Autoimmunity and Immune Mediated Disorders
GENERAL INFORMATION

VENUE
The Conference will take place at the:
San Raffaele Scientific Institute of Milan
Congress Centre
Aula “Caravella Santa Maria”
Via Olgettina, 58
20132 Milan, Italy

LANGUAGE
The official language of the Conference will be English.

TRAVEL INFORMATION
Milan is to be considered Italy’s door to Europe. Financial capital of Italy with its stock exchange and large industrial sector, Milan is regarded also as one of the fashion capitals of the world, along with New York, Paris, Rome and London. Most of the major Italian fashion brands, such as Valentino, Gucci, Versace, Prada, Armani and Dolce & Gabbana, are currently headquartered in the city. Historic sites such as the magnificent gothic Duomo Cathedral as well as Leonardo da Vinci’s Last Supper, housed at the Church of Santa Maria delle Grazie, are musts for every visitor. Not to mention La Scala, probably the world’s most illustrious Opera House. Milan was major artistic centre throughout the centuries. Numerous art institutes, academies and galleries (such as the Brera Academy and the Pinacoteca Ambrosiana) exist in the city. Splendid also is nearby Lake Como.
Serono Symposia International Foundation
Conference on:

AUTOIMMUNITY AND IMMUNE MEDIATED DISORDERS
Milan, Italy - April 23-24, 2010

AIM OF THE CONFERENCE
In last decades, the knowledge of immune system physiology and inflammation mechanisms has improved dramatically. Research on the genetics of cells involved in immune responses, cytokines and pharmacological targets of new compounds indicated in immune-mediated diseases has produced a huge amount of data. These data, on one hand, are clarifying some pathophysiological mechanisms and offering new treatment options. On the other hand, they depict an extremely complex scenario which stimulates further research. In this rapidly evolving field, a crucial point seems to be that different autoimmune diseases share similar mechanisms and may be responsive to analogous pharmacological approaches. The aim of this conference is to gather together experts in genetics, pathophysiology and treatment of several autoimmune diseases to give the opportunity to share knowledge and experience in order to discuss and propose new lines of research and management options.

LEARNING OBJECTIVES
This Conference will offer participants:
• A special focus on some of the pathophysiological mechanisms shared by autoimmune diseases.
• Exhaustive and updated reviews of genetics, pathophysiology and therapy of type 1 diabetes, multiple sclerosis and inflammatory bowel diseases.
• Insight into interesting aspects of other autoimmune diseases.

TARGET AUDIENCE
Clinicians, researchers, geneticists and pharmacologists working on autoimmune diseases.

ACCREDITATION
Serono Symposia International Foundation (www.seronosymposia.org) has submitted this program “Autoimmunity and Immune Mediated Disorders” (Milan, Italy - April 23-24, 2010) for accreditation by the European Accreditation Council for Continuing Medical Education (EACCME) and the Italian Ministry of Health.
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# List of Speakers and Chairmen

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SCIENTIFIC PROGRAM
FRIDAY - APRIL 23, 2010

11.00 - 13.00  Registration

12.30  Opening Lunch

13.30  Serono Symposia International Foundation (SSIF) Opening
Giancarlo Comi, Italy
President of SSIF Scientific Committee

13.45  Welcome and Introduction
Maria Grazia Roncarolo, Italy - Giancarlo Comi, Italy - David Hafler, USA

SESSION I

BASIC MECHANISMS OF IMMUNE MEDIATED DISEASES

Chairmen: Giancarlo Comi, Italy - David Hafler, USA

14.00  L1: Role of innate immunity and inflammation
Cecilia Garlanda, Italy

14.30  L2: Leukocyte trafficking
Ruggero Pardi, Italy

15.00  L3: Effector T Cells: Th1, Th2 and Th17
Sergio Romagnani, Italy

15.30  L4: B cells
Vito Pistoia, Italy

16.00  L5: Regulatory T cells
Maria Grazia Roncarolo, Italy

16.30  Discussion

16.45  Coffee Break

SESSION II

MULTIPLE SCLEROSIS

Chairmen: Francesco Cucca, Italy - Matthias von Herrath, USA

17.00  L6: Genetics
David Hafler, USA

17.30  L7: Pathophysiology
Hans Peter Hartung, Germany

18.00  L8: Therapy
Giancarlo Comi, Italy

18.30  Discussion

18.45  End of day 1
SATURDAY - APRIL 24, 2010

SESSION III
TYPE 1 DIABETES
Chairmen: Sergio Romagnani, Italy - Luciano Adorini, Italy

9.00 L9: Genetics
Francesco Cucca, Italy

9.30 L10: Pathophysiology and Therapy
Matthias von Herrath, USA

10.15 Discussion
10.40 Coffee Break

SESSION IV
INFLAMMATORY BOWEL DISEASES
Chairmen: Robert L. Coffman, USA - Hans Peter Hartung, Germany

11.00 L11: Genetics
Carl Anderson, UK

11.30 L12: Pathophysiology
Claudio Fiocchi, USA

12.00 L13: Therapy
Paolo Gionchetti, Italy

12.30 Discussion
12.50 Working Lunch

SESSION V
OTHER CHRONIC IMMUNE MEDIATED DISEASES
Chairmen: Maria Grazia Roncarolo, Italy - Claudio Fiocchi, USA

14.00 L14: Celiac Disease and Diabetes: common pathogenetic mechanisms
Riccardo Troncone, Italy

14.30 L15: LES: genetic and pathophysiology
Virginia Pascual, USA

15.00 L16: Bifunctional TLR7 and TLR9 Inhibitors: Potential for Treatment of Autoimmune Disease
Robert L. Coffman, USA

15.30 L17: Rheumatic Disorders: pathophysiology
Salvatore Albani, USA

16.00 Discussion
16.20 Closing remarks
16.30 End of Conference
DISCLOSURE OF FACULTY RELATIONSHIPS

Serono Symposia International Foundation adheres to guidelines of the European Accreditation Council for Continuing Medical Education (EACCME) and all other professional organizations, as applicable, which state that programs awarding continuing education credits must be balanced, independent, objective, and scientifically rigorous. Investigative and other uses for pharmaceutical agents, medical devices, and other products (other than those uses indicated in approved product labeling/package insert for the product) may be presented in the program (which may reflect clinical experience, the professional literature or other clinical sources known to the presenter). We ask all presenters to provide participants with information about relationships with pharmaceutical or medical equipment companies that may have relevance to their lectures. This policy is not intended to exclude faculty who have relationships with such companies; it is only intended to inform participants of any potential conflicts so participants may form their own judgments, based on full disclosure of the facts. Further, all opinions and recommendations presented during the program and all program-related materials neither imply an endorsement, nor a recommendation, on the part of Serono Symposia International Foundation. All presentations solely represent the independent views of the presenters/authors.

The following faculty provided information regarding significant commercial relationships and/or discussions of investigational or non-EMEA/FDA approved (off-label) uses of drugs:

Salvatore Albani
Declared also to be a member of Ardimeses Consulting.

Carl Anderson
Declared no potential conflict of interest.

Robert L. Coffman
Declared to be stakeholder in a company as employee of Dynavax Technologies.

Giancarlo Comi
Declared receipt of honoraria from Serono Symposia International Foundation, Bayer Schering, Merck Serono, TEVA Pharmaceuticals Ind. Ltd, Sanofi Aventis and Biogen Dompè.

Francesco Cucca
Declared no potential conflict of interest.

Claudio Fiocchi
Declared no potential conflict of interest.

Cecilia Garlanda
Declared no potential conflict of interest.

Paolo Gionchetti
Declared a participation in a company sponsored speaker’s bureau to Ferring Symposium.

David Hafler
Declared receipt grant and contracts NIH/NINDS R37 NS024247 NIH/NINDS P01 NS 038037 NIH/NINDS R01 NS 049477 NIH (U19 AI 070352) NIH (P01 AI 073748), receipt of honoraria or consultation fees from: Allozyne Inc, EISAI Research Institute, Xceed Corp (honoraria for consulting 2009).

Hans Peter Hartung
Declared receipt of honoraria or consultation fee from: Biogen Idec, Teva Sanofi Aventis, Merck Serono, Novartis, Bayer Health Care.

Ruggero Pardi
Declared no potential conflict of interest.

Virginia Pascual
Declared receipt of honoraria: contract with Medimmune.

Vito Pistoia
Declared no potential conflict of interest.

Sergio Romagnani
Declared no potential conflict of interest.

Maria Grazia Roncarolo
Declared no potential conflict of interest.

Riccardo Troncone
Declared no potential conflict of interest.

Matthias von Herrath
Declared no potential conflict of interest.

The following faculty have provided no information regarding significant relationship with commercial supporters and/or discussion of investigational or non-EMEA/FDA approved (off-label) uses of drugs as of April 12, 2010.

Luciano Adorini
ABSTRACTS
(L1 – L17)
ROLE OF INNATE IMMUNITY AND INFLAMMATION

Cecilia Garlanda
Department of Research in Immunology and Inflammation, Laboratory of experimental immunopathology Istituto Clinico Humanitas, IRCCS, Milan, Italy

Innate immunity is a first line of resistance against pathogens and it plays a key role in the activation and orientation of adaptive immunity and in the maintenance of tissue integrity and repair. Similarly to adaptive immunity, the innate immune system consists of a cellular and a humoral arm. Cell-associated pattern recognition molecules (PRM) are strategically located in different cellular compartments (plasma membrane, endosomes, cytoplasm) and belong to different molecular classes, such as the toll like receptors (TLR), the NOD and RIG like receptors, the scavenger receptors.

Fluid phase PRM belong to different molecular families, including collectins, ficolins and pentraxins. Humoral PRM represent functional ancestors of antibodies and form an integrated system of diverse molecules, with complementary specificity, tissue distribution and mode of production. Humoral PRMs share a basic, evolutionarily conserved mode of action (complement activation; agglutination and neutralization; opsonization). Moreover, a bidirectional interaction between the humoral and the cellular arm sustains and regulates innate responses. The prototypic long pentraxin PTX3, which will presented here, represents a case-in-point of this interplay. Gene targeting of this evolutionary conserved long pentraxin has unequivocally defined its role at the crossroads of innate immunity, inflammation and matrix deposition.

IL-1R like receptors (ILRs) and TLRs are key receptors involved in innate immunity and inflammation. They are members of a superfamily of phylogenetically conserved proteins. Their common characteristic is the presence in the cytoplasmic region of a conserved sequence, called the Toll/IL-1R (TIR) domain, which is involved in the activation of a stereotypical signalling pathway leading to NF-κB translocation to the nucleus and activation of protein kinases such as p38 MAPK and JNK. TLRs act as sensors for the presence of micro-organisms and tissue damage through the recognition of specific pathogen associated molecular patterns and of necrotic cell derived danger signals and activate a complex, multifaceted cellular response. The ILR subfamily includes the receptors and the accessory proteins for IL-1, IL-18, IL-33, and other IL-1 family members, which are involved in the initiation of an amplification cascade of innate resistance, contribute to the activation and orientation of adaptive immunity and play a key role in inflammatory conditions.

The activation of the signalling cascade leading to the production of proteins related to inflammation and immunity by ILR or TLRs is tightly regulated. An uncontrolled or deregulated activation of these receptors can be detrimental as they mediate potentially devastating local and systemic inflammatory reactions. For the IL-1 system the control is exerted at different levels, both extracellularly and intracellularly. TIR8, also known as SIGIRR, a member of the ILR family will be presented here. TIR8 inhibits signalling receptor complexes of IL-1 family members associated with Th1 (IL-18), Th2 (IL-33) and Th17 (IL-1) differentiation, and also TLR-mediated activation. The ability to dampen signalling from ILR family members and TLRs makes TIR8 a key regulator of inflammation, cancer-related inflammation, and autoimmunity.
LEUKOCYTE TRAFFICKING

Ruggero Pardi
Immunology, Transplantation and Infectious Diseases, Vita-Salute San Raffaele University and Scientific Institute San Raffaele, Milan, Italy

Chronic inflammatory diseases represent the greatest collective burden of suffering and economic cost in the developed world: collectively, one out of three individuals is estimated to be chronically affected at a certain time of her/his life span. Once established, a chronic inflammatory process appears to take on a momentum of its own. Chronic inflammatory diseases comprise a heterogeneous group of disorders with unrelated etiologies but shared pathogenic mechanisms. In diseases as diverse as atherosclerosis, rheumatoid arthritis, asthma, chronic hepatitis, inflammatory bowel disease and psoriasis, a variety of chemotactic cytokines, acting via G protein-coupled receptors (GPCR), ultimately affect the recruitment and activation of immune and inflammatory cells, thereby amplifying and perpetuating the inflammatory state.

Prior evidence in non lymphomyeloid cells suggests that, in addition to being involved in GPCR desensitization, beta-arrestins act as signaling scaffolds catalyzing the activation of several enzymatic pathways (e.g. MAPK, Src) that are known to be playing relevant roles in the onset and maintenance of directed cell migration. Hence, we have extensively characterized the role of the ubiquitous beta-arrestins 1 and 2 in CXCR2-driven signaling and motility using multiple approaches: by knocking down selectively either arrestin isoform, we determined that they are both required for full PI3K and ERK activation following CK stimulation. Concurrently, we have assessed if knockdown (KD) of beta arrestins impairs CK-induced directional motility, using a single cell-based time lapse imaging approach. Results show that downregulation of beta-arrestins partially impairs CK-driven directional motility of CK responsive cell lines. These results were confirmed in intravital MO assays performed in vivo in beta-arrestin knockout mice, and collectively suggest that beta arrestins are required to convey CK-driven signals leading to integrin affinity modulation and adhesion strengthening in leukocytes extravasating at sites of inflammation. To dissect the mechanisms underlying the role of arrestins in the early steps of CK signalling, we have assessed [Ca++], transients and Rap-1 activation (by Ral-GDS pulldowns), which are both related to the integrin activation steps: results show that while Go protein-dependent signaling leading to increased [Ca++], is conserved in the absence of beta-arrestins, the activation of the small GTPase Rap-1, a key step in integrin affinity modulation, is severely impaired. Notably, beta-arrestin 2-dependent Rap1 activity is selectively involved in stabilizing integrin adhesion, which suggests that this pathway affects sequential steps in the process, ranging from the subsecond adhesion that allows tethering and arrest of leukocytes to the later processes that occur minutes after the initial stimulus and are relevant to ensure shear-resistant spreading and crawling onto the inflamed endothelial surface. We are currently exploring the possibility of combinatorially targeting the above pathway with drugs that interfere with selected and interdependent steps in the signaling leading from chemokine stimulation to the modulation of leukocyte adhesion and directional migration.
CD4+ T helper (Th) lymphocytes represent a heterogeneous population of cells that play an essential role in adaptive immunity. In addition to type 1 (Th1) and type 2 (Th2) cells, a third subset of CD4+ Th effector cells has recently been discovered and named as type 17 (Th17) because of its unique ability to produce interleukin (IL)-17. Studies in mice have initially suggested that Th17 cells are the pathogenic cells in autoimmune disorders, whereas Th1 cells may behave rather as protective. Subsequent studies in humans have demonstrated the plasticity of Th17 cells and their possibility to shift to the production of IFN-γ (Th17/Th1) and, more recently, also to the production of IL-4 (Th17/Th2). The plasticity of Th17 to Th1 cells has recently been confirmed in mice, where it was found that Th17 cells appear to be pathogenic only when they shift to Th1 cells. We have recently shown the shifting of human Th17 to Th1 cells in the synovial fluid of children with juvenile idiopathic arthritis and the correlation between the presence of Th17/Th1 cells and the activity of the disease. Studies in humans have also shown that Th17 are different than in mice because all of them express CD161 and exclusively originate from CD161+ precursors present in umbilical cord blood and in newborn thymus, but never from the CD161-CD4+ naïve T cells. While murine Th17 cells originate from naïve T cells in response to IL-6 and transforming growth factor (TGF)-β, human Th17 cell originate from CD161+ precursors already expressing in vivo the transcription factor RORC and the IL-23 receptor (R) in response to the combined activity of IL-1β and IL-23. The addition in vitro of TGF-β was not critical and only indirectly favoured the development of human Th17 cells by inhibiting the proliferation of Th1 cells. Transduction of the RORC gene in human CD4+ CD161- naïve T cells enabled a noticeable proportion of them to express CD161, the IL-23R and IL-1R and, therefore, to become susceptible to the differentiation into Th17 cells in response to the combined activity of IL-1 and IL-23. Very recently, Th17 cells were also found in murine thymus and the in vitro addition of TGF-β was found to be dispensable for murine Th17 differentiation. Thus, we believe that studies in humans have depicted Th17 cells better than studies in mice.
B CELLS

Vito Pistoia
Laboratory of Oncology, Department of Experimental and Laboratory Medicine, G. Gaslini Institute, Genoa, Italy

In the post-natal life, B cells originate and differentiate in the bone marrow. Thereafter B cells migrate to the peripheral lymphoid organs where they become functionally competent upon interaction with antigens. A key step in the peripheral differentiation of B lymphocytes is the germinal centre reaction, whereby antigen-activated naïve B cells colonize the germinal centres of lymphoid follicles. Here B cell proliferation, somatic hypermutation of immunoglobulin variable region genes and selection take place, eventually leading to generation of memory B cells and plasma cells. All of the above events depend on B cell trafficking that is regulated by chemokines and their receptors.

The effector functions of B lymphocytes involve three fundamental mechanisms, i) antibody production, ii) antigen processing and presentation to T cells, and iii) production of pro-inflammatory and immunoregulatory cytokines. Over the last years there has been increasing awareness of the complex role played by B cells in the pathogenesis of autoimmune and immune-mediated disorders. Raised interest to B cells as major players in the latter pathological conditions is related to significant advances in the characterization of B cell subpopulations in terms of immunophenotypic features and gene expression profiles, the development of sophisticated animal models of autoimmune diseases and the discovery that B cells express different Toll-like receptors (TLR), whose triggering (e.g. TLR9) may contribute to the break of immune tolerance and the initiation of autoimmune diseases.

Quite recently, emphasis has been placed on novel B cell subsets producing the immunosuppressive cytokine IL-10. These “B-regulatory” cells hold promise for the treatment of autoimmune diseases since in different animal models have proven effective at down-regulating autoreactive immune responses.

These topics will be discussed in the course of the presentation.
Regulatory T (Tr) cells represent specific T cell subsets that play a key role in inducing and maintaining immunological tolerance. Most attention has been focused on the CD4+ CD25+ natural Tr (nTreg) cells and on the adaptive type 1 Tr (Tr1) cells. These two subsets of Tr cells, which are developmentally and functionally distinct, cooperate in suppressing activation of the immune system and thereby in maintaining immunological homeostasis and inducing tolerance to self and foreign antigens. nTreg cells arise from the thymus and their suppressor function is strictly dependent on high expression of the transcription factor FOXP3, whereas Tr1 cells are induced in the periphery upon chronic stimulation with antigen in the presence of IL-10. Tr1 cells secrete high levels of IL-10 in the absence of IL-4, and suppress antigen presenting cells and effector T cells through a cytokine-dependent mechanism. In humans, the presence of Tr1 cells is associated with tolerance, whereas defects in nTreg or Tr1 cells lead to autoimmune mediated diseases or to chronic inflammation, respectively.

We and others have dedicated much effort to establish methods to isolate and expand nTreg cells or to induce antigen-specific Tr1 cells ex vivo to be used as cell therapy to promote or rebuilt tolerance. We established a protocol to selectively expand CD4+FOXP3+ T cells with suppressive activity using rapamycin. Alternatively, we showed that exogenous IL-10 or IL-10-derived from tolerogenic dendritic cells promote the in vitro induction of Tr1 cells. Clinical studies in hematopoietic stem cell transplantation using Tr-based cell dendritic cell therapy to favor immune reconstitution without graft versus host disease (GvHD), demonstrated that infusion of Tr1 cells is well tolerated, safe, and does not result in severe GvHD.

Alternatively to the use of ex vivo expanded/differentiated Tr cells, these cells can be induced directly in vivo. We demonstrated that a combination therapy with depleting agents (i.e. anti-CD45 mAb) and rapamycin/IL-10 treatment is highly effective in inducing tolerance in pre-clinical models of transplantation and type 1 diabetes. In addition, a small molecular weight compound that specifically activates the aryl hydrocarbon receptor has been proven efficient to induce tolerance through a direct or DC-mediated effects on Tr cells.

These approaches represent the first step towards the definition of new therapeutic protocols aimed to suppress pathology and restore peripheral tolerance in immune-mediated diseases.
Common Risk Alleles Associated with Multiple Sclerosis

Autoimmune diseases exhibit significant heritability. The sequencing of the human genome has allowed large scale, replicated whole genome association scan aimed at identifying risk alleles associated with risk to developing autoimmune disease. We have employed a staged performing a series of whole genome association scans followed by replication to interrogate the allelic variation associated with disease susceptibility in patients with MS and compare these data to other human autoimmune diseases. We used a fixed-effect meta-analysis strategy to combine four genome-wide scans for MS susceptibility. This new meta-analysis includes a total of 3957 MS cases and 10,210 controls of European ancestry. A number of new loci have emerged that enrich the network of genes that have now been implicated in the onset of central inflammatory demyelination. Functional analysis of the allelic variants indicate that although they have minor effects on disease risk, the biologic effects are major with allelic variants determining splice isoforms, receptor level expression, and other biologic functions. Investigation of these gene pathways offer intriguing new targets in the early immune dysregulation events that lead to MS.

References:

MULTIPLE SCLEROSIS - PATHOPHYSIOLOGY

Hans Peter Hartung
Department of Neurology, Heinrich-Heine-University, Dusseldorf, Germany

Abstract not in hand at the time of going to press.
MULTIPLE SCLEROSIS - THERAPY

Giancarlo Comi
Department of Neurology, Institute of Experimental Neurology, Vita-Salute University, Milan, Italy

First line treatment for multiple sclerosis (MS) include beta interferons and glatiramer acetate, both have a quite good safety profile and a moderate effect on disease activity. Since they do not cure MS, a long term administration is necessary in patients who show good response with some negative impact on the quality of life, because of injection site reactions and other possible adverse effects. In patients not responding to first line treatments and in patients with aggressive courses of the disease, both mitoxantrone and natalizumab can be used. However, the safety profile of both these treatments presents some problems, such as acute myeloid leukaemia and cardiomyopathy related to mitoxantrone and progressive multifocal leukoencephalitis related to natalizumab. More recently, some very interesting new putative treatments, having in common the oral route of administration, have reached an advanced phase of development, including successfully completed phase III trials for 3 of them, cladribine, fingolimod and teriflunomide. Two other drugs, laquinimod and dimethyl fumarate acid, have completed at least one phase II study and are undergoing an extensive phase III programme. The benefit to risk ratio of each new oral agent will be discussed in detail and the relative potential therapeutic role will be envisaged. Based on the available information, some of them could be used as first line treatment. These oral treatments open a new era in MS therapy and seem to be particularly attractive for patients.
Type 1 diabetes results from the T lymphocytes attack on the insulin-producing beta cells. It depends on the complex interplay between several co-inherited susceptibility alleles interspersed throughout the genome and unknown environmental factors. The disease is more likely to occur in close relatives of an affected person and in individuals from high risk populations. Notably, it is more common in Europe and in European-derived populations and, within Europe, in northern countries with a major exception: Sardinia which together with Finland has the highest incidence in the world.

Over 40 disease variants have been detected so far. The MHC/HLA region on chromosome 6p21 has been the first disease locus discovered in 1973 and contains the major genetic component of the disease predisposition and protection. Within the HLA-complex, variants of the HLA-DQB1 and -DRB1 loci have been shown to be primarily associated with the disease, but there is also evidence of additional modifying effects due to variation at other HLA loci. A second disease locus has been mapped on chromosome 11p15.5 to a minisatellite (VNTR) locus in the insulin gene (INS) promoter region. Other disease variants, encoding important regulators of T cell activation, have been subsequently detected at the CTLA4 gene on chromosomes 2q33, at the PTPN22 gene on chromosome 1p13 and at the IL2RA gene on chromosome 10p15.1. During the last few years Genome-Wide Association studies (GWAS) have provided convincing statistical evidence for many other disease variants. While these first GWA analyses have shown that denser maps and larger sample sets increase the chance of detecting genuine associations they captured only a fraction of the inherited risk of type 1 diabetes. Furthermore, most of the novel associations have been cryptic. This is largely due to the fact that current maps, albeit very dense, are constrained by the incomplete knowledge of genetic variation. Correcting this shortfall will require massively parallel DNA sequencing integrated with modern strategies of statistical imputation.

Still, the functions of the protein products of the various associated alleles identified so far highlight a coherent picture of disease pathogenesis. Accordingly, the affinity of the HLA class II molecules for a preproinsulin-derived peptide, the level of this peptide in the thymus and the degree of activation of key components of the T cell antigen receptor-signaling pathway, act all together as key factors of type 1 diabetes autoimmunity.
ASSOCIATING VIRAL INFECTIONS WITH T1D AND OTHER AUTOIMMUNE DISEASES HAS BEEN A DIFFICULT ENDEAVOR. THERE ARE SEVERAL REASONS FOR THIS: FIRST, IN EXPERIMENTAL MODELS, MULTIPLE MECHANISMS HAVE BEEN DEMONSTRATED AS TO HOW VIRUSES COULD ENHANCE OR CONVERSELY PROTECT FROM AUTOIMMUNITY. FOR EXAMPLE, MOLECULAR MIMICRY AND BYSTANDER INFLAMMATORY EFFECTS IN THE TARGET ORGAN HAVE BEEN DEMONSTRATED TO LEAD TO ACCELERATED T1D AND MS. CONVERSESLY, ENHANCED TREG FUNCTION AND DAMPENING OF AUTOAGGRESSIVE T CELLS VIA PD-1L AND IL-10 MEDIATED PATHWAYS CAN LEAD TO PROTECTION FROM AUTOIMMUNITY. DUE TO THESE COMPLEXITIES, FUTURE STUDIES IN HUMANS HAVE TO DIRECTLY ADDRESS SUCH PATHWAYS, WHICH ULTIMATELY COULD LEAD US TO PREDICTING, WHETHER A CERTAIN VIRUSES INFECTION CAN BE EXPECTED TO BE PROTECTIVE OR ENHANCE AUTOIMMUNITY. IN TYPE 1 DIABETES SOME EXPERIMENTAL EVIDENCE POINTS TOWARDS THE LEVEL OF VIRAL REPLICATION AS A DETERMINANT (HIGHER LEVELS OF REPLICATION LEAD TO ENHANCED AUTOIMMUNITY, WHERE AS LOWER LEVELS LEAD TO PROTECTION). THEREFORE VACCINATIONS, FOR EXAMPLE TO PREVENT HIGH LEVEL ENTEROVIRAL INFECTIONS MIGHT BE USEFUL IN THE FUTURE. IN ADDITION, ELEVATED LEVELS OF MHC CLASS I, INTERFERONS AND SOME ENTEROVIRAL PROTEINS HAVE BEEN FOUND IN HUMAN ISLETS, MORE FREQUENTLY IN THOSE FROM TYPE 1 DIABETES PATIENTS BUT NOT IN HEALTHY CONTROLS. THEREFORE, LOCAL INFECTION OF ISLET WITH ENTEROVIRUSES MIGHT BE A NECESSARY FACTOR CONTRIBUTING TO SOME CASES OF HUMAN T1D.
INFLAMMATORY BOWEL DISEASES - GENETICS

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Crohn’s disease is one of few common human diseases where risk loci have been robustly mapped using both linkage and association methodology.

The mapping of a Crohn’s disease (CD) susceptibility locus to chromosome 16 by Jean-Pierre Hugot and colleagues in 1996 placed the disease at the forefront of common human disease genetics. In 2001, two missense mutations (Arg702Trp and Gly908Arg) and one frameshift mutation (3020insC) in NOD2/CARD15 were identified which account for the linkage signal seen at this locus. This remains one of the few success stories for linkage mapping and positional cloning of a common human disease. Linkage analysis, which is only sufficiently powered to identify rare highly penetrant disease loci, was only able to robustly identify one further CD locus (IBD5 on chromosome 5).

The hypothesis that common diseases are underpinned by common variants with modest to small effect on disease status has been around for some time. However, it was not until the advent of genome-wide association studies (GWAS) that this hypothesis could be vigorously tested. In December 2006 the first GWAS of CD was published and IL23R, a perpetrator of organ-specific inflammation, was robustly associated with disease status. Over the following 12 months a further four GWAS were published and 11 loci were confirmed as conferring CD risk, some of which implicated previously unsuspected biological pathways in disease aetiology. For example, association of disease status with SNPs in ATG16L1 and IRGM first highlighted the role of autophagy.

In August 2008 a meta-analysis of three previously published GWAS was published in Nature Genetics by the newly formed International IBD Genetics Consortium (IIBDGC). Not only did this unequivocally confirm association to the 11 previously published loci but it also identified (and replicated) a further 21 susceptibility loci – bringing the total number of CD risk loci to 32 (once more putting CD at the forefront of common disease genetics). Again, insight into disease pathogenesis was gained – the association of SNPs within STAT3 and JAK2 highlighted the role of the Th17 pathway that is involved in T-cell differentiation. It is estimated that these 32 loci account for 10% of the overall variance in disease risk (or 20% of the heritability). The IIBDGC has recently expanded and is currently undertaking a new meta-analysis of 6 GWA studies.

Efforts at identifying non-HLA variants associated with Ulcerative Colitis (UC) (the other common form of IBD) have followed somewhat behind those of CD. Linkage studies of UC failed to robustly identify any such loci and it was not until November 2008 that the first GWAS of UC identified the IL10 risk locus. Subsequently five further GWAS have identified an additional 10 risk loci, including IL23R, IL26, KIF1A and HNF4α. Interestingly, none of the autophagy genes have thus far been implicated in UC susceptibility and it has been known for some time that NOD2 variants do not increase risk. A meta-analysis of the six UC GWAS studies is currently underway through the IIBDGC, and it is hoped that this work will further elucidate the genetic and biological differences between CD, UC and other autoimmune diseases.
INFLAMMATORY BOWEL DISEASES - PATHOPHYSIOLOGY

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Inflammatory bowel disease (IBD), which includes two major clinical forms, e.g., Crohn’s disease (CD) and ulcerative colitis (UC), has become an increasingly common condition in the world, and one that clinicians must now seriously consider in the differential diagnosis of any patient presenting with abdominal pain, diarrhea and bloody stools. In addition, once the diagnosis of CD or UC is confirmed, clinicians must be familiar with current therapeutic options that include not only traditional anti-inflammatory medications, like steroids and immunosuppressants, but also a whole series of novel biological agents that target cytokines, adhesion molecules, chemokines, chemokine receptors, and signaling molecules.

Which are then the key components of IBD pathogenesis, and what clinical opportunities may they offer to the clinician responsible for treating CD and UC patients? There is currently general agreement that four basic components are involved in the causation of CD and UC: environmental changes, genetic susceptibility, the enteric commensal flora, and the intestinal immune system. The combined action of these components determines the type and probably the course of IBD in each patient.

Studying the environmental changes linked to the worldwide increasing incidence and prevalence of CD and UC has proven quite difficult. This is mostly due to the need to prospectively follow large populations of patients and unaffected relatives over long periods of time. Consequently, knowledge in this area is limited, and the therapeutic opportunities that could eventually emerge would entirely depend on establishing a close direct association of specific environmental factors with an enhanced risk of IBD in selected populations in various areas of the world. At the moment, this appears an unlikely prospect, but one that cannot be excluded. In fact, if such specific association(s) were to be discovered, then the elimination of specific factors from the environment (as in the case of known pollutants), their avoidance, or lifestyle modifications by individuals at risk for IBD could have practical therapeutic implications by preventing disease or altering the clinical course.

The investigation of genetic susceptibility in the pathogenesis of IBD has gained a major impetus with the implementation of genome-wide association studies (GWAs) in large patient groups. GWAs have replicated and confirmed findings achieved with positional cloning strategies based on linkage analysis followed by linkage disequilibrium mapping, like in the case of NOD2 variants. More importantly, GWAs have enabled variants at different loci to be associated with particular diseases, and this has had a major impact, with the discovery of more than 30 genetic variants associated with IBD. So far, the majority is associated with CD, or both CD and UC, but several variants exclusively associated with UC have also been recently reported. The genetic variants identified by GWAs have confirmed suspected associations with innate and adaptive immune pathways, like for TLR4 and IL-23/IL-17, respectively. Additionally, they uncovered previously unsuspected disease pathways related to handling or disposing of bacteria - like the ATG16L1- and IRGM-dependent autophagosome pathways, epithelial cell function - like the PTGER4-dependent pathway, apoptosis - like the TNFRSF6B-dependent pathway, and immune suppression - like the PTPN2-dependent pathway, in addition to several others.

What are the clinical implications of these findings? Because the number of genetic variations associated with CD and UC is probably quite high and multiple but small patient populations will display rare associations, the hope that the detection of a single genetic abnormality might help in the diagnosis of CD or UC seems remote. On the other hand, associations of multiple genetic variations, the so-called “genetic signatures”, are possible with particular subtypes of IBD, as it has been the shown in certain forms of cancer. Although the precise diagnosis of CD or UC appears improbable based on genetics alone, gene variations may be informative or predictive of clinical course, response to therapy, or need for surgery. This can theoretically be accomplished with the use of “IBD chips”, where all known IBD-linked genetic variations can be tested using the patient’s DNA to assess risk and predict outcome.

The analysis of the enteric commensal flora in IBD has been surprisingly challenging. This is primarily due to the fact that its complex composition in normal humans has yet to defined, that each person seems to carry an “individualized” sets of microorganisms, and that reported variations in the types of bacteria populating the IBD intestine have been inconsistent, perhaps with the only exception of an increased number of E. coli. On the other hand, accumulated evidence leaves little doubt that the host’s immune response toward the gut flora is a major determinant of health or disease, not only in regard to IBD, but many other conditions. It is widely accepted that the chronic inflammatory response in the gut of CD and UC patients is directed against local microbial components, and its range, intensity, and duration impact on its clinical course. This has two major implications for the clinician: the first is that modulation of host-bacteria interactions creates therapeutic opportunities because it may decrease or eliminate inflammation; the second is that measuring the antibody response against specific microorganisms may provide useful clinical tools. The first possibility has lead to the use of antibiotics.
and probiotics in the management of IBD. The second possibility has already been amply explored, resulting in the assessment of antibodies against specific bacterial components to differentiate CD and UC, or prediction of clinical course. The true value of assessing pANCA, ASCA, anti-I2, anti-OmpC, anti-Cbr1, and the new ACCA, ALCA and AMCA for diagnostic purposes is debatable, but it is becoming accepted that the higher the titers and/or the broader the spectrum of antibacterial antibodies present in IBD sera, the more severe the clinical course and the higher the chance of complications.

The most investigated and best understood component of IBD pathogenesis is the intestinal immune system, in regard to both the innate and adaptive immune responses. This improved understanding has led to the identification of IBD type-associated T cell responses and of specific key regulators of mucosal immunity and inflammation. Until recently CD was considered a prototypical Th1 response with elevated IFN-γ production, but more recent reports show evidence that CD may be actually represent a mixed Th1/Th17 response, where Th1 or TH17 cytokines predominate depending on the stage of the disease. On the other hand, UC appears to be an atypical Th2 response, with elevated levels of IL-5 and IL-13, the latter being produced primarily by NKT cells. Immune regulators are multiple and diverse, and include cytokines, receptors, adhesion and signaling molecules, and their major appeal resides in the fact that all represent potential targets for therapeutic intervention. The prototypical example is the pro-inflammatory cytokine TNF-α, whose neutralization with a variety of different antibodies has been proven to be clearly beneficial. The same can be said for some receptors (e.g. CCR9), adhesion molecules (e.g. α-integrins), as well as other molecules, whose blockade is clinically effective in humans or animal models of IBD. Therefore, the development of modern anti-inflammatory therapeutic strategies is the direct result of an improved understanding of how immunity and inflammation develop and are regulated at the systemic and mucosal levels.
Current guidelines advocate a step-up approach to treatment, with the addition of more powerful therapies as the severity of disease or refractoriness to therapy increases. However, biological therapy has opened new therapeutic horizons and novel treatment goals. Improvement of symptoms can no longer be considered sufficient and strategies to modify the clinical pattern of disease are needed. Altering the clinical course of CD is only likely when healing of the bowel mucosa is achieved and maintained. This, in turn, is expected to avoid complications and improve the quality of life of patients. Anti-TNF therapies induce mucosal healing and cease fistula drainage, although there is as yet only tentative evidence that this alters the course of the disease.

In contrast to the cautious, conventional step-up approach, a proactive top-down approach to treatment has been proposed. This regimen advocates biological and immunomodulator therapy at an early stage, shortly after diagnosis of CD.
Celiac disease (CD) is a T cell-mediated chronic inflammatory disorder with an autoimmune component. Altered processing by intraluminal enzymes, changes in intestinal permeability and activation of innate immunity mechanisms precede the activation of the adaptive immune response. Immunodominant epitopes from gliadin are highly resistant to intraluminal and mucosal digestion; incomplete degradation favor the immunostimulatory and toxic effects of these sequences. Some gliadin peptides (p31-43) are able to activate innate immunity, in particular they induce IL15. Others activate lamina propria T cells in the of HLA-DQ2 or DQ8 molecules. Gliadin-specific T-cell responses have been found to be enhanced by the action of tissue transglutaminase; the enzyme converts particular glutamine residues into glutamic acid, which results in higher affinity of these gliadin peptides for HLA-DQ2 or HLA-DQ8. The pattern of cytokines produced following gliadin activation is clearly dominated by IFN\(\gamma\) (Th1 skewed); IFN\(\alpha\), IL18 and IL21 are also upregulated. Downstream T cell activation, a complex remodeling of the mucosa takes place, involving increased levels of metalloproteinases and growth factors, which leads to the classical flat mucosa. Increased density of CD8\(^+\) cytotoxic intraepithelial lymphocytes are a hallmark of celiac disease. IL15 is implicated in the expression of natural killer receptors CD94 and NKG2D, as well as in epithelial expression of stress molecules, thus enhancing cytotoxicity, cell apoptosis and villous atrophy. The most evident expression of autoimmunity is the presence of serum antibodies to tissue transglutaminase. However, the mechanisms leading to autoimmunity are largely unknown, as well as their pathogenetic significance.

A strong link has been observed between type-1 diabetes (T1D) and CD. Susceptibility to many autoimmune disorders is strongly associated with particular variants of HLA molecules. CD and T1D are associated with HLA-DQ2 and HLA-DQ8. Non-HLA genes (many of which encode proteins with immune functions) also contribute to the genetic susceptibility of both diseases, suggesting that the pathogenesis of these diseases involves common immune pathways. In fact, in both CD and T1D tissue cells are targeted by the immune system (enterocytes in coeliac disease and \(\beta\)-islet cells in type 1 diabetes). Both CD4 and CD8 subsets are involved. A role for interferon-\(\alpha\) (IFN\(\alpha\)) in the development of various immune-mediated diseases is suggested by the finding that treatment with IFN\(\alpha\) (for example, to treat chronic infection with hepatitis C virus) is associated with the onset of both conditions. A more direct role of gluten could be envisaged in the pathogenesis of T1D. Alteration in the intestinal environment induced by gluten could result in the induction of innate molecules that promote the development of T1D. This hypothesis is supported by the following observations: in 3-week old NOD mice only lymphocytes isolated from gut-associated lymphnodes have a diabetogenic potential, suggesting that the initial priming takes place in the gut; gluten free diet was shown to decrease the incidence of T1D in NOD mice; finally, the small intestinal mucosa of T1D patients (without serum CD associated autoantibodies) shows signs of mucosal immune activation and altered intestinal immunity to gliadin. Taken altogether present evidence suggest that T1D patients (or at least a subset) can be considered gluten-sensitive.
LES: GENETIC AND PATHOPHYSIOLOGY

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LES: Genomic clues to pathogenesis

The past decade has seen an explosion in the use of genomic approaches to help understanding the pathogenesis of human diseases, including autoimmune diseases such as LES. Among them, blood microarray analyses permit to assess RNA abundance on a genome-wide scale. Microarray-based research faces significant challenges, including noise and inconsistent reproducibility across laboratories and platforms. We recently proposed a novel microarray data mining strategy emphasizing the selection of coordinately expressed genes or transcriptional modules. Once these transcriptional determinants have been characterized, changes in gene expression between study groups can then be assessed on a module-by-module basis. This strategy allowed the identification of disease-specific leukocyte transcriptional fingerprints in patients with LES. Importantly, we demonstrate that modular transcriptional data can be reproduced across microarray platforms and laboratories and is widely applicable to generate robust and interpretable module-level diagnostic and disease activity biomarkers.

More quantitative and sensitive high throughput RNA profiling methods are starting to be available and will be discussed. These assays will make it possible in the foreseeable future for transcriptome analyses to become a routine test in the clinical setting.
Recognition of self nucleic acids by the innate immune system is increasingly being appreciated as an important contributor to many autoimmune and inflammatory diseases. The clearest example is Systemic Lupus Erythematosus (SLE), in which immune complexes of containing autoantibody and DNA or RNA are a potent stimulus for plasmacytoid dendritic cells (PDC), leading to chronic overproduction of IFN-α. This signaling is mediated through Toll-like receptor-7 (TLR-7) for RNA containing complexes and TLR9 for DNA containing complexes Efficient entry of RNA and DNA into TLR-containing endosomal vesicles is facilitated by Fc receptor-mediated uptake. TLR7 and TLR9 are expressed in a restricted number of cell types in the blood, principally PDC, B cells and activated neutrophils - all important to the pathogenesis of SLE.

We have developed short, synthetic oligonucleotide inhibitors of TLR7 and TLR9 as a novel targeted therapy for SLE. These oligonucleotides, termed IRS (immunoregulatory sequences) are bifunctional molecules, using different parts of the nucleotide sequence for TLR7 and TLR9 inhibition. These molecules are active in animal models of lupus and are potent inhibitors of the stimulation of PDC by immune complexes isolated from SLE patients. They are active in a number of acute tissue injury models as well, suggesting that there are multiple mechanisms of efficient endosomal uptake of nucleic acids released during many forms of tissue injury.
Molecular immunology has provided tools for improved knowledge of the mechanisms contributing to the pathogenesis of rheumatoid arthritis and other rheumatic diseases (RD herein). The chronic inflammatory nature of the disease is now better understood and many of the mediators and pathways which amplify inflammatory processes and lead to tissue damage have been identified. This progress has been translated into clinical practice with the introduction of biologic agents that effectively interfere with the inflammatory cascade by blocking one of its key components. Direct biological interference with cytokines, such as TNFα, and accessory molecules on the surface of immune cells, such as antibodies interfering with CTLA-4 or CD3 on T cells or CD20 on B cells, is increasingly replacing generalized pharmacological immune suppression via induction of immune suppression or immune tolerance. The mechanisms underlying the clinical effects for these therapies are not yet fully understood. However, as evident from several recent reports, biologics aimed at one immune pathway affect other arms of the immune response. These effects are most likely primary, not simple byproducts. A complete understanding of the mechanisms of action for biologics on the diverse but interlacing components of the immune system cannot be left out of consideration for the optimization of therapy with these drugs.

Altogether, the focus of RD therapy has shifted from the induction of immune suppression to the development and maintenance of immune tolerance. This approach will lead to the development of novel therapies able to induce disease remission and maintain it with minimal continued therapy. As with any important field in rapid development, there is a need for a better understanding of how tolerance can be measured and induced in the therapy of human autoimmunity. Our program is developing a novel approach that directly addresses the need of a tolerogen as a complement to currently used biologics.