogeneity of the participants at this meeting and in order to facilitate informal contacts between students and faculty, we provide full addresses, picture and short biosketches of the invited speakers. More information including references may be found on the Conference Website www.eji-efis.com as well as at the institutional or personal websites of the speakers

Dr Mario Abinun
Honorary Clinical Senior Lecturer and Consultant in
Paediatric Immunology, Institute of Cellular Medicine
Children's BMT Unit Ward 23
Newcastle General Hospital Westgate Road
Newcastle upon Tyne NE4 6BE
Phone:+44-191-2563735, Fax: +44-191-2730183
E-mail: mario.abinun@ncl.ac.uk

Dr Abinun obtained his MD from University of Sarajevo, PhD from University of Belgrade and postgraduate training from University of London. His research interests include Primary Immunodeficiencies (PID), Haematopoietic Stem Cell Transplantation (HSCT) and Paediatric Rheumatology/Autoimmune Disorders

Professor Nebojsa Arsenijevic
Professor and Chair, Department of Microbiology & Immunology
Faculty of Medicine, University of Kragujevac, Belgrade
E-mail: arne@medf.kg.ac.yu

Professor Arsenijevic obtained his M.D and PhD degrees from University of Belgrade and moved back to his native Kragujevac where he was appointed through ranks of Assistant Professor to Associate and Full Professor. His research interest is in cancer immunology. In 2002 he obtained an MS degree from International Center for Health Management at the "Sapience" University in Rome. He is Dean of the Medical School from year 2005.



Dr Vladimir P. Badovinac
Department of Microbiology
University of Iowa
Iowa City, IA 52242, USA
Phone: 001-319-384 2930
E-mail: badovinacv@healthcare.uiowa.edu

Dr Badovinac received his Ph.D. degree working in the laboratory of Marija Mostarica Stojkovic (University of Belgrade, Serbia), and did postdoctoral work with John T. Harty (University of Iowa, USA). He is currently an assistant professor of pathology at the University of Iowa. His research focuses on antigen-specific $CD8^+$ T-cell homeostasis after infections and/or vaccinations.

Prof. Miodrag Colic
Corresponding Member, Serbian Academy of Sciences and Arts
Head, Experimental Medicine
Medical Military Academy, Belgrade
E-mail: vmaimi@EUnet.yu

Professor Colic obtained his MD and PhD degree from University of Belgrade. In the last decade he established a highly active research group at the Medical Military Academy. His main research interests are in physiology and Pathophysiology of dendritic cells and more recently in the immunopathogenesis of Periapical lesions.

Professor Ratko Djukanovic
Professor in Medicine and Head of the
Inflammatory Cell Biology Group
Division of Respiratory Cell & Molecular Biology
University of Southampton School of Medicine
Mailpoint 810, Level F, South Block
Southampton General Hospital
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Ratko Djukanovic is Professor of Respiratory Medicine and Honorary Consultant Physician, Director of the Division of Infection Inflammation and Repair and Director of the Southampton Respiratory Biomedical Research Unit.(NIHR BRU). His own translational research is conducted within the Inflammatory Cell Biology Group of which he is the head.

The basic research studies in Professor Djukanovic's group have led to a better understanding of mechanisms of T cell recruitment (requirement of co-stimulatory molecules and NFkappaB), chemotaxis and survival of eosinophils and neutrophils and the close communication between epithelial cells and inflammatory cells.

Professor Charles A. Dinarello
Professor of Medicine, Department of Medicine
Division of Infectious Diseases,
University of Colorado Health Sciences Center
Member, National Academy of Sciences, USA
Denver, Colorado 80262, U.S.A.
Phone: 001-303-315-3589, E-mail: cdinare333@aol.com

Charles A. Dinarello is Professor of Medicine at the University of Colorado School of Medicine in Denver. Until 1996, he was Professor of Medicine and Pediatrics at Tufts University School of Medicine and a staff physician at the New England Medical Center Hospital in Boston. Dr. Dinarello received his medical degree from Yale University and his clinical training at the Massachusetts General Hospital. From 1971 to 1974, he was a clinical associate and from 1975 to 1977 a senior investigator at the National Institutes of Health in Bethesda.

Dr. Dinarello serves on the editorial board of several scientific journals and has published over 450 original research articles on cytokines, particularly interleukin-1. The Institute for Scientific Information lists him as the world's third most cited life scientist (1981-1994). He was elected into the National Academy of Sciences in 1998 and has received several international awards for his contributions to medicine.

Professor Peter Doherty, FRS
Laureate Professor, Department of Microbiology and Immunology,
University of Melbourne, Melbourne
Victoria 3010, Australia.
Phone: +61 3 8344 7968/Fax: +61-3 8344 7990
E-mail: pcd@unimelb.edu.au

Professor Peter Doherty, AC is an Australian-born immunologist who works in the general area of immunity to viruses. He shared the 1996 Nobel Prize for Physiology or Medicine with his Swiss Colleague, Rolf Zinkernagel, for discovering »the nature of the cellular immune defense«. He was Australian of the Year in 1997, and has (since 1998) been commuting between St Jude Children's Research Hospital (SJCRH) in Memphis, Tennessee, and the Department of Microbiology and Immunology at the University of Melbourne.

He has recently returned to spend the majority of his year in Australia, holding appointments as Laureate Professor at this university and as a Burnet Fellow of the National Health and Medical Research Council. Over the subsequent 25+ years he has led substantial research efforts in viral immunology at the Wistar Institute, Philadelphia, SJCRH, and is now developing a new program in Melbourne.

Professor Anna Erdei
Treasurer, EFIS
Professor and Chair, Department of Immunology
University Eötvös Lorand
Pazmany s. 1/C, H-1117 Budapest, Hungary
Phone: (36)1381-2175, Fax.: (36)1381-2176
E-mail: anna.erdei@freemail.hu

Professor Erdei obtained her PhD and DSc from Hungarian Academy of Sciences and postdoctoral training at major laboratories in Europe and Israel. Her research is on interaction of innate and specific mechanisms in immunopathology with particular emphasize in complement functions.

Professor Olivera J Finn
Professor and Chair, Department of Immunology
University of Pittsburg School of Medicine E1040, PA 15262,
Phone: 412-648-8916/Fax:412-648-7042
E-mail: ojfinn@pitt.edu

Olivera J. Finn, Ph.D., is Founding Chair and Professor in the Department of Immunology at the University of Pittsburgh School of Medicine, and director of the immunology program at the University of Pittsburgh Cancer Institute (UPCI). Dr Finn obtained her PhD degree from Stanford University.

Dr. Finn's research interests and expertise are in the areas of tumor immunology, transplant immunology and T-cell biology. In particular, her group has identified a novel immune response to a tumor-associated antigen, MUC1. This work has led to the development of a potential cancer vaccine currently being tested in clinical trials.

Dr Finn is 2007/2008 President of American Association of Immunologists.

Professor Alexander Gabibov
Associate Member Russian Academy of Sciences
Professor, Lomonosov Moscow State University
Head, Laboratory of Biocatalysis Shemyakin & Ovchinnikov
Institute of Bioorganic Chemistry, Russian Academy of Sciences
117984, Miklukho-Maklaya Street, 16/10
Phone: (Mobile) - +7-9166835307/ Office: +7(495)-7273860
Phone (Lab): +7095 429-8269/Fax: +7095 3307329
E-mail: gabibov@ibch.ru

Professor Gabibov is Head of the Department of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry and Professor at the Moscow State University. His major research interest is in biological consequences of catalytical activities of antibodies, in particular its importance in monitoring and therapy of clinical autoimmunity syndrome

Professor Steffen Gay
University Hospital, Clinic of Rheumatology
Professor and Head, Center for Experimental Rheumatology,
Gloriastrasse 25, CH-8091 Zurich, Switzerland
Phone:+41-44-255-5737/Fax:+41-44-255-4170
E-mail: Steffen.Gay@usz.ch

Professor Steffen Gay graduated from Medical School at the University in Leipzig. Holding office from 1976-1996 at the Department of Medicine at the University of Alabama in Birmingham AL, he served there as Professor of Medicine from 1984-1996. At present he is Director of the WHO Collaborating Center for Molecular Biology and Novel Therapeutic Strategies for Rheumatic Diseases and Professor of Experimental Rheumatology at the University Hospital of Zurich, Switzerland.

Dr Dragana Jankovic
Senior Staff Scientist
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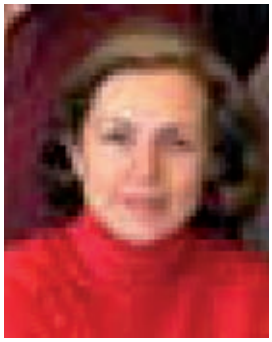

Dr Jankovic obtained her MD and PhD degree from University of Belgrade. She moved to NIH where she developed a distinguished scientific career in laboratory for Parasitic Diseases, NIAID. Her main research interest is host resistance and immune regulation in parasitic infections.

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Professor Jonjic obtained his MD and PhD degree from University of Rijeka (Croatia). For many years he has leading one of the most distinguished research groups in Croatia. He also developed a long standing and highly productive collaboration with research groups in Germany. His main research interests are immunology and immunopathology of viral infections.

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Professor Mitchison received his PhD degree from New College Oxford with Sir Peter Medawar and hold professorial and leadership positions at University of Edinburgh, National Institute of Medical Research, London, University College London and German Institute for Research in Rheumatology. His contributions, now textbooks matter include requirement of antigen presentation by cell, cell mediated graft rejection, low zone tolerance and 'hapten' – carrier effect. His influence in the development of immunology is hard to overestimate. His present interest is in immunoregulation and genetics.

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Sergio Romagnani is Professor of Internal Medicine and Head of the Section of Clinical Immunology, Allergy and respiratory Diseases of the Department of Internal Medicine at the University of Florence (Italy). In 1995, he was identified among the first 25 authors (the only European) in Immunology over the period 1990-94 (Science Watch 6: 1-2 May, 1995; Current Contents 38: 3-6, 1995), the first in the field of Human Immunology. He has been the most quoted Italian scientist between 1995 and 1998 and has recently been included by the Institute of Scientific Information among the "Highly Cited Researchers" in the field of Immunology for the last twenty years. Professor Romagnani is Past President of EFIS.

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The focus of Dr. Vukmanovic's research is studying development and function of T lymphocytes with the goal to manipulate the immune system in diseases requiring either enhancement (cancer, infectious diseases) or dampening (autoimmune diseases, allergies, transplantation) of the immune functions

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Professor Wekerle's scientific research is focused on the underlying reasons and mechanisms of diseases which arise due to a conflict between the immune system and the nervous system, his main focus being on multiple sclerosis

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In 1975, he was appointed Professor and Chairman for Pathophysiology and Immunology at the University of Innsbruck Medical School, where, from 1991 – 2003, he also was Director of the newly founded Institute for Biomedical Aging Research of the Austrian Academy of Sciences.

ABSTRACTS OF ORAL PRESENTATIONS

HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR SEVERE RHEUMATIC DISORDERS IN CHILDREN

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Since the year 2000, 8 children in the UK fulfilled the inclusion criteria and underwent haematopoietic stem cell transplantation (HSCT) for severe rheumatic disorders. Seven children with juvenile idiopathic arthritis (JIA) had autologous T cell depleted (TCD) HSCT. A child with JIA who failed autologous TCD HSCT and a child with systemic lupus erythematosus (SLE) had allogeneic HSCT from unrelated donor and HLA-identical sibling, respectively. We are reporting on the outcome, complications and long-term follow-up.

Outcome/Long-term Follow-up. JIA: 4/7 are well and off all treatment 4-8 years post, 2/7 relapsed 1-6 months post, requiring further anti-inflammatory therapy and allogeneic HSCT (1), and 1/7 died 4 months post HSCT. SLE: 2 years post HSCT alive and well, with limited skin chronic graft versus host disease (GvHD). The beneficial clinical response to HSCT can be dramatic, allowing stopping of all the immunosuppressive and anti-inflammatory treatment, catch-up growth and immense improvement of the quality of life.

Complications. Following immunosuppressive conditioning, adenovirus reactivation (2/8) with dissemination proved to be treatment-resistant and eventually lethal in 1, whilst EBV and CMV reactivation-driven haemophagocytic syndrome was life-threatening, but responded to antiviral and immunomodulatory treatment in 3 patients. Transplant-related infectious complications should be balanced against the life-threatening infections that occur in children with chronic disease refractory to (and with multiple, well-known side effects from) long-term immunosuppressive and anti-inflammatory therapies: 2 children with JIA referred for assessment for HSCT, whilst on methyl prednisolone, methotrexate and infliximab, developed fulminant central venous line related bacterial infections and died before conditioning for HSCT was started.

Even in the era of rapid development in novel therapies, there will be patients who do not respond and need other treatment options. How to identify these patients, how to appropriately clinically assess them, and how to optimize the timing for and decide about the 'auto vs. allo' HSCT procedure are some of the important questions awaiting answers from the on-going collaborative European study in which we participate.

CD8 T CELL MEMORY

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The ability to develop and sustain populations of memory CD8 T cells after infection and/or immunization is a hallmark of the adaptive immune response and a basis for protective vaccination against infectious disease. In response to infection, antigen (Ag)-specific CD8 T cells undergo massive expansion in numbers, acquire effector mechanisms, and disseminate throughout the body. The expansion phase is followed by a contraction (death) phase, where 90-95% of Ag-specific CD8 T cells are eliminated. The remaining Ag-specific CD8 T cells form the initial memory pool, which can be stably maintained for life. Major challenges for the future include identification of the precise input signals that shape CD8 T cell memory and determination of the molecular basis for how the responding CD8 T cells decode the myriad of signals encountered during immune responses to generate effective memory. The focus of this presentation will be on use of mouse models of infection and/or vaccination to examine and manipulate the characteristics of memory CD8 T cell populations after multiple antigen exposures.

IMMUNOGLOBULINS AND INFLAMMATION: SWITCHING BETWEEN STIMULATION AND SUPPRESSION

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When it comes to inflammation, immunoglobulin molecules exert a dual function. They can either stimulate or suppress the inflammatory response by exerting an opposing action on the complement system. Specific pathogenic and autoantibodies are known for their ability to activate complement upon interaction with their antigens on the surface of invading microorganisms or host's altered cells. This event sets in motion the effector, or "antibody-complementing" function of the complement system, leading to inflammation and immune damage. The remaining immunoglobulin pool, on the other hand, has the ability to quench the inflammatory reaction if it is exaggerated and potentially harmful, by scavenging active complement fragments. In doing so, immunoglobulins engage different portions of their molecules - F (ab)'2 to neutralize anaphylatoxins and Fc to bind C3b, C4b and iC3b. The ability to scavenge complement fragments, and thereby suppress inflammation, is not associated with any known phenotypic marker used to classify immunoglobulins, including allotypes (glycoforms). In the murine system, quantitative changes of the carbohydrate residues within the Fc IgG fragment and corresponding Fc receptors may play a role in switching between pro and anti-inflammatory functions. The exact mechanism of this phenomenon and its confirmation in the human system remains to be demonstrated.

MYELIN BASIC PROTEIN EPITOPE LIBRARY: PATH TO DIAGNOSTICS AND TREATMENT OF MULTIPLE SCLEROSIS

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The pathologic role of autoantibodies in autoimmune disease is widely accepted. Direct penetration of autoantibodies through the blood-brain barrier and their co-localization with neural tissue-specific autoantigens may explain their possible contribution in the neurodegeneration. The aim of this study is to determine myelin basic protein (MBP) epitopes specific for the autoantibodies in MS and compare these data with those from other neuronal disorders (OND) and rodent models of MS leading to the generation of new diagnostic and prognostic criteria.

We constructed a MBP-derived recombinant "epitope library" covering the entire molecule. We used ELISA to define the epitope binding/cleaving activities of autoantibodies isolated from sera of 26 MS patients, 22 OND patients, 11 healthy individuals and EAE mice and rats.

The levels of autoantibodies to MBP fragments 48-70 and 85-170 as well as whole MBP and myelin oligodendrocyte glycoprotein (MOG) molecules were significantly higher in sera of MS patients than healthy donors. In contrast, selective reactivity to the two MBP fragments 43-68 and 146-170 distinguished the OND and MS patients. In terms of binding DA rats were the most similar rodent model to MS. Rat's treatment by the respective peptides seems very promising and significantly reduces disease onset. Thus using myelin basic protein epitope library approach we determined specific characteristics of MS autoantibodies compared to OND and healthy donors. These data may serve as additional biomarker of disease progression and may open new paths in MS treatment.

SCHISTOSOMA MANSONI EGG ANTIGENS DIRECTLY AND INDIRECTLY INDUCE REGULATORY T CELLS THAT PREVENT DIABETES IN NOD MICE

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Schistosoma mansoni soluble egg antigens (SEA) profoundly regulate the infected host's immune system. SEA also prevents type 1 diabetes in NOD mice and splenocytes from SEA-treated mice have reduced ability to transfer diabetes to NOD.SCID recipients. We show the CD25 T cell depletion of splenocytes from SEA-treated donors restored their ability to transfer diabetes. Foxp3 T cells in NOD mouse pancreas increased with SEA-treatment, as did generation of Foxp3 cells from naïve NOD mice CD4 T cells in vitro. SEA polarization of T regulatory cells (Tregs) was TGFbeta-dependent, and SEA induced a range of phenotypic and functional changes in peritoneal macrophages, including induction of TGFbeta. SEA also increased expression of TGFbeta, integrin beta8 and galectins in CD4 T cell, suggesting that it had an additional direct effect on host T cells.

IMMUNOLOGY OF PERIAPICAL LESIONS

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Periapical lesions (granulomas and cysts) develop in response of periapical tissue to chronic stimulation caused by microorganisms that invade and destroy the dental pulp. The composition of infiltrating cells depends on the type of cellular and humoral immune response to bacterial antigens, the frequency of reinfection, duration of the inflammatory and immune processes, bone destruction and the immunocompetence of the host. However, the mechanisms involved in the development, progression and restriction of periapical processes are poorly understood. This paper summarizes our own research in this field, especially, related to the composition of infiltrating leukocytes (studied by immunocytochemistry and flow cytometry) and the balance of cytokines, produced by Th1, Th2, Th17 and T regulatory (Treg) cells. The levels of cytokines were correlated with the phenotype of inflammatory cells and clinical features of the lesions. The Th1 immune response, measured on the basis of IFN-γ production and the expression of IL-18R by T cells, was characteristic for the lesions with higher frequency of T cells and correlated with the proportion of dendritic cells, macrophages and IgG2+ B cells/ plasma cells. The Th2 immune response was predominant in a relatively small number of lesions in which the levels of IL-4 and IL-5 correlated with the number of mast cells and IgG4+ cells. However, neither Th1 nor Th2 response was associated with the clinical presentation of the lesions, including the extent of bone destruction. The production of IL-17 was higher in lesions with the predominance of T cells over B cells/plasma cells, and clinically symptomatic lesions. Since the level of IL-17 correlated with the production of IL-8 and proportion of neutrophils, one can assume its role in exacerbation of inflammation. Most CD4+ T cells in clinically symptomatic lesions coexpresed IFN-g and IL-17. However, the production of these cytokines did not correlate with the levels of their inducers, IL-12 and IL-23, respectively. The frequency of CD4+CD25hiFoxp3+ cells in the lesions was higher then in peripheral blood and correlated with levels of both TGF-β an IL-10. The ability of these cells to suppress the immune response was confirmed by using coculture experiments in vitro and the mechanism is associated with IL-10 production. The negative correlation between the frequency of Treg and IL-17 (Treg-hi/ IL-17-lo) was observed only in a small subgroup of clinically asymptomatic lesions with the predominance of B cells / plasma cells over T cells. These lesions were characterized by a low production of IL-6, high production of TGF- and low IL-12/ IL-10 and IL-23/ IL-10 ratios. In conclusion, our results suggest the complexity of immune response in periapical lesions and that both the proinflammatory and immunoregulatory mechanisms are involved in the immunopathology of these chronic inflammatory processes.

THE ROLE OF THE IL-1 FAMILY IN INFLAMMATION.

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Of the 11 members of the IL-1 family of ligands, some are clearly mediators of inflammation, some are also mediators of immune responses yet others are functioning to suppress inflammation. Angiogenesis is now one of the more important aspects of IL-1 family biology. Four members of the IL-1 family are under the control of caspase-1 and hence the regulation of the caspase-1 inflammasome affecets inflammation. For example, IL 1β and IL 18 are pro-inflammatory cytokines and suppression of the inflammasome results in less inflammation whereas activation of the inflammasome increses inflammation due to either IL 1β or IL 18. The ligand for ST2, an IL 1 receptor family regulating Th2 responses is IL 1 family member 11 (now called IL 33). IL 33 is also regulated by caspase-1. The IL 1 family member IL 1F7 binds to the IL 18 receptor alpha chain (IL 18Rα). However, this binding does not result in a pro-inflammatory response but rather an anti-inflammatory response. Over expression of IL 1F7 in cells of murine or human orgin results in suppression of Toll-like receptor mediated cytokine production as well as IL-1-mediated inflammation. Thus, within the IL-1 family, ligands fucntion to increase as well as decrease inflammation. In the case of the IL 1 receptor antagonist (IL 1 family member 3), the reduction in inflammation is highly specific and targets only IL 1α and IL 1β responses. In the case of IL 1F7, the reduction in inflammation is global and not restricted to IL 1 or IL 18. but rather to several challenges.

MECHANISMS OF T CELL ACTIVATION AND RECRUITMENT IN ASTHMA

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Asthma is an inflammatory disease where T cells play a central role, orchestrating other inflammatory cells and modulating tissue remodeling. Our group has been investigating the evidence and mechanisms of T cell activation and chemotaxis and has to that effect developed an ex vivo (explant) model whereby bronchial biopsies from volunteers are placed in culture and stimulated with appropriate stimuli (e.g. allergen). This has enabled experimental agents to be applied in a safe manner in order to investigate the production of relevant cytokines and chemokines, the utilization of transcription factors and signaling pathways. Thus, initially a role was identified for the CD4+ cell chemotactic cytokine IL-16 and RANTES, but subsequent studies showed a prominent role of chemokines (TARC and MDC) acting upon their receptor CCR4, the expression of which was shown to increase within both the asthmatic airways and the peripheral circulation where the numbers of CCR4+ cells correlated with the clinical severity of asthma. Application of CCR4 specific blockers in chemotaxis experiments using supernatants of explant cultures as the source of chemotactic activity abrogate most of the chemotactic activity for CD45RO+ T cells, strongly pointing to the CCR4/MDC-TARC axis as central to the accumulation of T cells in asthma. More recent studies have identified a role for PI3K in both activation and chemotaxis of T cells. Together these studies provide pre-clinical proof of concept for mechanisms and targets for which novel drugs could be developed. Importantly, our phenotypisation of T cells present in the airways of patients with asthma did not find large numbers of iNKT cells (Vijayanand, NEJM 2007) previously reported to be high (as much as 80% of T cells) in severe forms of asthma (Akbari, NEJM, 2006); this argues against CD1d-restricted T cells being key and favours the traditional view that asthma involves MHC-class restriction.

THE ROLE OF THE COMPLEMENT SYSTEM IN THE PATHOGENESIS OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is the most common inflammatory and demyelinating disease of the central nervous system. The histopathologic hallmarks of the disease include focal infiltration of lymphocytes and other inflammatory cells into white matter that cause demyelination and axonal damage. It is thought that in both MS and its animal model experimental autoimmune encephalomyelitis (EAE), that infiltrating CD4+ T cells initiate an inflammatory process and collect other immune effectors to mediate tissue damage. However, the pathophysiology of the disease remains unclear. We focus on the role of the complement system in the pathomechanism of the disease.

Female C57BL/6 mice were immunized with myelin oligodendrocyte glycoprotein (MOG) peptide 35-55 emulsified in complete Freund's adjuvant and pertussis toxin. Clinical signs of EAE were rated daily using a standard scale of 0 to 5. Our results show that in animals with transiently depleted complement activity (achieved by injecting CVF ip) the onset of the disease is significantly delayed, while its severity is not changed compared to mice with normal complement system. Histology of the spinal chords, isolated 2 weeks after the onset, showed no difference between the CVF-treated and non-treated group. Regarding the level of MOG specific antibody – as measured by ELISA – no correlation was found with the clinical scores. We investigated the in vitro response of antigen-specific T cells isolated from the lymph nodes of MOG-immunized animals at the onset of EAE. As antigen presenting cells bone marrow-derived dendritic cells were used. Our results show that the proliferative capacity of MOG-specific T cells derived from CVF treated animals is significantly lower than in the control group. Our data suggest that complement has a modulatory effect in the pathogenesis of EAE; i.e. lack of complement activity at the time of induction delays the onset of the disease and results in the generation of MOG-specific T cells with significantly decreased activity.

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CANCER IMMUNOSURVEILLANCE AND IMMUNOTHERAPY

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Numerous human tumor antigens have been characterized and a large number of them have been shown effective in preventing tumor growth in animal models. Based on in vitro data and animal models, some have been tested as components of cancer vaccines in Phase I and II clinical trials.

We have previously reported on two human tumor antigens, MUC1 and cyclin B1 (CB1), and on specific differences between their expression on normal cells versus tumor cells. We have shown that these differences are key to their immunogenicity and that in transgenic animal models, immune responses induced to their tumor forms protect from tumor challenge. MUC1 has also been tested in several Phase I/II trials by us and other investigators around the world. In order to pave the way for use of MUC1 and CB1 cancer vaccines for cancer prevention, we have focused on obtaining information and will report on the following topics: 1) Expression of these antigens on cancer stem cells; 2) Expression of these antigens on premalignant lesions; 3) Evidence for successful immunosurveillance of these antigens in spontaneous mouse tumor models; 4) Evidence for successful immunosurveillance of these antigens in humans.

CATALYTIC ANTIBODIES AND AUTOIMMUNITY

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Discovery of catalytic antibodies (abzymes) was a revolutionary event that created new junctions between chemistry, biochemistry, immunology and pathology. The general concept of complementarity introduced in life sciences by Emil Fisher explains the driving force of various biological processes including genetic machinery, enzyme catalysis, ligand-receptor interaction, antibody-antigen recognition etc. Creation of abzymes as a new class of biocatalysts is based upon the intrinsic properties of immunoglobulin superfamily to produce complementary "molecular imprints" using the hypervariability of CDRs. These "catalytic imprints" could be made from the stable chemical analogs of transition state (TSA) of the enzyme reaction. This approach was successfully developed by Richard Lerner group. The alternative way to create abzymes was proposed by author. It is generated on the basis of the immunological network hypothesis of Niels Jerne and declares the formation of anti-idiotypic antibody repertoire razed against the active site of corresponding enzyme. This approach allowed us to generate abzymes with acetylcholinesterase and protease activities. In both cases one try to mimic the highly evolved enzymatic function by selection of antibody catalysts from the vast repertoire of immunoglobulines. This may give rise to biocatalysts with new functions, previously unknown for common enzymes, which may be very profitable for fine organic synthesis. This method stimulated our attempts to make antibody-like acceptors for phosphorus-based poisons. Recombinant antibodies with such functions were obtained recently in this lab using chemical selection of "naïve" phage-display library. The second advantage of abzyme field is the opportunity to make "catalytic vaccines". Traditional drugs including antibiotics and other small-molecule compounds developed in the pre-biotechnology era showed the limited success in a number of sever bacterial and viral infections. Numerous attempts to combat HIV infection using drug therapy as well as classical vaccination turned out to be ineffective. One of the targets for the novel therapeutic approach may be the main surface antigen, viral envelope protein gp120. The specific cleavage of this protein can lead to the dramatic changes in the immune response toward virus and decrease binding of HIV to CD4 receptor. This task impossible to be solved by enzyme therapy may have an effective abzyme alternative

A novel approach for creating catalytic antibodies against pathogens is described. This involves utilizing the autoimmune disorder of SJL mice induced by myelin basic protein as a background for raising a protein-specific catalytic response toward gp120. Site-specific abzyme-mediated cleavage of gp120 is demonstrated. This approach developed in this laboratory can be considered as a general strategy to obtain a catalytic vaccine to proteins of interest.

In our studies we firstly showed that catalytic antibody formation has the strong intrinsic and still enigmatic links with autoimmune diseases. The existence of DNA-specific abzymes in scleroderma, systemic lupus erithematosus (SLE), rheumatoid arthritis and AIDS was described in this laboratory. Very recently the input of abzyme activity in neurodegeneration process was demonstrated. Autoantibody-mediated tissue and cell destruction is among the main features of organ-specific autoimmunity. We have described abzyme contribution to neural tissue-specific antigens (Ag) degradation. AutoAb to myelin basic protein (MBP) from humans with multiple sclerosis (MS) and SJL mice with experimental autoimmune encephalomyelitis (EAE) exhibited site-specific antigen degradation. AutoAb from patients with the secondary progressive MS and highest scores on the expanded disability status scale (EDSS) demonstrated augmented catalysis. An established MS therapeutic Copaxone® inhibited reaction in vitro. autoAb catalysis thus appears to be a specific feature associated with MS pathogenesis and potential marker of disease progression.

INNATE IMMUNITY, EPIGENETICS AND AUTOIMMUNIY IN RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by the progressive destruction of the affected joints.

Although the new biologicals have made a major breakthrough in targeting and/or eliminating the immune cells, including T cells, B cells and monocytes/macrophages from the joints, the disease cannot be cured yet. The latter observation is based on the fact that the synovial fibroblast (SF) is endogenously activated and not targeted by any current therapeutic regimen.

Most interestingly, we could show that RA-SF are part of the innate immune system by expressing Toll-like receptors 2-5 resulting in the production of numerous powerful chemokines and cytokines. Thereby these factors are responsible for the repopulation of immune cells in the joints after ceasing cell depleting therapies.

To characterize the molecular mechanisms of synovial activation, we explore at present the pattern of acetylation, methylation and cell expression regulating microRNA.

To more comprehensively study the contribution of auto-antibodies to the disease, we utilized a cDNA library from RA tissue and the SEREX method to identify novel auto-antigens. Indeed, we could identify 18 new auto-antigens and found, for example, that a plasmin-inhibiting auto-antibody is functional.

SELF CONTROL BY TH1 LYMPHOCYTES

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While Th1 responses are critical for host resistance to many intracellular pathogens, they must be carefully regulated to avoid bystander immunopathology. Interleukin-10 is thought to be a major regulator of Th1 function. A dramatic example of this type of regulation occurs during murine Toxoplasma infection. In this model wild-type mice readily control infection through the induction of a potent IL-12-dependent Th1 response while similarly exposed IL-10-/- mice succumb to acute disease characterized by excessive proinflammatory and IFN-g cytokine production and tissue necrosis. We analyzed the source of regulatory IL-10 in mice infected with Toxoplasma gondii. Unexpectedly, conventional T-bet+ Foxp3- Th1 cells were found to be the major producers of IL-10 in these animals. Further analysis revealed that the same IL-10+IFN-γ+ population displays potent effector function against the parasite while paradoxically also inducing profound suppression of IL-12 production by APC. Although at any given time point only a fraction of the cells appeared to simultaneously produce IL-10 and IFN-g, IL-10 production could be stimulated in IFN-γ+IL-10- cells by further activation in vitro, indicating that the IFN-g+IL-10+ population does not represent a specialized Th subset. Thus, IL-10 producing Th1 lymphocytes have a distinct profile of immunoregulatory properties that allows their expansion in the context of strong Ag-specific Th1 priming and serve the primary purpose of limiting collateral host damage while avoiding sustained suppression of effector function. The relevance of these findings extends beyond T. gondii infection, since a similar populations of IL-10+ producing Th1 cells in mice infected with Leishmania major and Trypanosoma cruzi, as well as in Th1 clones from Mycobacterium tuberculosis infected donors. We speculate that IL-10+IFN-g+ cells, may have broad regulatory function in multiple setting involving highly polarized Th1 responses to infection agents.

ACTIVATION AND INHIBITION OF NK CELLS BY MURINE CYTOMEGALOVIRUS

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Natural killer (NK) cells play a crucial role in the resistance to several viruses. They are among the first cells to sense the release of proinflammatory cytokines as well as the perturbations in the expression of MHC class I molecules and other surface molecules induced by viral invasion of cells. In recent years, a remarkable progress has been made in understanding of NK cell biology and their role in the control of viral infections. Various viral functions have evolved to counter NK cell response, illustrating the evolutionary struggles between viruses and NK cells.

Cytomegaloviruses (CMV) are species specific viruses and there is no animal model for direct studies of human CMV (HCMV) infection in vivo. Infection of mice with murine CMV (MCMV) as a model of HCMV infection has been particularly informative in dissecting the role of innate and adaptive immune response mechanisms. With regard to the early MCMV control by NK cells, laboratory mouse strains fall into two main categories: a minority of strains is resistant to the virus and they can mount a strong NK cell response, whereas other mouse strains, including wild mice, are susceptible and develop only weak NK cell response. Notably, some of MCMV resistant strains possess NK cell receptors specific for the viral proteins. However, even if the host is lacking activation NK cell receptors that can recognize viral proteins, ligand engagement by NKG2D should be able to activate NK cells, especially keeping in mind that the cellular ligands for NKG2D are inducible by infection.

Our laboratory has pioneered the work on the characterization of several MCMV proteins which prevent NK cell activation by down-modulating the expression of cellular ligands for the NKG2D receptor. We have described four MCMV proteins, m138, m145, m152 and m155, which are involved in the down-modulation of the expression of NKG2D ligands MULT-1, RAE-1 and H60. The significance of these viral inhibitors on NK cell activation was also demonstrated in vivo using the mutant viruses possessing the deletions of these MCMV genes. The primary focus of my talk will be the MCMV evasion of NKG2D during the early days post infection, but also their role in chronic/latent infections. The significance of other MCMV mechanisms involved in the activation or inhibition of NK cells will be discussed as well.

DEFECTS OF DENDRITIC CELL, CYTOKINE AND TH-17 IMMUNITY IN HUMAN CHRONIC CANDIDIASIS

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Patients with Chronic Mucocutaneous Candidiasis (CMC) suffer persistent infections with the yeast Candida. CMC is a heterogeneous group and includes patients with AIRE gene mutations who have Autoimmune Poly Endocrinopathy Candidiasis Ectodermal Dystrophy (APECED), and patients without known mutations (non-APECED). The immune defect(s) remains unknown, but dysregulated cytokine production has been documented (Lilic et al, Infect Immun 2003).

To investigate dendritic cells (DCs) as central orchestrators of cytokine production, we generated monocyte-derived DCs (moDCs), stimulated them with Candida albicans, Toll-like receptor 2/6 ligand and lipopolysaccharide, to assess cytokine production (IL-12p70, IL-23, IFNg, IL-2, TNFa, IL-6, TGFb, IL-10, IL-5, IL-13) and cell-surface maturation marker expression (CD83, CD86, HLA-DR). In both APECED and non-APECED CMC patients, we demonstrated impairment of DC function: 1) both groups over-produced IL-2, IFNg, TNFa, IL-13 and demonstrated impaired DC maturation. 2) Only APECED patients showed DC hyper-activation. 3) In contrast, only non-APECED patients showed markedly decreased Candida-stimulated production of IL-23 and markedly increased production of IL-6, suggesting impairment of the IL-6/IL-23/Th17 axis (Ryan et al, Eur J Immunol 2008, submitted).

As Th-17 cells were recently shown to be involved in protective immune responses against Candida, we assessed generation of Th-17 producing cells by intracellular staining, following stimulation with Candida and non-Candida antigens in APECED and non-APECED CMC patients. We found that non-APECED patients had markedly lower percentages of total CD4+ IL-17+ cells and significantly lower percentages of CD4+CCR6+CCR4+ IL-17+ cells of the Th-17 lineage, while in contrast, percentages of neither CD4+IFNg+ nor CD4+CCR6+CXCR3+ cells of the Th1 lineage, were decreased. Surprisingly, patients with APECED did not show this impairment and had percentages of IL-17 cells comparable to controls. Lastly, we demonstrated (Meloni et al, J Clin Endocrinol Metab, submitted) that all our APECED patients (but none of the non-APECED) had high titers of auto antibodies to type1 interferons.

In summary, these are the first reports of different pathogenic mechanisms on the same immune response pathway, underlying increased susceptibility to Candida infection involving DC, cytokine and Th-17 defects in APECED versus non-APECED patients.

NOVEL CYTOKINES IN INFECTIOUS AND INFLAMMATORY DISEASE

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Cytokines are the hormones of the immune system and play a pivotal role in the induction and regulation of immune response. Cytokine targeting is arguably the most important contribution of immunology to clinical practice. Thus there is a considerable interest in the search for novel cytokines. The latest members of the cytokine family are IL-33 and IL-35.

IL-33, a member of the IL-1 family, is the newly discovered ligand for the orphan receptor ST2 which is expressed on a subset of Th2 cells (but not on Th1 cells) and mast cells. IL-33 is also able to skew a predominantly Th1 cell population to a mainly Th2 cells phenotype in vivo. IL-33 could attenuate an on going atherosclerosis in ApE-/- mice on high fat diet. The disease attenuation was accompanied by the elevation of IL-5 and IL-6 production and the reduction of IFN synthesis in vitro and in vivo. Furthermore, the IL-33 -treated mice produced increased level of anti-oxLDL antibody which is known to be protective against atherosclerosis. The effect of IL-33 on atherosclerosis was reversed by the co-treatment of the mice with sST2 (a decoy receptor of IL-33) and anti-IL-5 antibody. Hence it appears that IL-33 may be a potential therapeutic agent against atherosclerosis via the induction of IL-5 produced by Th2 cells and consequently enhances the production of anti-oxLDL antibody by B cells. However, IL-33 is a double-edged sword. It can also enhance allergic reaction and inflammatory disease, such as arthritis and asthma.

IL-33 is expressed in synovial fibroblasts from patients with rheumatoid arthritis (RA). Expression is markedly elevated in vitro by inflammatory cytokines, such as IL-1 and TNFα. Mice lacking ST2 developed attenuated collagen-induced arthritis (CIA) and reduced ex vivo collagen-specific induction of proinflammatory cytokines (IL-17, TNFα and IFN-γ), and antibody production. Conversely, treatment of wild type (WT) but not ST2-/- mice with IL-33 exacerbated CIA and elevated proinflammatory cytokine and anti-collagen antibody production. Mast cells express high levels of ST2 and responded directly to IL-33 to produce a spectrum of inflammatory cytokine and chemokines in vitro. In vivo, IL-33 treatment exacerbated CIA in ST2-/- mice engrafted with mast cells from WT but not from ST2-/- mice. The disease exacerbation was accompanied by elevated levels of proinflammatory cytokine expression. Thus IL-33 is a critical pro-inflammatory cytokine for inflammatory joint disease.

IL-33 mRNA is expressed early during parasite infection of the intestinal-dwelling nematode Trichuris muris in mice. Susceptible BALB/c mice can be induced by IL-33 to expel the parasite. Thus IL-33 may be evolutionally preserved for the host defense against intestinal parasitic infection.

IL-35 is the latest cytokine of the IL-12 family. It is formed by pairing Epstein-Barr virus-induced gene 3 (EBI3) and the p35 subunit of IL-12. The Fc fusion protein of IL-35 induced proliferation of murine CD4+CD25+ and CD4+CD25- T cells when stimulated with immobilized anti-CD3 and anti-CD28 antibodies in vitro. The IL-35-expanded CD4+CD25+ T cell population expressed Foxp3 and produced elevated levels of IL-10, whereas the IL-35-induced CD4+CD25- T cells produced IFNγ but not IL-4. The in vitro-expanded CD4+CD25+ T cells retained their suppressive functions against CD4+CD25- effector cells. Furthermore, when cultured with soluble anti-CD3 and antigen-presenting cells, IL-35 suppressed the proliferation of CD4+CD25- effector cells. Moreover, IL-35 inhibited the differentiation of Th17 cells in vitro. In vivo,

IL-35 effectively attenuated established CIA in mice with concomitant suppression of IL-17 production but enhanced IFNγ synthesis. Thus IL-35 is a novel anti-inflammatory cytokine suppressing the immune response through the expansion of regulatory T cells and suppression of Th17 cell development.

GALECETIN-3 PROMOTE AT MULTIPLE LEVELS DEVELOPMENT OF T CELLS MEDIATED AUTOIMMUNITY

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Galectin-3 (Gal-3) is one of 15 members of a β galactoside –binding lectins conserved through animal evolution. Recent studies suggested that by its effects on cell growth, apoptosis, cell migration and functions, Gal-3 may be important in inflammation. However, roles of Gal-3 in autoimmunity (in contrast to Gal-1 and Gal-9) have not been studied. We have used two models of T cell-mediated diseases; multiple low dose(MLD-ST2) induced diabetes and MOG_{35-55} peptide induced experimental allergic encephalomyelitis (EAE) in susceptible C_{57} BL/6 mice. We analyzed the susceptibility to these experimental diseases in "wild type" (GAL-$3^{+/+}$) mice and "knockout" (GAL-$3^{-/-}$) mice on C_{57} BL/6 background, as evaluated by clinical, histological and biochemical criteria.

Gal-$3^{-/-}$ deficiency significantly reduced the severity of MLD-STZ induced diabetes and EAE. This attenuation of disease correlated with lower expression of proinflammatory cytokines in the islet of pancreas and CNS. Flow cytometric and histochemical analysis indicated that Galectin-$3^{-/-}$ mice contained fewer monocytes and macrophages but more apoptotic cells in the target organs.

Further analysis in EAE model revealed that following antigen stimulation in vitro, lymph node cells from the GAL-$3^{-/-}$ mice produced less IL-17 and IFN-γ^{+} than that of the "wild type" mice. In contrast, GAL-$3^{-/-}$ mice produced more serum IL-10, IL-5 and IL-13 than the WT mice. Furthermore, GAL-$3^{-/-}$ mice contained higher frequency of $Foxp3^{+}$Treg cells in the CNS. Bone marrow derived dendritic cells (BMDC) from Gal-$3^{-/-}$ mice produced more IL-10 in response to LPS or BLP than Gal-$3^{+/+}$ BMDC. Moreover, Gal-$3^{-/-}$ DC induced antigen-specific T cells to produce more IL-10 and IL-5, but less IL-17 than WT mice DCs. Together, our data demonstrate that Gal-3 plays an important disease-exacerbating role in T cell mediated autoimmune diseases through its multiplefunctions in enhancing cell migration, preventing cell apoptosis and increasing Th 17 and Th1 cell polarization.

TH1 AND TH17 CELLS - PARTNERS OR FOES IN CNS AUTOIMMUNITY?

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Numerous cytokines are implicated in all phases of the autoimmune responses leading to CNS pathology. The traditional concept of both experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS) pathogenesis suggests the major pathogenic role of Th1 cells and their signature cytokine, IFN-γ as evidenced by the Th1 nature of CNS infiltrates and their adoptively transferred encephalitogenic potential. However, Th1 paradigm was seriously questioned by the discovery that inactivation of molecules involved in IFN-γ mediated signaling exacerbated EAE. Therefore, it is postulated that IFN-γ is not only redundant, but could even be restricting for the pathogenesis of EAE. IL-17 has emerged as a crucial pathogenic factor in EAE and presumably MS. A pivotal pathogenic role for IL-17 is substantiated by attenuation of EAE in mice deficient in IL-17. Recent studies have defined Th17 cells as a unique effector lineage, distinct from Th1 and Th2 effectors and promoted them into key effector cells in EAE. IFN-γ was shown to inhibit Th17 differentiation and consequently IL-17 production, which may be added to the list of its putative suppressive mechanisms in EAE. However, the crucial data supporting the concept of Th1-Th17 antagonism in EAE are obtained from studies in mice which were exposed to complex manipulations, such as gene knockout technology or

systemic treatment with antibodies. Therefore, IFN-γ might be an essential factor for the initiation of disease in more physiological conditions. Moreover, evidence demonstrating not only coexistence of Th1 and Th17 cells in the inflammatory infiltrate in the CNS, but also co-expression of IFN-γ and IL-17 by a same cell indicated that a concept of mere antagonism between these cytokines and respective cytokine-producing cells is an oversimplification. Whether IL-17 and IFN-γ single producers and IL-17+IFN-γ+ double producers represent distinct subsets, or are developmentally related, is not clear. To test the relationship between IFN-γ and IL-17 producing cells in EAE, and to find out the role for cells expressing both cytokines in the CNS autoimmunity we immunized DA rats with encephalitogenic emulsion and examined the kinetics of IFN-γ and IL-17-producing cells during EAE. Infiltrating mononuclear cells (inMNC), isolated from CNS in different phases of EAE (onset, peak and recovery), were analyzed for cytokine mRNA expression by real time PCR and for production of proteins by ELISA and intracellular staining, measured by cytofluorimetry. Our results show that both IL-17 and IFN-γ were expressed in the CNS of DA rats in the course of EAE with the highest production at the onset of the disease. Further, number of IL-17+ cells, but not of IFN-γ+ cells, declined among in MNC during EAE. Interestingly, among cells expressing IL-17 or IFN-γ, there was a significant proportion of cells capable of expressing both cytokines, and their percentage among in MNC also decreased from the onset till the resolution of the disease. These results, showing specific patterns of IFN-γ and IL-17 co-expression in DA rats, suggest that IL-17 might direct the initial inflammation, whereas IFN-γ might be important in prolonging and/or resolving tissue inflammation. Therefore, it is reasonable to argue that no single dominant cytokine, or effector cell population, will uniquely regulate the overall process of tissue damage. Further investigations which should explain the exact roles of IFN-γ and IL-17 in the CNS autoimmunity are necessary.

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TUMOR NECROSIS FACTOR AS MEDIATOR OF INNATE IMMUNITY AND INFLAMMATION: IMPORTANCE OF MOUSE MODELS.

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TNF is a critical mediator of innate immune reactions and inflammation, being activated downstream of many pattern-recognition receptors. We want to understand the consequences of TNF ablation in vivo, as it occurs in patients on continuous anti-TNF therapy. To do so, we have engineered animal models to explore the balance between beneficial and deleterious effects of TNF. Firstly, we generated a panel of mice with cell-type specific TNF ablation, allowing assignment of distinct TNF functions to distinct type of immunocytes producing TNF. Secondly, we have generated models tailored to study TNF blockade in adult immunocompetent organism, in particular, mice that are “humanized” for TNF. In such mice, which possess apparently normal immune and protective functions, human TNF compensates the loss of its murine counterpart in several TNF-dependent pathophysiological models. All blockers of human TNF can now be compared with mice`s. Additionally, a mouse model with inducible genetic TNF ablation has been generated, in which case TNF gene deletion occurred with high efficiency in hematopoietic cells, yet these mice retained some residual TNF signaling, similarly to patients placed on TNF blockers. These models of regulated TNF ablation are helping us to define the thresholds for distinct TNF functions.

T-CELL RESPONSE TO VIRAL INFECTIONS IN THE OLD AGE: HOMEOSTATIC AND FUNCTIONAL ASPECTS

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T-cell aging is marked by drastically reduced production and increased consumption of T-cells, resulting, amongst other changes, in the reduced TCR repertoire diversity and the appearance of T-cell clonal expansions (TCE). We recently showed that in specific pathogen-free mice, TCE most likely arise due to increased homeostatic and/or bystander proliferation, and are antigen-independent (e.g. are not responding to pathogens). Moreover, we showed that TCE, which accumulate regularly in aging rodents, primates and humans, have the potential to impair immune defense.

Latent persistent pathogens present a unique challenge to the immune system, and the immune system devotes a very large portion of its resources to contain these pathogens over the lifespan. Presence of latent herpesviruses, and, in particular, of CMV, correlates with increased exhaustion of the immune system and shorter lifespan in octogenarians. However, the relative roles of persistent antigenic stimulation and of the virus-independent senescence of the immune system were not conclusively addressed. We showed that animals infected with HSV-1 exhibit dysregulation of CD8+ T-cell memory pool with aging, in the form of "memory inflation" (increase in percentage of virus-reactive T-cells long after primary infection). Memory inflation was directly proportional to the spread of primary infection, and could be completely prevented by drugs that interfere with viral replication. Therefore, periodic, subclinical reactivation of HSV-1 is necessary to precipitate dysregulation of homeostasis of the memory CD8 compartment, suggesting that therapy of persistent viral infections may be beneficial in ensuring the functionality of the aged immune system. Finally, the results will be presented to demonstrate the existence of effector T-cell differentiation defects in aged T-cells which impair immunity against the West Nile virus.

INCREASED ATHEROSCLEROTIC LESIONS IN INTERLEUKIN-18 DEFICIENT APOLIPOPROTEIN E-KNOCKOUT MICE FED HIGH-CHOLESTEROL DIET REVEAL A ROLE FOR TH17 CELLS IN ATHEROSCLEROSIS

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Background: Atherosclerosis is a chronic immunoinflammatory disease elicited by accumulation of lipids in the artery wall. Interleukin-18 induces Th1 cells to produce IFN-γ, which stimulates vascular inflammation in atherosclerosis and inhibits IL-17 production by newly identified pro-inflammatory Th17-cell subset. Mice deficient in IL-18 develop obesity, hypercholesterolemia and arterial lipid deposits. Cholesterol, a major atherogenic agent, is known to stimulate production of PGE2, a strong regulator of IL-23 that is required for survival and expansion of Th17 cells.

In this study, we investigated the role of IL-18 in the development of atherosclerosis during high-fat diet in IL-18-deficient apoE-knockout mice. We hypothesized that in the absence of IL-18, severe hypercholesterolemia arising from persistent high-fat diet could modulate pro-inflammatory responses and enhance atherosclerosis with the preferential induction of pathogenic Th17 cells.

Methods and Results: IL-18-knockout (IL-18-/-) and apoE-knockout (apoE-/-) mice on C57BL/6J background were crossed to obtain IL-18-/-apoE-/- mice. Male IL-18-/-apoE-/- and IL-18+/+apoE-/- mice, aged 5 weeks (n=6/group), were fed high fat diet for 12 weeks. At termination, cryosectioned aortic arches were stained for atherosclerotic plaque measurement and immunohistochemistry. Ex-vivo vascular smooth muscle cells (VSMC), isolated from murine aortas, were

treated in vitro with cholesterol and homocysteine and IL-23 production assayed. Total serum cholesterol, LDL, HDL and triglyceride levels were significantly higher in IL-18-/-apoE-/- compared to IL-18+/+apoE-/- mice. IL-18-/-apoE-/- mice had increased the number of plaques and significantly bigger total plaque size; mean size of 0.244 ± 0.04μm2 for IL-18-/-apoE-/- and 0.113 ± 0.03μm2 for IL-18+/+apoE-/- (p=0.028). The increased atherosclerosis in IL-18-/-apoE-/- mice correlates with enhanced Th17 cells and IL-23-, producing VSMC in the plaques. In vitro, cholesterol induced VSMC from IL-18-/-apoE-/- mice aortas to produce increased amounts of PGE2 and IL-23, the latter enhanced by homocysteine.

Conclusion: In IL-18-deficiency high-fat diet induced greater dyslipidemia, which correlated with enhanced atherosclerosis via the alternative IL-23/Th17 pathway.

SHORT ARM OF CHROMOSOME 6: GENETIC CONTRIBUTIONS TO INNATE AND ACQUIRED IMMUNITY

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Introduction: Polymorphisms in several genes on the short arm of chromosome 6 (6p), among them VEGF (6p12), HLA-DR (6p21.3) and TNF-α (6p21.3), have been associated with inflammation, autoimmune diseases and transplant outcome. Strong linkage disequilibium (LD), between the HLA-DR and the TNF regions, has been identified within the HLA gene complex, through studying the autoimmune-associated diseases, such as SLE, RA and IDDM. We studied associations between SNPs outside (VEGF) and inside (TNF and HLA-DR alleles) the HLA gene complex. Independent segregation of these genes is unproven, and we intended to investigate linkage between distant genes on chr 6p and the putative existence of evolutionarily-conserved long-range 6p pro- or anti-inflammatory haplotypes.

Methods: SNPs studies were VEGF-2578*C/A (rs699947) and TNF-α -308*G/A (rs1800629) in 206 random and 80 selected HLA-DR52 positive individuals. SNPs were detected by TaqMan allele discrimination assays (Applied Biosystems, USA). HLA-DR was typed by serology or SSP. To simplify the analysis, the HLA-DR genotypes were collapsed to the 5 human ancestral HLA-DR supertypes, namely: DR51 [DR15(2), DR16(2)], DR52 [DR11(5), DR12(5), DR13(6), DR14(6), DR17(3), DR18(3)], DR53 [DR4, DR7, DR9], DR1 [DR1, DR10] and DR8. Linkage between paired genotypes was determined using ARLEQUIN 3.01 software and significance was determined by Chi-square and Markov chain/Fisher's exact test analysis.

Results: Significant allelic associations were evident across the 6p region examined.

Two putative extended haplotypes were identified, associated with DR52 and DR1.

	VEGF	HLADR	TNFα
VEGF	*	P<0.05	P<0.05
HLADR	P<0.05	*	P<0.05
TNFα	P<0.05	P<0.05	*

	DR52	VEGF*C	TNF*A		DR1	VEGF*A
Chi sq		6.79	32.54			17.14
P value		<0.05	<0.001			<0.001

Within the HLA-DR52 supertype, TNF*A was associated with DR3.

Discussion: The interval between VEGF and TNF-ά is 12.31Mb. Therefore, allelic associations are surprising considering expected recombination and the evolutionary time since divergence of DR supertypes. This suggests that DR1 and DR52 haplotypes have a survival advantage. Within the DR52 supertype, VEGF*C-DR3-TNF*A is a 'high inflammatory' haplotype associated with acute and chronic rejection. HLA and antigen processing and presentation is a central point of any acquired cellular immune response. On the other hand TNF-alpha is one of the crucial molecules in the inflammatory response. VEGF has enormous inflammatory potential, but also plays important role as a cytokine in the specific immune response. Conversely, DR1 appears to be associated with a 'low inflammatory' haplotype.

Conclusion: Distant genes on chromosome 6p cosegregate. This has implications for in transplantation, many inflammatory conditions and in the definition of HLA-disease associations.

TRICHINELLA SPIRALIS – HELMINTH THAT HOLDS BACK AUTOIMMUNITY

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Chronic helminth infections are often associated with polarized Th2 response and the wide range of immunomodulatory effects. Shaping the host's immune system, down-modulating responsiveness to bystander (third part) antigens and preventing excessive inflammatory responses, helminths help themselves and protect the host organism at the same time. Current studies show that parasitic worms or their products can dampen or even prevent a number of autoimmune and allergic disease models in experimental animals. Underlying mechanisms are in a focus of extensive explorations today. It seems that Trichinella, like other parasitic worms, has a strong impact on host immune system. Its capacity to prevent immunological diseases in animal models, such as experimental colitis and Type 1 diabetes, has been indicated recently. According to our results, Trichinella spiralis infection in rats leads to amelioration of experimental autoimmune encephalomyelitis. The effect is, at least in part, based on the Th2 cytokine bias and the strong regulatory cytokine response. One of underlying mechanisms could be the incomplete dendritic cell maturation driven by the presence of antigens from all three Trichinella life stages, as observed in our experimental model system.

REGULATORY PATHWAYS IN T CELLS ANALYZED UNDER NANOMETER PRECISION AND MICROSECOND TIME RESOLUTION USING SINGLE MOLECULE IMAGING

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The knowledge of the molecular mechanisms underlying formation of the immunological synapse is a key to understand T cell activation and consequently to identify targets for diagnosis and therapy of immunological diseases. Little is known, however, about the dynamic of subcellular organization, interactions and functions of the underlying molecules because of the lack of appropriate technology. We have recently made technological advances with single molecule imaging, allowing mobility measurements of proteins, lipids and DNA-molecules in the living cell at length scales of a few nanometers and a sub-millisecond time scale. Further, by developing fluorescence recovery after photobleaching at the single molecule level, we can now detect molecular cluster formation in the cellular plasma membrane of living cells, determine the load of each cluster and its life-time. We were also able to employ live Förster resonance energy transfer (FRET) on a large number of cells to visualize spatio-temporal activation of key signaling molecules during T cell activation. For these studies we constructed Lck biosensors by incorporating both cyan and yellow fluorescent proteins into Lck, the key protein tyrosine kinase for T cell signaling. Using these techniques, we can now provide a model of sequential molecular interactions after T cell receptor engagement, identified novel binding properties between the coreceptor CD4 and Lck by quantification in living cells the contribution of individual domains of these molecules for the interaction, and by FRET analysis of Lck biosensors new insight into molecular modification and cellular positioning of Lck for regulation of its activation in T cell signaling. In addition to the gain of significant scientific results by these techniques we also have evidence that they will allow the set-up of novel diagnostic platforms: Potential differences in the dynamic patterns of molecules in cells derived from patients in comparison to healthy individuals might not only give rise to novel fine-tuned diagnostic and prognostic platforms, but also to individually-tailored therapeutic strategies.

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MIF – A CYTOKINE AT THE TOP OF THE INFLAMMATORY CASCADE

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Macrophage migration inhibitory factor (MIF) is a pluripotent cytokine that plays a major role in the induction of immunoinflammatory responses. It is already proposed that MIF acts upstream of other proinflammatory cytokines, such as IFN-γ, TNF-α and IL-10 thus determining the magnitude and duration of inflammatory processes. The importance of MIF for the onset of inflammation was confirmed in our investigations using different approaches. The first approach utilized a model of immunoinflammatory diabetes induced by multiple low doses of streptozotocin (MLD-STZ). Both MIF inhibition (by specific antibodies or chemical inhibitor) and deletion (MIF-KO mice) reduced clinical and histopathological features of diabetes and severely down-regulated the production of proinflammatory cytokines (IFN-γ, TNF-α, IL-10 and IL-17). This resistance to diabetes induction was in correlation with highly preserved viability of MIF-KO beta-cells or pancreatic islets in response to death signal generated by cytokines (IL-10+TNF-α+IFN-γ) or STZ. What is more, MIF-KO islets produced lower amounts of self-destructive molecules (IL-1β and nitric oxide) upon toxic insult than WT counterparts. Seemingly, since MIF absence down-regulated Th1 and Th17 arms of immune response, it could be postulated that MIF is a key trigger of inflammation during MLD-STZ diabetes. The second approach was based on inflammation induced by complete Freund's adjuvant (CFA). Although paws of CFA-treated MIF-KO mice displayed similar manifestations of inflammation (redness and swelling) to WT animals, the number of lymphocytes from draining lymph nodes was considerably lower and they possessed reduced potency for IL-17 production and secretion. Moreover, MIF absence suppressed in vitro stimulated lymphocytes to produce IL-10, IL-6, IL-23 and IL-17 production suggesting that MIF is also involved in development and action of Th17 immune response. Our findings clearly show that production of MIF is necessary for and precedes the production of other proinflammatory mediators. This feature positions MIF at the top of inflammatory cascade.

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ADULT AND EMBRYONIC STEM CELLS FOR TREATMENT OF HUMAN DISEASES

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The advantages of producing patient-specific stem cells are that these can be tailor-made (i.e. are autologous in nature) for the patient and may overcome the need to administer lifelong immunosuppression following stem cell transplantation. Although the rationale for using embryonic stem cells (ESC), derived from fertilised or nuclear transfer (NT) embryos or after genetic manipulation of somatic cells (induced pluripotency or iPS cells), is an improvement of regenerative medicine, these fields remain clouded in different controversies. Theoretically, ESC and ESC-like iPS cells have ability to differentiate into 220 different cell types of human body, including cells with neural characteristics, muscle cells and liver cells, and are potentially valuable for the development of cell transplantation therapies for the treatment of various human diseases. However, there are a number of factors which may limit the medical application of ESC and iPS cells including viral contamination, formation of teratoma and/or immunorejection. It has been proposed that immunorejection could be circumvented in non-patient-specific stem cell lines by replacement of the major histocompatibility complex genes with host-specific genes via homologous recombination technology. Immunosuppressive strategies, such as overexpression of Fas-ligand in ESC or knocking out B7 antigens such as CD40, may also overcome the inability to use non-compatible stem cell lines, although these remain a complex molecular challenge and raise issues which are not as straightforward in overcoming as originally proposed, as recent evidence suggests that immune rejection endures following suppression of the CD40 pathway. Furthermore, recent proposals to generate histocompatible ESC banks, comprised of

a limited number of ESC lines, may be an effective alternate strategy; however, developing stem cell banks comprised of histocompatible and clinical grade ESC but patient-specific cells including adult, NT or iPS lines may be even more advantageous.

SELECTIVE SILENCING OF DISEASE-ASSOCIATED AUTOREACTIVE B LYMPHOCYTES BY CHIMERIC ANTIBODIES TARGETING THEIR INHIBITORY FCGAMMAIIB AND CD22 RECEPTORS

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There is an urgent unmet need for therapeutics that specifically target disease-associated auto-reactive B-lymphocytes. We hypothesized that it should be possible to suppress selectively such B cells by using chimeric molecules that cross-link their BCRs with surface inhibitory FcγRIIb and CD22 receptors. A series of chimeric antibodies was constructed by coupling copies of a DNA-mimicking peptide (J.Immunol.2000; 164:2542) and of the STN epitope (with a free terminal sialic acid, ligand for CD22) to a mouse monoclonal IgG antibody backbone (that would bind to FcγRIIb).

The chimeric antibody, added to cultured spleen cells from diphtheria toxoid-immunized MRL/lpr mice, caused a reduction of the numbers of anti-DNA, but not of anti-diphtheria IgG antibody-producing cells, proving that only the disease-associated B lymphocytes were targeted. Lupus-prone MRL/lpr mice, aged 7 and 18 weeks, were injected twice weekly with 20ug of the chimeric antibodies, with the same amount of the control chimeras or with PBS. The treatment of the 7-weeks old animals prevented the appearance of IgG and of IgM anti-DNA antibodies and of albuminuria in the next two months. The administration of the same antibody chimera to 18 weeks-old mice with full-blown disease resulted in the maintaining of flat levels of the IgG anti-DNA antibodies, in the delaying the aggravation of the lupus glomerulonephritis and in prolonging survival. The use of chimeric antibodies, targeting inhibitory B lymphocyte receptors, represents a novel approach for the selective suppression of pathological auto-reactive B cells and for changing the natural course of a spontaneous autoimmune disease.

VIRUSES AND Tregs – TWO SIDES OF A COIN

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Regulatory T cells (Tregs) are important regulators of immune responses. There are many different types of Tregs, and two important subgroups are FoxP3+Cd127-TGF-b+ Tregs and IL-10+ Tregs. We wished to understand their roles in type 1 diabetes and in chronic viral infections. Our data indicate that IL-10+ Tregs are potent immune modulators and can prevent T1D long term when induced in response to islet antigens. In viral infections, in turn, IL-10-producing T cells can be deleterious by causing persistence of the virus, when induced by CD8a neg dendritic cells. In our diabetes studies, FoxP3+ Tregs can also play important roles in delaying the onset of disease, but are induced to a lesser degree after tolerogenic immunization with islet antigens and are not as potent as IL-10+ Tregs when generated in an antigen specific manner. Nevertheless, the polyclonal CD25+FoxP3+ Treg compartment appears crucial for maintenance of immune homeostatsis and for preventintion of autoimmunity. Interestingly, following acute viral infections, this polyclonal Treg compartment appears to be invigorated and incidence of autoimmunity is lowered. Thus, there are probbaly differential functions for IL-10+ versus FoxP3+ tregs: The former are potent mediators of tolerance following antigen specific therapies (i.e. oral or nasal insulin), whereas the latter are crucial fro maintenance of overall immune homeostasis.

ACTIVATION OF HUMAN NK CELLS BY ADENOVIRALLY-ENGINEERED DENDRITIC CELLS

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Recombinant adenoviral vectors (AdV) are an effective modality for antigen engineering of dendritic cells (DC) for clinical induction of tumor antigen-specific T cells. Natural killer (NK) cell immune functions are regulated via cross-talk with DC, but little is known about the capacity of AdV-transduced DC (Ad.DC) to activate NK cells and stimulate their cytotoxic and regulatory abilities. In a previous study, we have made the observation that NK cells are activated in cancer patients following their vaccination with Ad.DC, suggesting that Ad.DC might be able to cross-talk with, and activate NK cells. Here, we investigated this possibility in short term cell cultures. We showed that AdV transduction did not enhance DC sensitivity to lysis mediated by NK cells, but rather enhanced the level of DC maturation and survival. Furthermore, Ad.DC induced activation of $CD56^{hi}CD16^{-}$ and $CD56^{dim}CD16^{+}$ NK cell subsets as evidenced by their expression of CD69 activation marker and IFN-γ secretion, as well as by their acquisition of increased ability to recognize the K562 target tumor cell line. The ability of Ad.DC to cross-talk with NK cells was dependent on AdV dose used for DC transduction. Ad.DC-mediated NK cell activation was induced by cell-to-cell contact, and was mediated, at least in part, by membrane-bound tumor necrosis factor alpha. Together, this data demonstrates, for the first time, that Ad.DC can efficiently crosstalk with NK cells in vitro, which may be an important additional mechanism by which this vaccine platform induces effective anti-tumor immunity.

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TUMOR NECROSIS FACTOR ALPHA: THE MASTERKEY OF INFLAMMATION AND IMMUNE REACTIONS

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Inflammation plays an important role in pathogenesis of infective, autoimmune, and cancerous diseases, while adaptive immunity is central to host defense against infection and cancer. Inflammation and adaptive immune mechanisms are mediated and regulated, respectively, by the central effector cells of innate immunity natural killer (NK) cells and dendritic cells (DCs), and their crosstalk. The essential mediator of both inflammation and regulation of adaptive immunity is tumor necrosis factor alpha (TNF). TNF is produced as plasma membrane-bound (memTNF) and soluble (solTNF) molecules. Both forms of TNF as well as two TNF receptors (TNFR1 and TNFR2) that have structurally different intracellular domains and activate different signaling pathways are expressed by both NK cells and DCs. We will present our novel findings that in NK cell-DC crosstalk memTNF and solTNF selectively utilize different receptors and mediate different key immune functions. Bacterial infection, exemplified by the bacterial lipopolysaccharide (LPS), rapidly and sequentially induces in DCs expression of memTNF, activation of TNF alpha converting enzyme (TACE) that convert memTNF into solTNF, and secretion of solTNF. LPS-stimulated DCs potently crosstalk with NK cells and selectively mediate via memTNF-TNFR2 interplay enhanced secretion of Th1 (IFN-Q and IL-12p70) cytokines that are essential for generation of effective adaptive immunity against pathogens and cancers. memTNF also down-regulates secretion of the potent immunosuppressive mediator PGE2 in NK cell-DC crosstalk. In contrast, solTNF selectively stimulates secretion of Th17 (IL-17) and Th3 (transforming growth factor beta and IL-10) cytokines in NK cell-DC crosstalk. Clinical studies with TACE inhibitors have previously indicated that solTNF might mediate autoimmune inflammation. We conclude that memT-

NF is a selective mediator of Th1 immune mechanisms, while solTNF is a selective mediator of Th17, inflammatory and immunosuppressive immune mechanisms, which are all critical to induction and regulation of immune reactions.

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THE KINETICS OF CD4+ FOXP3+ REGULATORY T CELL ACCUMULATION AND PROLIFERATION DURING AN ANTIGEN-SPECIFIC MEMORY RESPONSE IN HUMANS

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Although human naturally occurring regulatory T cells (nTregs; CD4+ Foxp3+) are highly proliferative in the blood, the kinetics of nTreg accumulation and proliferation during a localized antigen-specific T cell response is not known. After injection of tuberculin purified protein derivative (PPD) in the skin, CD4+Foxp3- (memory) and CD4+Foxp3+ (putative nTreg) T cell populations increased in parallel and proliferated equally over time. In contrast to CD4+ Foxp3- populations, CD4+Foxp3+ T cells in skin expressed typical regulatory T cell markers (CD25hi, CD127lo, CD27+, CD39+) and did not synthesize IL-2 or IFN-g after re-stimulation in vitro, indicating that they were not recently activated effector cells. To address the possibility that the CD4+Foxp3+ T cells in skin could be induced from memory CD4+ T cells in situ, we expanded skin-derived memory CD4+ T cells in vitro. When energized, these cells expressed high levels of CD25 and Foxp3 and suppressed the proliferation of skin-derived responder T cells to PPD challenge. Memory and regulatory CD4+ T cell populations are therefore regulated in tandem during a secondary response to antigen. Furthermore these studies highlight the possibility of inducing regulatory T cells from inflammatory CD4+ T cell populations isolated from tissues in vivo.

ALLOIMMUNIZATION IN PATIENTS WITH SICKLE CELL DISEASE: AN EXPERIMENTAL MODEL TO STUDY "IMMUNE RESPONSE GENES" IN HUMANS

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Patients with sickle cell disease (SCD) that receive therapeutic red blood cell (RBC) transfusions have a higher rate of anti-RBC antibody development than other transfused subjects. We have taken three approaches in attempt to uncover genetic basis thought to underlie the increased rate of anti-RBC antibody formation. Firstly, we hypothesized that an allele of a gene encoding a molecule with an immunomodulatory function may be in linkage disequilibrium with in the hemoglobin beta S (HbS) allele. To address this possibility, we are analyzing association of anti-RBC production and polymorphisms in five genes in the neighborhood of hemoglobin beta locus. Secondly, to test whether overproduction of a cytokine(s) may predispose SCD patients to develop anti-RBC antibodies, we are testing plasma cytokine levels and cytokine gene(s) polymorphisms in antibody-producing and non-producing SCD patients. Finally, to address a potential role of mechanism other than the former two, we are performing comparative gene profiling analysis in peripheral blood cells of antibody-producing and non-producing SCD patients. These studies will allow us to identify genetic marker(s) and perhaps uncover the pathogenesis of anti-RBC production in SCD patients. This, in turn, may teach us about genetic regulation of immune responses in human population.

AUTOIMMUNE T CELL MIGRATION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS: A JOURNEY THROUGH MULTIPLE MILIEUS.

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Myelin specific T cells are regular residents in the healthy immune repertoire. They become autoaggressive and mediate EAE upon activation either in vivo, by autoimmunization again myelin antigen in adjuvant, or in vitro, through recognition of the antigen presented by antigen presenting cells.

We explored the migratory pathway of activated encephalitogenic T cells from the injection site to their brain tissue destination. Our studies revealed that before reaching the CNS target, encephalitogenic cells have to pass through a scaled series of different tissue milieus, each of them inducing specific functional gene expression patterns that are required for the cells to reach the next location. Activated T cells injected i.v., which initially reach the lung, travel to the spleen, where they develop a "migratory" phenotype by down-regulating their activation markers, but up-regulate genes required for migration (chemokine receptors etc.). After the re-differentiation, T cells leave the spleen via the bloodstream and break through the endothelial blood-brain barrier. Using two-photon in vivo imaging, we observed that invasion of the CNS tissue also occurs stepwise. The migratory T cells attach to the inner surface of BBB vessel and crawl along and against blood flow before they abruptly pass the endothelial wall and continue to crawl on the outer surface. The contact with perivascular phagocytes communicates signals, which finally enable the T cells to penetrate into the CNS parenchyma, where they interact with local MHC class II positive cells presenting autoantigen.

The identification of stepwise interactions of encephalitogenic T cells, with different tissue milieus, may offer an opportunity to determine new therapeutic targets which could be of use in the treatment of multiple sclerosis.

CLASSICAL RISK FACTORS AS INDUCERS OF ANTI-ENDOTHELIAL CELL IMMUNE REACTIONS IN ATHEROSCLEROSIS

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The Autoimmune Hypothesis of Atherogenesis postulates that, preexisting cellular and humoral immunity to either microbial heat shock protein 60 (HSP60) or bona fide autoimmunity to biochemically altered autologous HSP60, leads to an attack on stressed arterial endothelial cells (ECs) (G. WICK et al, Annu. Rev. Immunol. 22: 361-403, 2004). We have shown previously in various animal models, with spontaneously occurring autoimmune diseases, that two essential sets of genes have to be present for an autoimmune disease to develop, i.e. genes that code for autoimmune reactivity of the immune system and genes that are responsible for target organ susceptibility (G. Wick et al Immunol. Lett., 16: 249-257). In the case of atherosclerosis, the target arterial ECs express HSP60 that is also transported to the cell surface after being subjected to classical atherosclerosis risk-factors. We have demonstrated the HSP60-inducing effect of most of these risk-factors, including mechanical stress (hypertension), oxygen radicals, oxidized low-density lipoproteins (oxLDL), proinflammatory cytokines (TNFα), and cigarette smoke constituents. Exposure to these classical atherosclerosis risk-factors entails the simultaneous expression of HSP60 and various adhesion molecules (ICAM-1, VCAM-1, P-selectin). Most recently, we were able to provide experimental evidence that infection of ECs with Chlamydia pneumoniae also represents a potent HSP60-inducing factor. Due to their lifelong mechanical pre-stress by the arterial blood pressure, arterial ECs have a lower threshold for the HSP60 inducing effect of atherosclerosis risk-fac-

tors as compared to venous ECs. However, when veins are subjected to arterial blood flow conditions, e.g. after arterial-venous bypass operations, HSP60 expression and intimal infiltration with mononuclear cells with subsequent restenosis occurs similar to the pathogenetic events in classical atherosclerosis.

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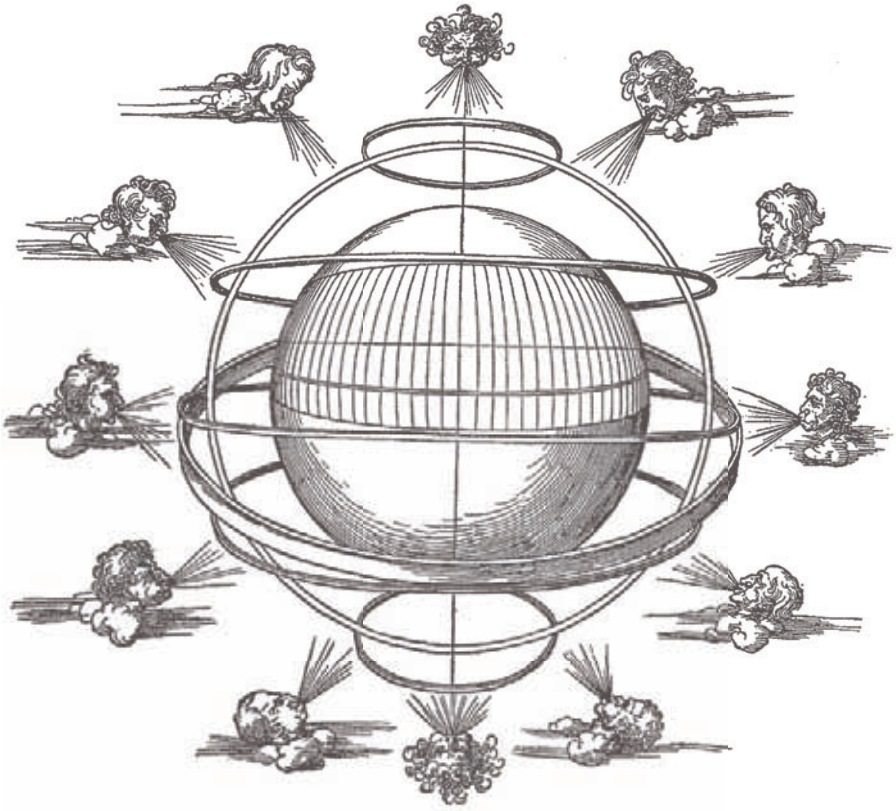

ABSTRACTS OF POSTER PRESENTATIONS

SILISA© – A NEW TEST SYSTEM FOR DETECTION OF PROTEIN SIGNATURE ON SILICONE SURFACES

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An inflammatory response with subsequent fibrotic reactions is the most common side effect in carriers of implanted active and passive medical silicones. This is evident from numerous mononuclear cells, including T-cells, dendritic cells and macrophages, which can be found in the surrounding fibrotic tissue[1]. However, the functional connection between the local inflammation processes and fibrosis remains poorly understood. In the present study, we focused our attention onto differential protein adhesion to the surface of medical silicones. 184 proteins adsorbed from the serum of silicone mammary implant (SMI) carriers to the surface of various types of silicone were identified, and their differential adsorption pattern analyzed in vitro[2]. We have developed a simple silicone linked immunosorbent assay (SILISA©) that can simultaneously detect the signature of the 14 differentially adhered proteins in a high throughput fashion. In a blinded cohort study of 100 SMI carriers, the SILISA successfully discriminated patients with adverse reactions to silicone implants. Furthermore, the same test can be used to assess various silicone types for the risk to induce fibrotic side effects, thus allowing the selection of the most biocompatible implant material for individual patient. Both large scale prospective and retrospective blinded studies are being conducted to validate these preliminary results.

The project has been supported by the CEMIT and the Lore and Udo Saldow Foundation.

Reference List

1. Wolfram D, Rainer C, Niederegger H, Piza H, Wick G. Cellular and molecular composition of fibrous capsules, formed around silicone breast implants with special focus on local immune reactions. J Autoimmun 2004 Aug;23(1):81-91.

2. Backovic A, Huang HL, Frari BD, Piza H, Huber LA, Wick G. Identification and Dynamics of Proteins Adhering To the Surface of Medical Silicones in Vivo and in Vitro. J Proteome Res 2007 Jan 5;6(1):376-81.

THE ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN PALMITATE-INDUCED DYSFUNCTION OF β CELLS

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Obesity-associated type 2 diabetes (T2D) has been recently characterized as a state of persistent, low-grade inflammation. It is suggested that during the final stage of T2D, pro-inflammatory mediators along with fatty acids have a role in β cell apoptosis. Since macrophage migration inhibitory factor (MIF) is increased in circulation of obese and T2D patients, our aim was to investigate the role of MIF in nutrient-induced β cell apoptosis, in vitro.

Treatment of pancreatic islets from C57BL/6 mice or MIN6 cells with palmitate, induced apoptosis (measured by histone-DNA ELISA and MTT) and caused elevation of MIF secretion after 24 h of incubation. However, the addition of exogenous recombinant MIF did not further promote islet apoptosis because of high endogenous concentration of this cytokine. Consequently, MIF treatment did not change bax/bcl-2 ratio or caspase 3, 8 and 9 expressions as determined by real-time PCR. Also, MIF did not impair islet glucose-induced insulin release, while glucose oxidation was slightly inhibited suggesting a mild islet dysfunction. In contrast, when MIF was blocked either by anti-MIF antibody or pharmacological inhibitor ISO-1, palmitate-induced apoptosis was significantly suppressed. This was accompanied by up-regulation of mitochondrial function (measured by JC-1 and rhodamine 1,2,3) and down-regulation of endonuclease G and AIF expression. On the other hand, palmitate-induced caspase 3, 8 and 9 expression remained unaffected during MIF neutralization. Although MIF inhibition did not improve already impaired glucose oxidation and insulin release, insulin and PDX-1 mRNA expression was

strongly increased. Finally, islets isolated from MIF knock-out mice (MIF-KO) displayed significant resistance to palmitate-induced apoptosis in comparison to wt islets. Taken together, these results suggest that MIF is probably involved in nutrient-induced apoptosis of pancreatic islets through promotion of mitochondrial dysfunction in caspase-independent fashion. Therefore, MIF inhibitors could be used as a novel therapeutic tool for preventing/blocking β cell failure.

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PALMITIC ACID AND LYMPH NODE CELLS COLLABORATE IN RAT INSULINOMA CELLS DESTRUCTION

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Diabetes is characterized by progressive failure of insulin producing β cells. Immune mediators produced by leukocytes and fatty acids are considered to be main actors in β cell destruction. In order to investigate their possible collaboration in β cell disruption, we co-cultivated rat insulinoma cells (RIN), as β cell model, with concanavalin A (ConA)-stimulated rat lymph node cells (LNC), in the presence or absence of high concentration of palmitic acid (PA, 100 μM). ConA-stimulated LNC and PA acted synergistically in RIN cell number reduction, as detected by MTT test after 24 hours of cultivation. The observed decrease was present when transwells were used to separate RIN and LNC, thus suggesting the role of soluble mediators. This assumption was confirmed by the finding that cell-free supernatants, collected from cultures of ConA-stimulated LNC or spleen cells (ConSn), acted with PA in disruption of RIN cells. Interestingly, similar effects were observed for ConSn from various strains of rats or mice, thus suggesting that the phenomenon was dependent on species- and strain-unspecific soluble molecules. Further, there was a significant accumulation of early apoptotic, Annexin V-positive RIN cells treated with ConASn and PA, determined by cytofluorimetry, as well as a rise of caspase-3 activity in these cells, detected by enzyme-activity assay, after 2 hours of cultivation. This rapid induction of apoptosis was accompanied with activation of Jun and p53 transcription factors, as determined by cell-based ELISA and was abrogated by pretreatment of RIN cells with SB202109, an inhibitor of p38 signaling molecule which acts up-stream of Jun and p53. Thus, we could conclude that soluble mediators from activated LNC and PA cooperate in activation of p38-Jun-p53 signaling and induction of apoptosis in RIN cells. In summary, these results imply that stimulated LNC could synergize with PA in destruction of β cells during pathogenesis of diabetes.

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THE ANTI-INFLAMMATORY EFFECT OF NEUROPEPTIDE Y (NPY) IN RATS IS DEPENDENT ON DIPEPTIDYL PEPTIDASE IV (DPIV) ACTIVITY AND AGE

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A sympathetic neurotransmitter neuropeptide Y (NPY) plays an important role in regulation of the immune and inflammatory responses. The pleiotropic action of NPY is accomplished by the multiplicity of NPY receptors, of which at least five subtypes (Y1, Y2, Y4, Y5 and Y6) have been identified so far. In addition to tissue-specific receptor subtype expression, NPY actions may also be regulated by its processing by dipeptidyl peptidase IV (DPIV, also known as CD26) which cleaves NPY1-36 to an Y2/Y5 receptor preferring peptide, NPY3-36, therefore terminating its function on Y1 receptor. The present study investigated the age-dependent effect of NPY on Concanavalin A-induced inflammatory paw edema and peritoneal macrophage nitric oxide production in Dark Agouti rats exhibiting a high plasma DPIV activity, as acknowledged earlier. The receptor specificity was examined by use of NPY related peptides with different individual pharmaco-

logical specificity for Y receptor subtypes. The results showed that plasma DPIV activity decreased, while macrophage DPIV activity, as well as macrophage CD26 expression, increased with aging. NPY-induced suppression of paw edema in adult and aged rats was mediated via Y1 and Y5 receptors. In contrast to the in vivo situation, NPY stimulated macrophage nitric oxide production in vitro only in young rats, and this effect was mediated via Y1 and Y2 receptors. It can be concluded that age-dependant modulation of inflammatory reactions by NPY is, at least in part, determined by plasma and/or macrophage DPIV activity at different ages. This work was supported by grant (145049) from the Ministry of Science, Serbia.

THE ROLE OF ADIPOSE TISSUE AND MACROPHAGES IN CHRONIC INFLAMMATION ASSOCIATED WITH OBESITY

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We performed a critical review of last five years literature in order to better describe the impact of adipose tissue and macrophages interactions in low grade chronic inflammatory condition in the obese and their role in comorbidities pathogenesis.

In the last decades, life style modifications, particularly those related to food patterns and food choices, gave rise to a worldwide pandemic disease with no precedent in Human History: obesity.

Energy storing and free fatty acids production were the only adipose tissue functions. However, it has been well established other adipose tissue functions like low grade pro-inflammatory molecules production (cytokines, adipokines and chemotatic factors) evolved in obese inflammatory condition. In this inflammatory state, adipocytes active role is inflammatory mediators (adipokines) production and and cell to cell interaction with resident macrophages. Adipose tissue low vascularization is even lower in the obese; so, hypoxia can be a critical factor in inflammatory obese state manifestation.

Adipose tissue cytokines production and pre-adipocyte conversion into macrophage results in adipose tissue and macrophages interactions. One of the mononuclear phagocytic system components – macrophage, has an important role in obese-related inflammatory state. These cells have been found to be increased in number and shape proportional to Body Mass Index, rising to up to 60% of total adipose tissue components. The macrophages proportional accumulation could lead to an increase in pro-inflammatory molecules expression and contribute to the inflammatory state in a significant way.

The reversion of the low grade inflammation and the reduction of risk factors in obese individuals seem to occur when a reduction in Body Mass Index is achieved and loss of adipose tissue is observed.

We concluded that increasing evidence has emerged to support the theory that adipocyte and macrophages interact not only by inflammatory mediators' production amplify from adipose tissue but also by pre-adipocyte conversion into macrophages.

INFLAMMATION AS A DETERMINANT OF ENVIRONMENTAL TOXINS IMMUNOTOXICITY: EXAMPLE OF CADMIUM

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The immunotoxicity of cadmium in terms of suppression of both humoral and cellular adaptive immune responses is well documented. Recent immunotoxicological investigations point to inflammation as the newly recognized aspect of toxicity of xenobiotics to the immune system. Our previous studies demonstrated an acute systemic response by cytokines, acute phase proteins and white blood cells, concurrent with hepato- and nephrotoxicity following i.p. cadmium (Cd) administration to rats. As lungs are a frequent target organ in the settings of systemic inflammation, we investigated the existence of inflammatory response in this organ. The presence of cellular (lung tissue neutrophil infiltration) and soluble (IL-6 and IL-17)

indicators of lung inflammation was determined 24 hours following i.p. administration of 0.5 and 1 mg/kg cadmium (as cadmium chloride) to male DA rats. Atomic absorption spectrometry revealed the presence of microgram quantities of Cd in the lungs following i.p. administration. Increased myeloperoxidase (MPO) content was found in homogenates of lungs from Cd-treated rats, indicating tissue infiltration by neutrophils. Most of the neutrophils were observed in the histologically evidently widened interstitium. Increased levels of IL-6 and IL-17 were found in lung tissue homogenates. Both lung cells and cells from the alveolar compartment were responsible for cytokine production, as judged by increased levels in the medium conditioned by bronchoalveolar lavage cells and cells obtained by collagenase/DNAse digestion of lungs. Increased levels of malondialdehyde (MDA) were noted in lung homogenates, indicating pulmonary toxicity of cadmium. The observed oxidative stress might have resulted from cadmium itself and/or from lung cell activity. Both resident macrophages and infiltrating neutrophils might have contributed to peroxidative events. The presence of cytokines that are known to influence neutrophil influx and/or activity corroborates such considerations.

PHARMACOLOGICAL CHARACTERIZATION OF THE CONCANAVALIN A-INDUCED PAW EDEMA IN TWO INBRED RAT STRAINS

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Our previous study revealed that rats of Dark Agouti (DA) strain developed more pronounced inflammatory paw edema upon intraplantar (i.pl.) administration of 50 μl of Concanavalin A (Con A, 400 μg per paw) than rats of Albino Oxford (AO) strain. The aim of the present study was to pharmacologically define Con A-induced paw edema and to investigate contribution of immune- and neurally-derived mediators of inflammation to the differences observed between these rat strains. Seven months old male DA and AO rats were i.pl. treated with 100 μg of antagonists specific for different receptors: opiod (naloxone), beta and alpha adrenergic (propranolol and phentolamine, respectively), histamine H1 and H2 (chloropyramine and ranitidine, respectively), 5-hydroxytryptamine 5HT3 (granisetron), or calcium antagonist verapamil. Thirty minutes later rats were i.pl. treated with 400 μg of Con A. Intensity of inflammation was measured by the diameter of tarso-metatarsal joints with nonius before and 3, 6, 9, 12 and 24h after injection of Con A. Results revealed that naloxone and granisetron suppressed development of inflammatory edema in both rat strains, confirming involvement of opioid and 5HT3 receptors in Con A-induced inflammation in DA and AO rats. In contrast, propranolol, phentolamine, chloropyramine, ranitidine and verapamil oppositelly affected Con A-induced inflammation in DA and AO rats. It could be concluded that difference in the expression and/or regulation of mediators of inflammation, especially those acting via adrenergic and histamine receptors, as well as calcium ions, in the subcutaneous tissue of DA and AO rats, contribute to the differences in the inflammatory response between these rat strains. (Supported by Ministry of Science, Belgrade, Serbia, Grant No145049)

IMMUNOLOGICAL BACKGROUND OF RECURRENT AIRWAY OBSTRUCTION (RAO) IN HORSES

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Recurrent airway obstruction (RAO) in horses, formerly known as chronic obstructive pulmonary disease (COPD) is an inflammatory obstructive disease of the airways characterized by hypersensitivity of the airway tissues to various different allergens, most commonly the spores of the fungi – Saccharopolyspora rectivir-

gula, Aspergillus fumigatus and Thermoactinomyces vulgaris, contained in the poor-quality hay and straw bedding. The disease is clinically manifested in the middle-aged horses with reccurent episodes of dyspnea, chronic cough, and reduced athletic and working capacity of affected horses. Genetic predisposition, infectious noxae, effects of the endogenous proteases and endotoxins lead to desquamation of the epithelial cells of the airways and denudation of the basal membrane, which leads to the direct contact of the various antigens and the immunologically active tissues which increase lung tissue hypersensitivity. Pulmonary alveolar macrophages (PAM) and CD4+ Th1 produce IL-8, (MIP-2), LTB-4, ICAM-1, CD4+ Th2 lymphocytes produce IL-4, IL-5, IL-13, B lymphocytes produce immunoglobulins (IgE, IgA and IgG). Allergens with IgE play an important role in degranulation of the mastocytes and the releasing of the histamine, bradykinin, LTC-4, LTD-4, PAF, PGD2, PGF2α, and eosinophils are also present. All these cells and the substances they release are very important for the pathogenesis of the RAO in horses as well as asthma in humans. Histopathological findings in 70 examined horses with RAO are chronic bronchitis/bronchiolitis, with characteristic changes in lumen, mucosa, submucosa and smooth muscle layer and distensive and destructive form of alveolar emphysema. Increase immunoreactivity of the tracheobronchal lymph nodes is also present. Most common cytological finding, obtained both by endoscopy and necropsy are the thick, viscous, PAS-positive mucus which forms Curschmann's spirals and neutrophils, desquamated airway epithelial cells and eosinophils. These findings, together with etiopathogenesis of this disease indicate that horses could be model for invesigations of hypersensitive diseases in alergology and pulmology.

IMMUNE INTERACTIONS BETWEEN NEUTROPHILS AND LEISHMANIA INFANTUM

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Dogs constitute the main reservoir for L. infantum, the parasite that causes zoonotic visceral leishmaniasis, playing a crucial role in parasite transmission. Neutrophils or polymorphonuclear leukocytes (PMN) belong to the first wave of cells that are recruited to the sites of infection and seem to be the first host cells of Leishmania during the initial phase of infection. However, it is unclear whether the uptake of L. infantum by PMN at the beginning of infection plays a role in the innate defense against the parasite or in silencing the presence of the parasite by favoring its survival. This study aims to characterize the role of PMN during the early phase of L. infantum infection through the evaluation of immune mechanisms. C57BL/6 mice PMN were isolated and infected in vitro with L. infantum promastigotes and the effect of the parasite on several PMN related-immune mechanisms like phagocytosis, oxidative burst, apoptosis, chemotaxis, cytokines and Toll-like receptor-2 (TLR-2) expression were further assessed. It was shown that PMN can indeed phagocyte L. infantum promastigotes and that this event is associated with the production of large amounts of superoxide anion (O2-) and with the inhibition of PMN spontaneous apoptosis. The expansion of the lifespan of PMN and the release of leishmanicidal reactive oxygen species may contribute to improve the control of parasite replication. On the other hand, the expression of interleukin-12 and 10, tumor necrosis factor-Qtransforming growth factor-Qand TLR-2 were downregulated in PMN that were co-cultured with L. infantum. The presence of the parasite also decreases PMN's ability to migrate towards fMLP stimulus. These facts could indicate that the parasite is negatively disturbing some of the PMN functions, allowing them to escape earlier immune responses that might control their survival. In conclusion, neutrophils appear to have an essential role during the early phase of L. infantum infection, although their immune mechanisms should be further dissected.

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ANTICANCER AND IMMUNOSENSITIZING PROPERTIES OF TOTAL DRY OLIVE LEAF EXTRACT

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One of the primary problems facing the treatment of cancer is development of drug resistance. Although immunotherapy is much more specific and less organ toxic, cells resistant to chemotherapy frequently acquired cross-resistance to immune- mediated killing. Therefore, in order to overcome chemo- and immune- tumor cell unresponsiveness, new therapeutic approaches are needed. Total dry olive leaf extract (DOLE) presents natural mixture of compounds with confirmed anti-inflammatory, antiviral, antibacterial and fungicidal proprieties. Additionally, numerous biologically active constituents of olive leaf demonstrate strong anticancer potential. In this study, it was shown, for the first time, that treatment of B16 mouse melanoma cells, mainly resistant to induction of apoptosis by conventional cytostatics, resulted in significant decrease of cell viability mediated by induction of dramatic apoptosis. Typical early apoptotic cell death was determined by Anexin - Propidium Iodide double staining only 18 h after exposure of B16 cells to DOLE, while DNA fragmentation, as a sign of late stage apoptosis was confirmed by elevated proportion of hypodiploid cells after 24 h treatment. Moreover, cell cycle distribution analysis revealed the accumulation of cells in G0/G1 phase indicating that DOLE possesses cytostatic as well as cytotoxic properties. The cell cycle arrest and apoptosis could be ascribed to the strong upregulation of cyclin D1 and D3 as well as significant increase of p53 expression. Observed capacity of the extract to upregulate the proapoptotic signaling in B16 cells indicated its possible role in immunosensitization of tumor cells to TNF-α mediated cytotoxicity. Actually, a treatment with DOLE strongly sensitized B16 cell to TNF-α-triggered cell death. In summary, naturally occurring compounds isolated from olive leaves were capable to downregulate the growth of B16 melanoma directly – through cytostatic and cytotoxic action and indirectly – sensitizing them to natural immune response mediated by TNF-α.

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STRAIN DIFFERENCES IN PERITONEAL MACROPHAGE ACTIVITY IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN RATS

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There is evidence for the critical role of hydrogen peroxide (H_2O_2) and the nitric oxide (NO) produced by phagocytes in development of inflammatory processes and autoimmune inflammatory diseases. In the present study we have investigated both phagocytotic and secretory activity of resident peritoneal macrophages of two rat strains: Dark Agouti (DA), susceptible, and Albino Oxford (AO), resistant to the induction of experimental autoimmune encephalomyelitis (EAE). Macrophages were obtained from rats immunized with guinea pig spinal cord in complete Freund's adjuvant (GPSC/CFA) on days 1, 3 and 7 post immunization (dpi 1, 3 and 7), as well as from non-immunized control rats, and were tested for zymosan phagocytosis, phorbol myristate acetate-stimulated H_2O_2 production and lipopolysaccharide-stimulated NO production. Before immunization macrophages from AO, rats demonstrated lower phagocytosis capability and higher H_2O_2 production in comparison with macrophages from DA rats, while cells from both strains produced comparable amounts of NO. All three macrophage functions tested in immunized rats of AO strain were suppressed on dpi 1 and/or dpi 3 and returned to the control levels on dpi 7. Immunization with GPSC/CFA did not influence H_2O_2 and NO production in peritoneal macrophages from DA rats, but increased zymosan phagocytosis on dpi 1. Taken together, our results suggest that an early increase in macrophage phagocytosis before the development of clinical signs of the disease in EAE-susceptible rats, as well as an early and

transient decline of macrophage functions following immunization with GPSC/CFA in EAE-resistant rats is associated with rat strain differences in the sensitivity to EAE induction. (This work is supported by Ministry of Science, Belgrade, Serbia, Grant No 145049.)

TECHNICAL APPROACHES OF ANTIGEN-SPECIFIC RESPONSE ESTIMATION IN EXPERIMENTAL MODEL OF AUTOIMMUNE ENCEPHALOMYELITIS

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Experimental autoimmune encephalomyelitis (EAE) is an established animal model for the human disease multiple sclerosis (MS) mediated by activated T cells specific for various myelin autoantigens. Although artificial immunization may not appropriate reproduce all the pathogenetic mechanisms most therapies of MS patients are based on EAE rodent model. Peripheral blood mononuclear cells and splenocytes were obtained from 3 groups of Wistar rats: animals with established EAE, immunized rats without clinical signs and control group. Cells were examined for their in vitro proliferative responses to ConA after 3 days and myelin antigens (syngeneic spinal cord homogenate (SCH) or myelin peptide cocktail of myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP)) after 6 days of cultivation using 3H-thymidine incorporated assay in three independent experiments. The spontaneous proliferation was significantly higher in rats with EAE whereas in immunized rats it was the same as in control group. The proliferative response to ConA increased in control group (p=0,02) as well as in immunized rats (p=0,05) without any changes in rats with established EAE. The addition of autoantigens in the culture showed the increase of antigen-specific response to both SCH (p=0,04) and peptide cocktail (p=0,02) in healthy rats, the enhancement of SCH-induced proliferation in immunized rats (p=0,04) while in animals with established EAE the response to myelin antigens was at the same level as spontaneous proliferation. These controversial results may reflect the involvement of immunoregulatory mechanisms, e.g. anergy, by means of which the spontaneous recovery of animals with EAE may occur.

THE ROLE OF COLOSTRAL ANTIBODIES OF VACCINATED SOWS ON CLASSICAL SWINEFEVER (CSF) PATHOGENESIS IN EXPERIMENTALLY INFECTED PIGLETS

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Classical Swine Fever (CSF) is a highly contagious disease of domestic pigs and wild boars caused by the virus CSFV that belongs to genus Pestivirus of the Flaviviridae family. During CSFV infection lymphoid organs are depleted while in peripheral blood severe lymphopenia develops which contributes to the immunosuppression. Tissue lesions and lymphocyte distribution depend on the virulence of the virus. CSFV E2(gp55) antigen localization corresponded with microscopic lesions. Endothelial cells, intravascular macrophages and mononuclear cells in the leptomeninges have been recognized to be infected with the virus. We investigated if colostral antibodies in piglets, originating from sows vaccinated with C-strain and challenged with CSF virus, affected the distribution of the B and T lymphocytes in mandibular lymph nodes. All of twenty 45 days old cross breed pigs of both sexes which were divided in three groups died until 22nddday post inoculation with Baker strain of CSFV. For the confirmation of detection of virus genome RT-PCR was used. LSAB immunohistochemistry method was performed for immunolabeling E2 (gp55) glycoprotein of CSFV in mandibular lymph nodes and brain, as well as CD3+ T lymphocytes and CD79+ B lymphocytes in mandibular lymph nodes. In the group of animals originating from unvaccinated sows against CSF, severe depletion of CD79+ B lymphocyte was detected. In the group of pigs originating from sows vaccinated with C-strain

(CSFV antibody positive group), a reduced number of CD79+ B lymphocytes was detected but B cells were still present in the periphery of the germinative centers of secondary lymph follicles. There was an increase of the number of CD3+ T lymphocytes in mandibular lymph nodes of pigs with or without maternal antibodies. Classical Swine Fever (CSF) is a useful animal model for biomedical research in pathogenesis of human virus infections prompting immunosuppression and hemorrhage.

A MASSIVE RETROPERITONEAL ABSCESS IN A PATIENT WITH INFLAMMATORY BOWEL DISEASE ASSOCIATED WITH COMMON VARIABLE IMMUNODEFICIENCY

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Common variable immunodeficiency (CVID) is a rare form of severe antibody deficiency defined by low serum immunoglobulins and recurrent infections. The impaired antibody secretion has been attributed to intrinsic B-cell defects and T-cell dysregulation. The presentation of CVID is highly variable and includes recurrent respiratory infections, autoimmune diseases, gastrointestinal disorders and malignancies. The current treatment for CVID patients is life-long administration of gammaglobulin.

We present a 40-year-old male patient with CVID-associated inflammatory bowel disease (IBD) who developed a massive retroperitoneal abscess. His past medical history was significant for recurrent respiratory infections, sinusitis, pulmonary tuberculosis and bronchiectasis. At age of 30, he was diagnosed with indeterminate colitis (IC), as colon biopsy revealed elements compatible with both, Crohn disease and ulcerative colitis. His immunodeficiency was overlooked for years and at age 40, his laboratory findings revealed panhypogammaglobulinemia with an IgG of 45 mg/dL (normal 800-1,700 mg/dL), an IgA of 55 mg/dl (normal 100-490 mg/dL), and an IgM of 15 mg/dl (normal 50-320 mg/dL), with increased ESR and CRP and low levels of testosterone. The patient was diagnosed with CVID-associated IBD and hypogonadism and was started on intravenous immunoglobulin (IVIG) with mesalasine and prednisolone therapy. After three months of regular therapy, he was admitted with fever and abdominal pain. CT scan and US revealed a massive retroperitoneal abscess in both muscles illeopsoas, which caused compression of the ureter and subsequent left kidney hidronephrosis. The 4000ml of purulent liquid collection was evacuated by puncture and cytological examination revealed predominant neutrophils, while microbiological stains and cultures remained sterile. Ureteral stenosis was treated with balloon dilatation and “double J” endoprotesis insertion with satisfactory results. Presently, at 5-years of follow-up, the patient continues to be asymptomatic and his pulmonary function remains stable. His current therapy includes monthly IVIG infusion at a dose of 400-500 mg/kg.

INTERSTITIAL LUNG DISEASE AMONG FILIPINOS WITH CONNECTIVE TISSUE DISEASE

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Aim: To describe the clinical features, radiographic findings, therapies and clinical course of Interstitial lung disease (ILD) in Filipinos with connective tissue diseases (CTDs).

Methods: We retrospectively reviewed the records of patients diagnosed with ILD by Chest radiograph and High resolution CT scan. All patients had underlying CTDs, defined by respective American College

of Rheumatology (ACR) criteria, and were seen at 2 tertiary referral centers (St. Luke's Medical Center and University of Santo Tomas Hospital) in the Philippines.

Results: Of the 35 patients (32 women), 7 had systemic lupus erythematosus (SLE), 7 with scleroderma, 5 with mixed connective tissue disease (MCTD), 5 with dermato/polymyositis (DM/PM), 6 with rheumatoid arthritis (RA), 3 with undifferentiated connective tissue disease (UCTD) and 2 with overlap syndrome. The average age at ILD diagnosis was 48 ±14 years (mean ±SD), and mean duration of illness from CTD to ILD diagnosis was 26 ±42 months. Dyspnea was the most common manifestation (29, 83%), 29% (n=10) had concomitant pulmonary hypertension (PAH), 43% (n=15) developed serositis, 23% (n=8) had intermittent cyanosis and 60% (n=21) had chronic cough. Radiographic findings included the following: 17 usual interstitial pneumonia (UIP), 19 nonspecific interstitial pneumonia (NIP), 3 broncholitis obliterans organizing pneumonia (BOOP), 5 diffuse alveolar damage (DAD), and 4 lymphocytic interstitial pneumonia (LIP). Five patients developed malignancies: 2 breast, 1 thyroid, 1 liver and1 non-Hodgkin's lymphoma. Two patients had died at the time of this report, 1 UCTD and 1 DM, both with 1 month duration from ILD diagnosis to mortality. Therapies used in this group of patients for both ILD and CTD included: oral and pulse steroids, hydroxychloroquine, azathioprine, cyclophosphamide, penicillamine, colchicine, methotrexate, sildenafil, iloprost, and leflunomide. Three patients received biologics (2 infliximab and 1 rituximab) and 1 was treated with intravenous immunoglobulin.

Conclusions: We have described the clinical profile, radiographic findings of ILD in a group of Filipino patients with CTD. Varied forms of pharmacologic therapy were used for these patients. Early recognition and aggressive therapy especially during the "inflammatory" stages of ILD is crucial to more favorable outcomes.

IMMUNOREGULATORY MECHANISMS DURING CHRONIC HERPES VIRUS INFECTION IN CHILDHOOD

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Introduction: An important characteristic of the herpes group viruses (HSV, CMV, EBV) is their ability to persist in the tissues of their hosts for many years after initial infection as intracellular viruses. Characteristic life of virus (hronic persistent and ciclic replication) in organisms is often followed by immune dysregulation (Th1 or Th2 tip immune response). Chronic stimulation of immune system and immunodeficienty are development by virus persisting in organisms.

Materials and Methods: Clinically manifestations in patients with herpesvirus infections were examined. We analysed: white blood cell count, hemoglobin level, serum immunoglobulins level, enzymes of cell destruction (LDH, CPK, AST and ALT), oxidative metabolism of the peripherial blood phagocytes as ability of NBT reduction, ELISA test of antibody for one of the viruses: HSV, CMV and EBV. Serum level of IFN-γ, IL-4 and DHEAS, cortisol were measured by ELISA test.

Results: Our patients had and all of them had positive ELISA test on one of viruses (CMV, HSV or EBV). These were the initial parametars for separate our patients in our analysis. Our parameters approved low level of hemoglobin, monocytosis, lymphocytosis, virocytosis and leukopenia. Our patients had high level LDH, CPK, low ability of NBT reduction and hypergamaglobulinemia. High levels of IFNQ(70%) followed high levels of LDH, CPK, GOT and GPT. Decrease levels of DHEAS and cortisol opposite control grupe were evident.

Conclusion: Chronic activation of immune system is background of patogenetic mechanisms during herpes virus infection. Different level of DHEAS and cortisol are part of regulatory mechanisms of immune response across endocryne system. Increase levels of DHEAS in our patients can display chronic inflammation. The absence of increased level of cortisol may suggest that our patients had a little "acute" faze of infection opposite a lot of chronic disorders. Increase level of IFN-γ can suggestion on dominant Th1 response in our patients. Analysis of immunoregulatory mechanisms is essential to order level and place of damage cells, tissue and organs. It is important for therapy and prognosis of disease.

STRAIN DIFFERENCES IN THE EFFECT OF ACUTE RESTRAINT STRESS ON PERITONEAL MACROPHAGE ACTIVITY IN RATS

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We have previously shown that restraint stress (RS) potentiated and suppressed paw edema in Albino Oxford (AO) and Dark Agouti (DA) rat strains, respectively. The objective of the present study was to investigate: (a) the effect of RS on peritoneal macrophage functions in these two rat strains, and (b) the involvement different neuroendocrine mediators of stress and inflammation in the effect of RS. Rats were subjected to RS for 2 h and elicited peritoneal macrophages were harvested 24 h later. Cells were tested for adherence and zymosan phagocytosis in the absence or presence of 10^{-12} –10^{-6} M of antagonists specific for different receptors: opioid (naloxone), beta and alpha adrenergic (propranolol and phentolamine, respectively), histamine H_1 and H_2 (chloropyramine and ranitidine, respectively), 5-hydroxytryptamine $5HT_3$ (granisetron), or calcium antagonist verapamil. Exposure to RS diminished adherence and increased phagocytosis in macrophages obtained from rats of DA strain, but did not influence macrophage functions in rats of AO strain. The effect of RS on macrophages in DA rat strain was mediated via $5HT_3$ receptors and Ca++ (adherence) and $5HT_3$, H_1 and H_2 receptors (phagocytosis). Besides, exposure to RS abolished stimulating effect of opioid receptor blockade and alpha-adrenergic receptor blockade on macrophage adherence. In contrast, antagonists of opioid and alpha- and beta- adrenergic receptors potentiated phagocytosis in macrophages from DA rats regardless of RS. In AO rat strain RS did not modulate macrophages' functions, but increased their sensitivity to blockade of H_2 receptors resulting in the suppression of both adherence and phagocytosis. It can be concluded that: (a) the effect of RS on rat macrophage functions depends on genetic background, and (b) in vivo exposure to neuroendocrine mediators released during stress creates a hormonal milieu that change macrophage reactivity to mediators of inflammation. (Supported by Ministry of Science, Belgrade, Serbia, Grant No 145049).

A NOVEL ROLE FOR ST2 AS AN INHIBITORY REGULATOR OF APOPTOSIS AND INFLAMMATION IN CON A-INDUCED HEPATITIS

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ST2 is a member of the interleukin-1 receptor family implicated to play a role in the regulation of Th2 response. Moreover ST2 is described as negative regulator of Th1 cell response. To study cellular and molecular mechanisms of immune-mediated hepatitis, concanavalin A (Con A) induced hepatitis model was established in mice. Administration of Con A induces Th1 immunological response, hepatocyte apoptosis and severe liver injury that is considered to be an experimental model for human autoimmune hepatitis. Disease is characterized by marked increase in plasma level of inflammatory cytokines, AST and ALT, and an activation of proapoptotic signals, such as caspase 3. In the present study we investigated the role of ST2, in Con A-induced hepatitis by using of ST2 knock-out mice. All animals (wild type BALB/c and ST2 knock-out mice) were administered Con-A intraperitonealy (single dose of 16 mg/kg). Plasma and liver samples were prepared after 24 hours. Plasma levels of AST, ALT, GST were evaluated spectrophotometrically, whereas the level of active caspase 3 in hepatocytes was determined by Western blot analysis. ST2-deficient mice were more susceptible to Con A treatment than wild-type mice, showing significantly higher level of plasma AST, ALT and GST, as well as higher expression of the active caspase 3 in hepatocytes. These data revealed a novel role for ST2 as a suppressor of inflammatory response and apoptosis in the Con-A induced hepatitis.

Keywords:

ST2, Con-A induced hepatitis, caspase 3, AST, ALT, GST

RETINOIDS ATTENUATE THE DEVELOPMENT OF AUTOIMMUNE DIABETES IN MICE INDUCED BY MULTIPLE LOW DOSES OF STREPTOZOTOCIN

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Retinoids have a variety of biological activities, including immunomodulatory action in a number of inflammatory and autoimmune conditions. Considering the pathogenesis of type 1 diabetes, in this study we examined the potential role for retinoids in the experimental model of diabetes induced in susceptible CBA/H mice by multiple low doses of streptozotocin (MLD-STZ). Continuous 7 day treatment with synthetic retinoid, etretinate (30 to 90 mg/kg daily) during the induction of the disease markedly suppressed the development of hyperglycemia. The protective effect was even more pronounced when vitamin A metabolite all-trans-retinoic acid (ATRA, 2 to 20 mg/kg) was used. Accordingly, insulin expression was significantly higher in the pancreas of ATRA-treated mice than controls treated with MLD-STZ only, suggesting that drug administration preserved the islets from autoimmune attack. To investigate immunomodulatory mechanisms that may contribute to the beneficial effect of ATRA we performed ex vivo analysis during early diabetes development, i.e. on day 15 and day 22 post induction (pi). In accordance with well known increase in cellularity of relevant lymphoid tissue after disease induction, in control MLD-STZ mice cellular content of pancreatic draining lymph node cells (PLNC) was high on day 15 pi, but decreased on day 22 pi. In contrast, in ATRA-treated mice total PLNC number was significantly lower in comparison to control diabetic group on day 15 pi, and was the same on day 22 pi, suggesting that ATRA is capable to limit (autoreactive) lymphocyte proliferation and/or recruitment. Moreover, as evaluated by real time RT-PCR, ATRA down-regulated the ex vivo PLNC expression of proinflammatory cytokines IFN-gamma and IL-17, while augmenting that of the anti-inflammatory cytokine IL-4, as well as of the FoxP3. Therefore, through the regulation of various gene transcriptions ATRA trends to favor the inhibition of proinflammatory mediators typically associated with development of the disease thus shifting the balance towards the anti-inflammatory phenotype. Retinoids may thus be considered as a candidate drugs in developing a new therapeutic strategy for pharmacological intervention against T-cell-mediated injury of endocrine pancreas.

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TREATMENT OF WEGENER'S GRANULOMATOSIS WITH RITUXIMAB – CASE

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Wegener's granulomatosis is a systemic vasculitis of small blood vessels. Respiratory tract and kidneys are usually involved in this disease. Standard therapy for the induction of the remission is the combination of corticosteroids and cyclophosphamide. A 48-year-old male was treated in our clinic from 22.01 - 07.03.2007. He had 3 months history of pains of ear, throat, muscles, joints, weakness and fewer. He was hospitalized in Institute of lung diseases Vojvodina, under the doubt of reactivation of sarcoidosis, which was diagnosed earlier. Sarcoidosis diagnose was excluded with bronchoscopy and clinical and laboratory tests. High resolution CT of the lunges verified intraalveolar hemorrhage without reticulonodular lesions. Serum urea and creatinine started raising Pulmonary infiltrates appeared on RTG. Patient started having haemoptysis and haemoptoas. Corticosteroid therapy was started with the pulse doses. Patient was transferred to the Clinic of nephrology and clinical immunology because of the pulmo-renal syndrome. Anti-glomerular basement membrane antibodies were negative, but c-ANTA positive. Renal biopsy showed rapidoprogressive glomerulonephritis, with crescent formations in more than 90% of the glomerulous. RTG findings included infiltrations and cavernous lesions of the lungs. Intravenous pulses of cyclophosophamide were started, 1000mg monthly. He had 4 plasma exchanges and he was dialyzed. No benefit of this therapy was seen. Patient received Rituximab in a dose of 375mg/m2 of body surface (4 weekly sessions with 500mg, cumulative dose of 2 gr). Regression of lung infiltrates with progressive deterioration of creatinine was seen, as an

effect of the therapy. Patient is in a stabile remission six months after Rituximab therapy. There are no lung infiltrates on RTG or CT scan. Renal function is almost normal, with a significant regression of proteinuria For

Wegener's granulomatosis, Rituximab is promising alternative for patients' refractory to the standard therapy, or ones who can not receive cyclophosphamide.

ANTIGEN-SPECIFIC RESPONSE OF SPLENOCYTES IN CO-CULTIVATION WITH AMSC FROM EAE RATS

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Current theories on the induction and progression of multiple sclerosis (MS) place autoreactive T cells in the centre of autoimmune pathogenesis. Our previous data showed that autoreactive response in MS is driven by both CD4+ and CD8+ memory T cells. Experimental autoimmune encephalomyelitis (EAE) is an autoimmune inflammatory disease of the CNS and represents the paradigmatic model for MS.

In MS, therapeutic approaches targeting T cells have been successfully used, leading to immunosupression or tolerance. Mesenchymal stem cells (MSCs) are multipotent progenitor cells with great promise for pathogenic therapy of MS following immunomodulatory and neuroprotective properties.

In present study adipose tissue-derived MSCs (aMSCs) were co-cultured with splenocytes to compare immunosuppressive properties of aMSCs from rats with established EAE and aMSCs from healthy ones. The majority of these cells were able to differentiate into adipocytes and osteoblast-like cells. We showed that aMSCs of 1st and 2nd passages derived from EAE rats, as well as aMSCs from healthy one, inhibit ConA- and myelin-antigen stimulated splenocytes proliferation. Moreover, supernatants from aMSC cultures showed inhibitory effect on mitogen- and antigen-specific response of splenocytes comparable with immunosuppressive effect of direct co-culture of aMSCs with stimulated splenocytes.

Our data demonstrate that aMSC from EAE and healthy rodents exert their immunosuppressive effects through both soluble factors and cell-cell contact, these effects were similarly and didn't depend from initial status of rats.

THE ROLE OF SMOOTH MUSCLE CELLS AND VASCULAR DENDRITIC CELLS IN THE INFLAMMATORY RESPONSE IN ATHEROSCLEROSIS

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Introduction: Arteriosclerosis is a very complex disease. A complex interaction exists between the critical cellular elements of the atherosclerotic lesion. These cellular elements are macrophages, lymphocytes, neutrophils, endothelial cells, vascular smooth muscle cells, vascular dendritic cells, platelets and bone marrow circulating endothelial stem cells.

Objectives: The purpose of this work was to determine of the role of vascular dendritic cells and the role and phenotype state of smooth muscle cells during the pathogenesis of atherosclerotic lesion.

Materials and Methods: During the course of this study, 30 samples of atherosclerotic aortic aneurysms have been analyzed, all of them excised during surgery. Sections 5μm thick were stained by DAKO LSAB+/

HRP technique to identify α-smooth muscle actin-α-SMA, vimentin, myosin heavy chains-MHC, desmin, CD3, CD45, CD68, S100 protein and PCNA (DAKO specification). Sections were also stained for electron microscopy.

Results: The results of this study have shown that aortic atherosclerosis is characterized by the presence of a huge number of CD68-immunoreactive cells with lipid inclusions in the cytoplasm. This finding indicates the process of monocytes transition into foam cells. The finding of vimentin-immunoreactive foam cells (which points to their smooth muscle origin), suggests that these cells express scavenger receptors and competitively take part with macrophages in the accumulation of lipids and creation of foam cells. In the atherosclerotic lesion, there is also a huge number of cells which are immunoreactive to S-100 protein, which is generally characteristic of vascular dendritic cells.

Conclusions: Foam cells originate from macrophages (express CD68) and smooth muscle cells (express vimentin). At the earliest stage of atherosclerosis, monocytes and macrophages represent the main precursors of foam cells. From the stage of fatty streak, in parallel with synthetic activity, smooth muscle cells start to accumulate lipids. Antigen presenting dendritic cells in atherosclerotic aorta could play an important role in immune mechanisms during atherosclerotic lesion formation.

REGULATORY MECHANISMS IN LOW DOSE STREPTOZOTOCIN DIABETES INDUCTION

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Experimentally induced insulin dependent diabetes mellitus in rodents with multiple low dose of streptozotocin (MLD-STZ) has clinical and immunohistological features similar to those of human disease with T cells and macrophages playing a major pathological role. In bred strains of mice differ in their susceptibility to MLD-STZ diabetes induction, C_{57} BL/6 male mice being susceptible while BABL/C mice are partially resistant. It had been demonstrated recently that low dose cyclophosphamide sensitive $CD4^+$ $CD25^+$ $Foxp3^+$ cells downregulate spontaneous onset of diabetes in non-obese diabetic mice. There is also ample evidence that Th1/Th2 balance affect diabetes development.

We investigated whether the cyclophosphamide regimen enhance diabetes in susceptible C_{57}BL/6 mice and abrogate the resistance to disease induction in BALB/C mice and, we analyzed whether the effect of the lack of ST2 dependent signaling in Th-2 cells my affect disease induction.

Two injections of 20mg/kg body weight at the time of disease induction led to significant enhancement of diabetes induction in C_{57}BL/6 mice with significant increase of TNF production as evaluated by serum TNF level. However, this stimulatory effect of low doses of cyclophosphamide was not seen in BALB/C mice. In addition disease was enhanced in $ST2^{-/-}$ BALB/C mice in comparison with wild type BALB/C mice but cyclophosphamide did not have an additional disease enhancing effect as seen in C_{57}BL/6 mice.

Thus it appears that both Th1-Th17/Th2 balance and Treg cells maybe involved in susceptibility to diabetes depending on genetic back ground of the mice.

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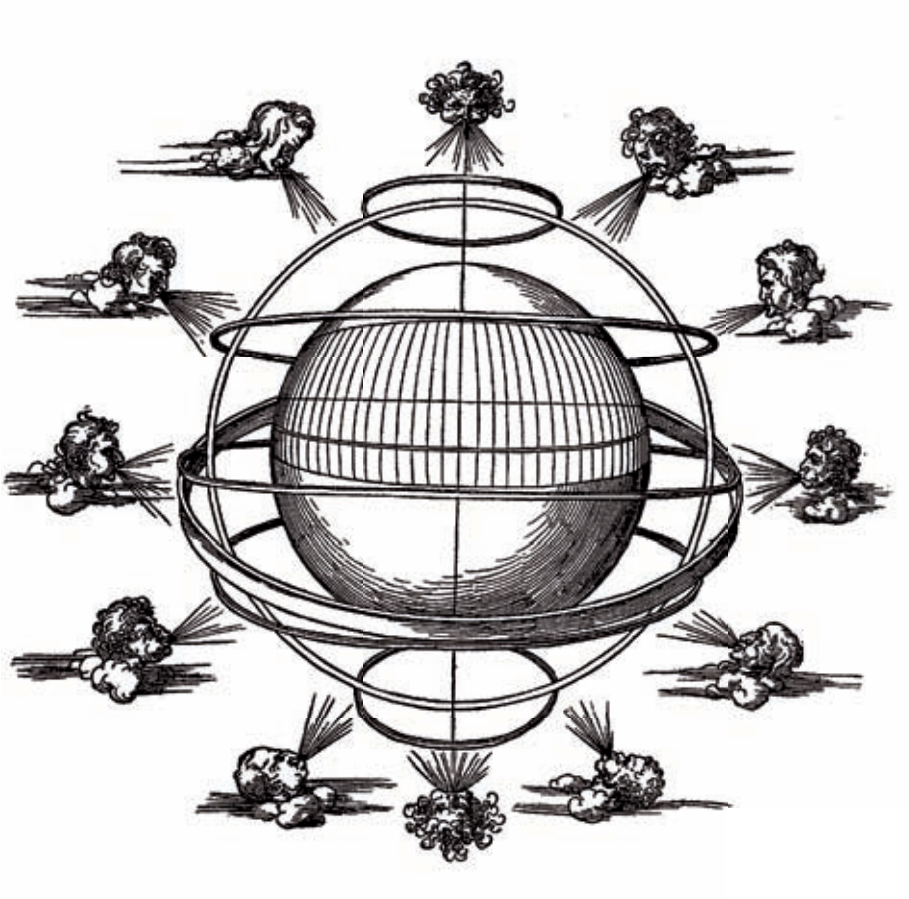

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