Plenary and parallel sessions

ANCA-ASSOCIATED VASCULITIS, DIAGNOSIS, PATHOPHYSIOLOGY AND TREATMENT

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The idiopathic small vessel vasculitides have been recognized as autoimmune disorders characterized by the presence of anti-neutrophil cytoplasmic antibodies (ANCA) directed to proteinase-3 (Pr3) and myeloperoxidase (MPO). Anti-Pr3/anti-MPO are both sensitive and specific markers for the disorders, and rises in their levels proceed relapses in about 78% of cases although these rises are not highly specific for ensuing relapses. In vitro and in vivo experimental data suggest that the autoimmune response contributes to the immunopathology of the lesions but additional factors seem to be required. These supposedly exogenous factors may include chronic nasal carriage of Staphylococcus aureus, silica exposure, and others. ANCA-testing has contributed to the (early) diagnosis of the associated diseases as well as to the insight into their pathophysiology, but treatment, generally, still consists of steroids and cyclophosphamide. However, new and less toxic treatment modalities are currently being tested by the European Vasculitis Working Party. It has already been shown that azathioprine is as effective and less toxic than cyclophosphamide for maintenance of remission. Other studies will be discussed.

VASCULITIS IN CHILDHOOD

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The childhood vasculitides can be considered under a number of headings but to formally classify them has proved difficult. A classification of sorts that has had some practical value divides the childhood vasculitides into the following groups: polyarteritis (macroscopic, microscopic, cutaneous), Kawasaki disease, granulomatous vasculitis (Wegener's, Churg-Strauss, primary cerebral angiitis), leucocytoclastic vasculitis (Henoch-Schonlein, hypersensitivity angiitis, hypocomplementaemic urticarial vasculitis), "Rheumatic" vasculitis (SLE, dermatomyositis, juvenile chronic arthritis, mixed connective tissue disease), giant cell arteritis (Takayasu) and miscellaneous (Cogan's etc). This is far from perfect and criteria for inclusion into the various categories have not been fully established. This is compounded by the considerable overlap that occurs between various vasculitic syndromes causing most clinicians who see a lot of vasculitis to become "lumpers" rather than "splitters" in terms of categorization. ACR criteria can be applied to some vasculitides but require adjustment for children and the initial Chapel Hill modifications, although helpful in many ways, caused confusion rather than clarity particularly in relation to how polyarteritis nodosa, a disease of the young, was considered. Some attempts have been made to produce appropriate criteria that are applicable in childhood but there is a considerable way to go before these will be acceptable. Correct classification has relevance in a number of ways including aetiopathogenesis, natural history, therapeutic approach and choice of outcome measure when evaluating particular treatment regimens. More work is necessary to facilitate our journey through this etymological quagmire.

RELEVANCE OF IGG RECEPTORS (FCγR) FOR PATHOGENESIS AND TREATMENT OF AUTOIMMUNE DISEASES

W.-L. van der Pol. *Department of Immunotherapy and Neurology, University Medical Center, Utrecht, Netherlands.*

Leukocyte IgG receptors (FcγR) constitute the natural link between the cellular and humoral branches of the immunesystem, and confer potent cellular effector functions to the specificity of antibody. Engagement of leukocyte FcγR may induce such diverse responses as phagocytosis, respiratory burst, cytotoxicity, antigen presentation, degranulation, cytokine production, and regulation of antibody production. Both murine and human FcγR families encompass three classes (FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16)), which differ genetically, biochemically, strucurally, and functionally. The extracellular part consists of two or three immunoglobulin-like loops and contains the ligand binding domain. FcγR classes differ in ligand specificity and receptor binding characteristics. The intracellular tails of FcγR and associated signaling subunits contain either of two FcγR-class specific signaling motifs, which trigger distinct signaling routes, and determine the nature of the cellular response.

FcγR may be crucial molecules for both efficient host defense against microorganisms and pathological inflammatory reactions. The use of genetically modified animals in murine models for inflammatory disease permitted dissection of the role of individual FcγR in autoimmune disease. Moreover, FcγR were shown trigger molecules for several immune modulating therapies. Understanding of FcγR mediated inflammation may thus provide insight into basic aspects of pathogenesis of autoimmune disease, and help the development of novel treatment modalities.

HSP REACTIVE T CELLS AS INFLAMMATION REGULATORS

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Immunization with heat shock proteins has protective effects in models of induced arthritis. Analysis has shown a reduced synovial inflammation in such protected animals. Adoptive transfer and immunisation with selected T cell epitopes (synthetic peptides) have indicated the protection to be mediated by T cells directed to conserved hsp epitopes. This was shown first for mycobacterial hsp60 (JEM 181: 943;1995) and later for mycobacterial hsp70 (JI 163: 5560;1999 and 164: 2711; 2000). Fine specificity analysis showed that such T cells were cross-reactive with the homologous self hsp. Therefore protection by microbial hsp reactive T cells can be by cross-recognition of self hsp overexpressed in the inflamed tissue. Preimmunisation with hsp leads to a relative expansion of such self hsp cross-responsive T cells. The regulatory nature of such T cells may originate from mucosal tolerance maintained by commensal flora derived hsp or from partial activation through recognition of self hsp as a partial agonist (Altered Peptide Ligand) or in the absence of proper costimulation. Recently, we reported the selective upregulation of B7.2 on microbial hsp60 specific T cells in response to self hsp60 (Int Imm 12: 1041; 2000). Through a preferred interaction with CTLA-4 on proinflammatory T cells this may constitute an effector mechanism of regulation. Also, regulatory T cells produced IL10.

IMMUNOTHERAPY OF RHEUMATIC DISEASES

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Recent progresses in immunology and molecular biology have opened new avenues for understanding the complex mechanisms which trigger and modulate autoimmunity. Unfortunately, translation from basic science into useful therapy of autoimmunity, has been slow. In fact, with a few notable exceptions now in trial or planned, current immunotherapy approaches for the treatment of rheumatic diseases have little specificity for pathogenic immune-mediated pathways and their specific antigenic triggers. For most patients, therapy is broadly immunosuppressive and a source of significant toxicity. The need for effective and more specific therapy is particularly dramatic in pediatric rheumatic diseases, often neglected by research and general public alike. In particular, there is a gap between our knowledge of T cell mediated pathogenic events and the availability of specific reagents tailored to modulate such pathways for clinical benefit.

We will discuss our personal experience in combining state of the art Immunology and Molecular Biology techniques for the identification of antigen specific pathways, which we are now attempting to influence by immunomodulatory therapy in both clinical and experimental settings.

IMMUNODEFICIENCIES AS MODELS FOR AUTOIMMUNITY

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Several primary immunodeficiencies expose to autoimmunity. The underlying mechanisms are only partially understood in most cases. We will focus on 2 inherited immunodeficiencies that are responsible for autoimmune and inflammatory complications in early childhood.

Mutations in Fas, a transmembrane receptor involved in apoptotic cell death, or Fas-Ligand have been reported in patients with a lymphoproliferative syndrome and autoimmune disorders. Accumulation of "double negative" CD4- CD8- αβT cells and decreased in vitro Fas-mediated lymphocyte apoptosis are characteristics of these "autoimmune lymphoproliferative syndromes type I (ALPS I)". In most families, the disease has a dominant pattern of inheritance, due to a transdominant negative effect of heterozygous Fas mutations, with variable penetrance. These patients are also prone to develop lymphomas. Defective lymphocyte Fas-mediated apoptosis has also been reported in patients with no Fas or FasL mutation. Some of these "ALPS II" patients have mutations of the downstream enzyme caspase 10. "ALPS III" patients have a defect in lymphocyte apoptosis that is not Fas-mediated.

Familial hemophagocytic lymphohistiocytosis, an autosomal recessive disorder characterized by an overwhelming activation of T lymphocytes and macrophages, is due in one third of cases to perforin gene mutations. Perforin is an essential effector of the secretory cytotoxic pathway, which also plays an homeostatic role by allowing the induction of T lymphocyte apoptotic death in response to granzymes. Homeostatic role of this secretory cytotoxic pathway has been previously evidenced in perforin knock out mice. When challenged with certain strains of viruses these mice develop a very similar overeactive specific T cell response and die within a few weeks as a consequence of immune damages mediated by activated T lymphocytes and macrophages producing high quantity of IFN-γ and TNF-α. In humans, the disease is lethal unless treated by allogeneic hematopoietic stem cell transplantation.

SLEEPING WITH THE ENEMY: TOLERANCE INDUCTION IN CHILDHOOD AUTOIMMUNE DISEASES

B. J. Prakken. *University Medical Center Utrecht, Wilhemina Childrens Hospital, Department of Pediatric Immunology, Utrecht, Netherlands.*

Over the last 10 years, a enormous progress has been made in understanding immunity and autoimmunity. This has led to novel concepts for immunotherapy of autoimmmune diseases, aiming at deviation instead of elimination of potential auto-reactive T cell clones. Approaches such as the induction of mucosal tolerance, have been highly successful in virtual all models of experimental arthritis. However, to translate this knowledge into successful therapy of human autoimmune diseases, the following conditions have to be fulfilled. Firstly, the identification of a target antigen, preferable including peptide epitopes recognised in a majority of patients, irrespective of HLA background. The newly identified pan-DR binding peptides derived from human and mycobacterial hsp60 might provide such targets. Secondly, the route of administration of the antigen chosen is of vital importance. Whereas subcutaneous administration of target antigens holds a great risk of developing hypersensitivity, oral administration has been shown to be safe and effective. The latter was demonstrated in a recently completed Phase I trial in RA using a dnaJp1 peptide (Prakken&Albani, submitted). In experimental arthritis, we have shown that nasal administration might be even more effective then oral administration (PNAS 1997;57:139, Prakken & Wauben, submitted). Lastly, the local environment, both in the joint and at the site of tolerance induction will also be determining the fate of tolerance induction. We recently showed that in the model of Adjuvant Arthritis that Immune stimulatory DNA sequences (ISS) ultimately determine the severity of disease in experimental arthritis (Ronaghy et al, submitted). ISS can effectively be used as an adjuvant for mucosal vaccination, steering the immune response in the right direction.

Altogether, those new developments should allow us to set the framework for a first immunotherapy trial in children in the near future.

MECHANISMS OF JOINT DAMAGE BY T CELLS IN JUVENILE IDIOPATHIC ARTHRITIS: CAN THEY BE BLOCKED?

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Activated T cells are one of the hallmarks of chronically inflamed synovium in juvenile idiopathic arthritis (JIA), and disease associations with distinct MHC alleles remain the most powerful genetic risk genes demonstrated in JIA. The presence of persistent, expanded T cell clones within the joint, which are differentially distributed in the CD4 or CD8 populations depending on MHC allele association, suggests that specific peptides are driving a proportion of the inflammatory T cells. However evidence also suggests that many inflammatory cells may be cytokine driven, without the need for specific antigen recognition in situ. An understanding of the ability of these cells to enter the joint and survive there is crucial to strategies which target this arm of the inflammatory response. A large proportion of these T cells produce Th1 type cytokines and chemokines, and our data suggest that synovial endothelium provides continuing signals to the non-specific recruitment of these activated and polarised T cells.

In addition to local joint damage, in some cases with bone erosion, many children with JIA also develop osteoporosis. Bone remodelling is controlled in part by the balance between RANK (receptor activator of NF-kB) and its ligand, the TNF- like molecule RANKL. Data will be presented which suggest that RANK and RANKL are over - expressed by macrophages/dendritic cells and T cells respectively, in the JIA joint. The challenge in coming years will be to tailor combinations of biological therapies aimed at various components of destructive synovitis, to maximise benefit and prevent disability.

REDUCED PERFORIN EXPRESSION IN SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS IS RESTORED BY AUTOLOGOUS STEM CELL TRANSPLANTATION.

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Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder characterized by uncontrolled activation of T cells and macrophages and overproduction of pro-inflammatory cytokines. Clinical characteristics are fever, hepatosplenomegaly, pancytopenia, DIC and hemophagocytosis. Mutations in the perforin gene are the underlying cause of this disease. The Macrophage Activation Syndrome that occurs in patients with systemic Idiopathic Arthritis (sys-JIA) is clinically very similar to FHL.

Therefore we determined the perforin expression levels on CD8+ and NK cells from patients with Sys JIA before and after auto-SCT. Perforin expression on cytotoxic effector cells (CD8+CD28-CD45RA- and CD8+CD28-CD45RA+) and NK cells was determined by 3 or 4-color immunofluorescence.

Results: Cytotoxic effector cells of sJIA patients express significant lower levels of perforin as pJIA patients (sJIA 27.6 mean perforin fluorescence intensity, n = 13; pJIA MFI 98.0, n = 9) or control donors (MFI 124.6, n = 5). Also NK cells from sJIA patients expressed significantly less intracellular perforin than healthy controls or patients with other forms of JIA. Although mean perforin expression levels were decreased in sJIA, considerable variation did exist between individual patients. In 4 patients with sJIA who were treated with auto-SCT, perforin expression was analyzed at 12 months after Auto-SCT. In all 4 patients a clear increase in perforin expression was found both in NK cells as well as in cytotoxic effector cells (pre-AutoSCT MFI 13.2, 12 months post-AutoSCT MFI 172.3).

We conclude that perforin expression can be severely reduced in systemic JIA. This finding can explain why sys-JIA may be complicated by macrophage activation syndrome and hemophagocytosis. Auto-SCT leads to a reconstitution of the (T cell) immune system with a normal expression of perforin.

AUTONOMIC NERVOUS SYSTEM IN THE PATHOGENESIS OF JUVENILE IDIOPATHIC ARTHRITIS (JIA)

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The stress of inflammation induced by a variety of agents initiates a coordinated complex series of bidirectional adaptive reactions involving the immune, nervous and endocrine systems facilitated by shared common signal molecules and receptors. The cytokine, hormonal and

paracrine signals provide the molecular basis of this cross-talk. These adaptations are aimed at restoring homoeostatic balance and the return to the status quo ante. Defects in the neuroendocrine immune interactions (NEI) influence the susceptibility to developing chronic autoimmune inflammatory disease such as RA and SLE. The autonomic nervous system (ANS) affects immune function directly and via the activation of the HPA axis through the Locus Ceruleus. RA and MS are associated with a dysfunction of the sympathetic nervous system (SNS) activity evaluated by measurements of cardiovascular activity, pupil size, sweating responses and skin conductance.

Patients with JIA have defective NEI characterised by inappropriate low levels of cortisol for the degree of on-going inflammation, raised prolactin and arginine vasopressin levels, IL-6 and TNFα. Kuis et al have studied the SNS and immune cell responses in JIA. Patients with active JIA had a higher heart rate, with elevated levels of 3-hydroxy-4 phenoxy phenylglycol (a central metabolite of catecholamines), in comparison to normal controls. Cardiovascular responses (diastolic blood pressure, stroke volume) to orthostatic stress (tilt up) were reduced suggesting increased central noradrenergic outflow, leading to increased vasoconstriction and decreased orthostatic stress responses. Interestingly, the increased central adrenergic flow fails to increase cortisol secretion. When peripheral blood monuclear cells (PBMC) from JIA patients were incubated with β_2 agonists, they failed to increase intracellular cAMP. What is the functional significance of the altered function of the ANS ? The mechanisms associated with the dysregulation of the ANS are not fully known. Under normal physiologic conditions, the immune response is inhibited by catecholamines via an increase in intracellular cAMP level. β_2 adrenergic agonists inhibit T-cell proliferation, IL-2 receptor and IL-2 expression. The responses to β_2 agonists by PBMC from JIA patients are blunted. This is due to the high activity of the cAMP degrading enzymes CNP-ase in cells since inhibition of the CNP-ase restores the response to β_2 adrenergic agonists. In healthy controls, β_2 adrenergic agonists inhibit the activity of lymphocytes, and α_1 adrenergic drugs have no effect. Lymphocytes and macrophages from JIA patients posses functional α_1 adrenergic receptors. α_1 Adrenergic receptor agonists increase production of IL-6 and TNFα in PBMC from JIA patients and which is reversed by selective α_1 adrenergic receptor antagonists. Thus, these observations are of pathophysiologic significance in JIA.

NEUROENDOCRINE REGULATION OF CHRONIC ARTHRITIS

J. W. J. Bijlsma. *Department of Rheumatology & Clinical Immunology, University Medical Center Utrecht, Netherlands.*

Evidence is presented that defects or deficiencies in maintaining normal regulation of the neuroendocrine immune loop can contribute to chronic inflammatory or autoimmune diseases. Proinflammatory cytokines (such as interleukin-1, interleukin-6 and tumor necrosis factor α) are released in the acute phase response and act not only as local mediators but also stimulate the neuroendocrine response. In turn, these hormones, particularly glucocorticoids, are immunomodulatory. The complex orchestration of cascades of acute and chronic immunomediators in inflammation provokes neuroendocrine responses that are accompanied by manifold glucocorticoid anti-inflammatory mechanisms. The neuroendocrine immune loop constitutes essential integrated physiologic circuits for the maintenance of health and the regulation of inflammation. Dysregulation of this loop has important physio pathologic consequences in the systemic rheumatic diseases, including JIA.

Neuroendocrine immune studies in patients with rheumatoid arthritis show ineffective hypothalamic pituitary adrenal axis responses to inflammation, characterized by inappropriate low levels of circulating cortisol for the degree of ongoing joint inflammation. In JIA only a small number of data are available. However, the observation that disease activity seems to be worst in the morning, improves during the day and worsens at night suggests that neuro-endocrine immune mechanisms might be involved in the disease pathophysiology. Some studies indicate the existence of abnormal communications between the neuroendocrine and immune systems in these children as well. The possible role of an observed hypo-andro-genicity in the pathophysiology of JIA will be discussed.

ADAPTATION OF THE HYPOTHALAMIC RESPONSE AND PULSATILE HPA ACTIVITY TO ACUTE AND CHRONIC STRESS

Lightman SL *University of Bristol, UK*

The neuroendocrine response to stress is mediated through a neurally mediated activation of the hypothalamic pituitary adrenal (HPA) axis and the sympathetic nervous system. At neural level HPA activity is regulated by hypothalamic CRH and AVP both of which respond to acute stress with a rapid increase in hnRNA and mRNA. This hypothalamic response is transduced at the hormonal level to a frequency and amplitude modulated release of pulses of both ACTH and adrenal corticosteroids. There is a differential sensitivity of the HPA response to stress depending on the phase of activity of the endogenous HPA rhythm. Thus stress occurring during a basal or secretory phase (pulse) of the cycle results in a robust response, while stress occurring after a secretory phase shows little or no response. This inhibitory phase of the corticosterone response to stress is seen in all rat strains studies to date - with the single exception of the Fischer rat which shows a total loss of this refractory period.

During chronic stress there is a gradual change in hypothalamic response such that CRH levels fall and AVP becomes the predominant responsive HPA secretagogue. Coupled with this change in hypothalamic regulation, there is also a marked change in the pattern of corticosterone secretion with increased frequency of the corticosterone pulses.

REFRACTORY CHILDHOOD SYSTEMIC LUPUS ERYTHEMATOSUS TREATED WITH HIGH DOSE CYCLOPHOSPHAMIDE AND STEM CELL RESCUE

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To assess safety and efficacy of autologous stem cell transplantation (ASCT) in 2 children with SLE that was drug resistant or was associated with severe drug toxicity.

2 children with SLE with vital organ involvement that was prednison dependant (more than 1 mg/kg/24h) and who were treated with i.v. cyclophosphamide (CY) previously were selected for ASCT, because of their ongoing disease activity despite treatment and severe side effects due to steroids and CY. Clinical evaluation and laboratory measures were performed at a 3 months interval. Bone marrow was harvested 1 month before ASCT and T cell depletion was performed. The preparative regimen of the ASCT consisted of Anti Thymocyte Globulin (ATG), CY and Total Body Irradiation (TBI). The autograft contained 0.55 x 10^6 /kg (patient 1) and 1.2 x 10^6 /kg (patient 2) CD34+ cells.

The procedure was well tolerated and induced a lasting and drug free remission of 30 and 12 months follow-up. The SLEDAI score decreased from 28 and 20 to 0 and 8. Steroids could be discontinued after 6 and 3 months in patients one and two, respectively without a subsequent flare in SLE disease activity. The malar rash, glomerulonephritis and the pericarditis (in patient 2) resolved, as did the initial lymphopenia and hypocomplementaemia. However after 12 months he developed a a Coomb's positive autoimmune hemolytic anemia, while anti ds-DNA and ANA became persistently negative. His AIHA was steroid responsive. Patient 1 is now 24 months after prednisone withdrawal. Since then, a third patient was elected for ASCT. Here CD34+ stem cells were obtained by peripheral stem cell mobilisation using high dose CY (4.2gr) and GCSF. This CY dose induced a clinical remission of disease, of sofar 8 months duration. These pediatric data will be compared with data from 12 adult SLE patients, reported to the EBMT.

In conclusion, ASCT was performed safely in 2 pediatric SLE patients. It remains to be determined whether high dose Cy on its own is sufficient or that a more profound immunosuppression followed by stem cell rescue is necessary to induce prolonged drug free remission.

DO ANTIBODIES REALLY CAUSE DISEASE IN PATIENTS WITH SLE?

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Autoantibodies to some 40/50 different specificities have been identified in the serum of patients with SLE. In very few cases, however, have these antibodies been identified in more than 30% of the patients which fact alone renders the importance of the majority of these antibodies in the pathogenesis of lupus overall most unlikely. There is however strong circumstantial evidence linking some autoantibodies with particular clinical subsets. For example, anti-Ro antibodies are clearly associated with photosensitivity, vasculitis and neonatal lupus syndrome in patients with lupus. Antiphospholipid antibodies are equally strongly associated with those lupus patients who give a history of thrombosis, recurrent miscarriages, livedo reticularis and thrombocytopenia.

By far the strongest autoantibody link with lupus is that of anti-DNA/nucleosome antibodies. A variety of mechanisms from the deposition of circulating immune complexes containing DNA and anti-DNA antibodies to the direct binding of these immunoglobulins to renal and other structures have been proposed. Irrespective of the mechanism, however, there are very strong circumstantial and direct links which strongly indicate that some anti-DNA antibodies at least are indeed directly pathogenic. In family studies for example, whereas anti-single stranded DNA antibodies are commonly found in family relatives, anti-double stranded DNA antibodies though present in 60–90% of patients with lupus, are virtually never present in their healthy family members. Older studies by the groups of Madaio and Eilat, using murine monoclonal antibodies injected into healthy or pre-immune mice, or using an isolated kidney perfusion system, respectively, have provided direct evidence that murine anti-DNA antibodies are truly pathogenic in some, but not all cases. My colleagues and I have extended these studies to human immunoglobulins using a set of hybridoma derived human monoclonal anti-dsDNA antibodies. Using SCID mice, we have demonstrated that though on an ELISA pklate these antibodies may appear equal, some in an Orwellian sense are more equal than others, since individual immunoglobulins may or may not bind to the SCID mouse tissues—some demonstrating an in vivo penetrating ANA-like pattern in all the organs examined, whilst others deposit exclusively to the kidney. Those antibodies which do bind, also induce varying amounts of proteinuria and electron microscope studies have shown the capacity of one of these immunoglobulins (RH14) to induce thickening of the renal basement membrane and flattening of the foot process, two early features of lupus nephritis. Our current studies have involved the development of antibody expression systems to increase the yield of these monoclonals for detailed structure/function analyses.

PATHWAYS IN CHRONIC ARTHRITIS, CONSEQUENCES FOR IMMUNOTHERAPY?

T. W. J. Huizinga. *Department of Rheumatology, Leiden University Medical Center, Netherlands.*

Chronic arthritis is caused by dysregulation in the immune system that leads to presence of inflammatory cells in the synovium of the joints. Histological studies of joints have revealed that T-cells, B-cells and monocytes/macrophages are the cells that contribute to the perpetuation of inflammation. Strategies aimed at reducing levels of cytokines produced by monocytes such as TNF have revealed that very effective reduction of inflammation can be achieved in the majority of patients. However the effects of such therapies is relatively short thereby necessitating ongoing administration of TNF-binding agents. Therefore current research is aimed at influencing more proximal pathways of inflammation in order to reach a more pronounced disease modifying effect. Results will be highlighted that such disease modifying effects can also partly be reached by timely administration of traditional DMARD therapy.

Apart from the proximal pathways that lead to production of cytokines such as TNF by monocytes/macrophages, new insight has been generated in distal pathways of joint destruction. Long-lasting inflammation leads to changes in the fibroblast-like synoviocytes, that allow these are cells to invade bone and cartilage thereby leading to joint destruction. The recent demonstration of ungoing joint destruction in a subset of patients with RA in remission (meaning RA without inflammation) demonstrates the relevance of this pathway in-vivo. The potential ways to interfere with this pathway of joint destruction will be discussed.

IL-1 AS A TARGET FOR INTERVENTION: LESSONS FROM ANIMAL MODELS

W. B. van den Berg. *Rheumatology Research and Advanced Therapeutics, Institute of Molecular Life Sciences, University Medical Center Nijmegen, Netherlands.*

Rheumatoid arthritis is characterized by chronic inflammation of multiple joints and concomitant destruction of cartilage and bone. Although the disease is considered as an autoimmune process, distinct antigens have not been identified. The inflammatory process in the synovial tissues of the joint shows abundant macrophage activation and generation of a pleithora of cytokines and growth factors. It is now evident that the cytokines TNFa (tumor necrosis factor) and IL-1 (interleukin-1) are pivotal mediators in this process. Studies with neutralising antibodies and scavenging receptors mice made it clear that TNF is crucial in joint swelling, whereas IL-1 is a key mediator in cartilage and bone erosion. TNF alone is hardly destrcutive, but may cause erosion through IL-1 generation. Erosive arthritis can still be induced in TNF deficient mice, whereas general arthritis models are nondestructive in IL-1b deficient mice.

IL-1 causes cartilage destruction through inhibition of chondrocyte matrix synthesis and generation of metalloproteinases. In addition, Il-1 is a potent upregulator of RANK-L (OPG-L), which is the crucial mediator of osteoclast activation and bone erosion. More recent studies identified a distinct role of the T cell cytokine IL-17 in erosive arthritis. Control of arthritis can be achieved with cytokine inhibitors and with modulatory cytokines such as IL-10 and IL-4. The latter is a potent inhibitor of IL-1 and IL-17 in vivo. Advanced therapeutic approaches include local transfer of gene constructs of cytokine inhibitors, using retroviral and adenoviral vectors.

EMERGING NEW THERAPIES

F. C. Breedveld. *Leiden University Medical Center, Department of Rheumatology, Leiden, Netherlands.*

Pharmacotherapy is the corner stone of the treatment of patients with chronic arthritis. With the development of biotechnology and computational chemistry dramatic changes have occurred in the possibilities to treat patients with chronic arthritis. In addition solid clinical research have shown the optimal application of novel therapies. These can be categorized as follows:

Early treatment: it has become clear that irreversible joint damage occurs early in the disease course and can be prevented by early and aggressive therapies.

Application of combinations of presently available slow acting rheumatic drugs results in unprovements of the efficacy/toxicity balance.

Targeted therapies already have created a break through in the treatment of arthritis in the form of TNF antagonists and further developments are underway.

TNF blocking agents work fast and induce a clinical response in more than half of the patients. It was shown that these therapies have the potential to stop structural joint damage. From a practical point of view several questions need an answer. How is the efficacy and safety during long-term treatment? What is the ideal treatment regimen that provides optimal care for the patient for acceptable costs?

Besides TNF, also other pro-inflammatory cytokines are involved in the rheumatoid inflammation. Blockage of these molecules or the signaling events following receptor binding could provide potential therapeutic benefit in chronic arthritis. In this presentation the strategies that target signals upstream or downstream of these cytokines will be discussed.

COMBINATION THERAPY IN JIA: HAS THE SERPENT SPOKEN?

M. Boers. *Department of Clinical Epidemiology & Biostatistics, Vrije Universiteit Medical Center Amsterdam, Netherlands.*

The speaking serpent refers to one of the most vicious conflicts in the Dutch reformed church that led to a split in the 1920s. As you may but need not know, the Dutch reformed church ('gereformeerd') itself was a split faction from the main Calvinist church in Holland with a similar name ('hervormd'). As these Calvinists were (and often still are!) believers of the Word, the conflict arose over whether Genesis (and in particular the 'speaking snake') should be understood as literal truth or not. A mini-inquisition erupted and thousands of believers were evicted or chose to depart and form a new faction. Notably, yet another split occurred while Holland was occupied by the nazi's in World War 2!

My personal experience in the design and report of the COBRA trial bears some resemblances to the above events: it firmly challenged dogma and made explicit the dilemmas of corticosteroid therapy in RA. The COBRA combination of step-down prednisolone, methotrexate and sulfasalazine was challenged in the funding phase by referees who predicted I would be responsible for the death of RA patients; in the presentation and publication phase by the same referees who now questioned the low dose of methotrexate, but also by experts who disbelieved the low toxicity rates ('because they were not logical'). Being one of the few trials to publish one-year rates for radiographic damage, the referees nevertheless found the follow up time too short. When we expanded the follow up to 1.5 years, the relevance of damage (until that time undisputed) was put into question. Even last year, I was attacked by a physician over my viewpoints on Corticosteroids while I was presenting a poster on a totally different subject that made use of COBRA data!

Although physicians treating JIA have traditionally had much less qualms applying Corticosteroids, they have also seen the horrible spectrum of side effects that can accompany this therapy; compared to 'adult' rheumatologists, they have embraced the new biologicals with as much or more enthusiasm.

This presentation will summarize the main findings from the COBRA trial, including the new and highly favorable results of the 5-year radiological follow up, and compare these with data from other recent trials of anti-TNF, leflunomide and combination therapy. It will explore the relevance of these findings for JIA.

PATHOGENESIS OF SPONDYLOARTHROPATHIES

J. Sieper. *Department of Rheumatology, University Hospital Benjamin Franklin, Berlin, Germany.*

The spondyloarthropathies (SpA) comprise ankylosing spondylitis, reactive arthritis or Reiter's syndrome, arthritis/spondylitis with inflammatory bowel disease and arthritis/spondylitis with psoriasis. The main link between each other is the association with HLA-B27, the same pattern of peripheral joint involvement and the possible occurence of sacroiliitis, spondylitis, enthesitis and uveitis, predominantly in the HLA-B27-positive patients.

Crucial for the pathogenesis of the SpA is the interaction between bacteria and HLA-B27. This is also supported by the two major animal models for SpA, the HLA-B27 transgenic rat and the HLA-B27-transgenic mouse lacking β2-microglobulin, which do not get disease if raised in a germ (bacteria) free enivironment. Encounter of the immune system with bacteria can occur because bacteria persist in vivo in ReA patients or because of damage of the gut mucosa (barrier) in patients with inflammatory bowel disease. It has also been suggested that streptococci could be crucial for the initiation and maintainance of psoriasis and that this trigger could also be responsible for arthritis.

A likely explanation for the persistence of bacteria in reactive arthritis is the presence of a T cell response insufficient to eliminate the reactive arthritis-associated bacteria. This seems to be mediated by cytokines such as interleukin-10. In healthy persons gut-derived T cells are tolerant against bacteria found in the gut. In contrast, this tolerance is lost in patients with IBD. About 20% of the HLA-B27 patients with ReA and about 50% of IBD patients move on to ankylosing spondylitis while these manifestation occurs only in a small proportion of HLA-B27-negative patients. Presently there is no evidence that bacterial antigen persists in ankylosing spondylitis and therefore it seems to be more likely that in AS an autoimmune response is present, most probably triggered by bacteria.

Related to the question about autoantigens is the question about the primary tissue involved in spondyloarthropathy. There is now good evidence based on immunohistological and imaging studies that inflammation is found at the interphase between bone and cartilage and that the synovium is not primarily affected in the spinal manifestations Therefore, candidates for crossreacting self antigens are cartilage-derived proteins. Based on results from animal and human experiments there is evidence that the proteoglycan aggrecan could be of major importance for the pathogenesis of SpA

Identification of immunodominant T cell epitopes both for CD4- and CD8+ T cells seems to be crucial for a better understanding of the patogenesis and for the development of new treatment. By applying antigen-specific cytometry, the cytokine secretion assay and the tetramer-technology we have been able to identify bacteria and autoantigen-derived epitopes relevant for both T cell subsets. These powerful new techniques are an important step forward for the investigation of immune-mediated diseases.

Juvenile SpA and adult SpA show many similarities and many SpA starting in childhood end up in a full blown picture of AS later on. In countries such as Mexico or China a diagnosis of AS is made mostly below the age of 20 in contrast to countries in the West where the age is well above 20 years suggesting that bad hygiene and repeated infections in childhood is a major contributing factor for the pathogenesis of SpA in less developed countries.

NOVEL THERAPIES IN JUVENILE IN JUVENILE DERMATOMYOSITIS

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The outcome of juvenile dermatomyositis (JDM has improved over the last three decades. This change has likely been due to the empirical application of new therapies proven to be useful in other autoimmune diseases, and better use of existing therapies, rather than controlled trials. There has in fact only been a single prospective controlled therapeutic trial in JDM. This is due to JDM being rare and the lack of validated disease outcome measures.

Substantial changes in treatment include the use of intravenous pulse steroids particularly in the early phases of disease, and less reliance on maintenance of high dose oral steroids. Early introduction of specific DMARDs and minimising exposure to steroids is likely to be valuable. For severe chronic or ulcerative/resistant disease, immunosuppressive drugs such as cyclophosphamide or IVIG may be useful. New "biologics" such as anti-tumour necrosis factor agents are being trialled, as TNF is evident in muscle biopsies, and certain alleles of the TNF gene are linked to overproduction of TNF, and more severe disease.

Autologous stem cell transplantation is being pioneered in severe rheumatic disorders including dermatomyositis, with the potentially for curative effects. Therapy for complications of JDM such as calcinosis remains difficult, with bisphosphonates, calcium channel blockers, warfarin and probenecid all being claimed as useful. Early aggressive treatment in general may prevent calcinosis in some patients. The management of osteoporosis is important as JDM patient are at particular risk of pathological fractures due to disease or steroid induced osteoporosis.

Physical therapies and psychosocial interventions are important in JDM but the techniques and outcomes are unproven. Prospective controlled trials in JDM are essential, The definition of predictors of outcome and specific outcome measures are also critical. Understanding genetic predispositions and specific immunopathogenesis, will in time allow more specific and effective treatment protocols be designed.

PATHOGENESIS OF POLYMYOSITIS AND DERMATOMYOSITIS

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Polymyositis (PM) and dermatomyostis (DM) are acquired muscle diseases with inflammatory infiltrates in the muscle biopsy specimens. In both, the frequent association with other autoimmune diseases and the response to immunotherapy support an autoimmune pathogenesis. Muscle biopsy specimens in PM show evidence for a T cell mediated immune response against an unknown antigen expressed by muscle fibres. The endomysial inflammatory exudate contains large numbers of cytotoxic (CD8 +) T cells, macrophages, and only sparse B cells. The cytotoxic T cells surround and invade non-necrotic muscle fibres expressing MHC class I antigens. Presumably, destruction of the muscle fibers is mediated by these cytotoxic T cells. In DM the inflammatory exudate is located predominantly perivascular and perimysial and to a lesser extent endomysial. B cells are abundant, and the majority of the T cells are T helper cells (CD4 +). Furthermore, light and electron microscopy studies of muscle tissue in these patients show evidence for injury in the intramuscular blood vessels mediated by complement. These findings suggest a humurally-mediated microangiopathy in DM. Although the evidence that PM and DM are autoimmune in origin is nearly overwhelming, in neither of the disorders the factor that initiates self-sensitisation or the antigen to which self-tolerance is broken has been identified.