Meeting report

22nd European Workshop for Rheumatology Research, Leiden, The Netherlands, 28 February–3 March 2002

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Abstract

The European Workshop for Rheumatology Research met this year in Leiden, The Netherlands. The Workshop provided a platform to feast on new technologies and how they have taken research programmes forward. While there will be the inevitable delay during which mechanisms are devised for analysing the huge amount of information generated by these technologies, there is a lot already to look forward to. Highlights included genomic, reverse genomic and proteomic approaches to understanding disease pathogenesis and to identifying new therapeutic targets. Opportunities for exploring whether pharmacogenomics has a place in the clinic are now a reality, and phage display technology has been applied to *in vivo* arthritis models to identify human synovial microvascular 'post codes'.

Keywords: diagnostics, inflammation, prognostics, research, workshop

Introduction

Some 200 delegates convened for the 22nd annual European update on progress in research into the rheumatic diseases. This meeting has always been popular, since it brings together rheumatologists and basic scientists who share a common interest in exploring disease pathogenesis and identifying new therapeutic targets. It also provides opportunities for young investigators to present their unpublished work. This year was no exception, with nine plenary sessions ranging from technologies and genomics to cytokines and signal transduction pathways. In striving to meet its objectives, around 40% of all posters presented at the meeting were also discussed through oral presentations. Delegates were also treated to an array of outstanding research expertise based locally.

Review

Pathology and pathogenesis

Evolving concepts of autoimmune disease propose that the overwhelming default response of the immune system of the nonsusceptible host is immune regulation. According to this model we are all exposed to 'danger signals' (P Matzinger, Bethesda, MD, USA), but the key to understanding why some individuals are disease susceptible lies in the mechanisms whereby the immune response is not only initiated, but also perpetuated.

In her keynote address, P Matzinger proposed an approach to understanding the different mechanisms for the development of rheumatic autoimmune diseases. Diseases were used to illustrate each of five possible mechanisms for autoimmunity and chronic inflammation: (i) infection, illustrated by Lyme disease; (ii) molecular mimicry, illustrated by rheumatic fever and possible flares of inflammatory arthritis; (iii) 'bad death' as a result of mutations in 'clean-up' genes, illustrated by systemic lupus erythematosus; (iv) immune impressionism where injury or insult leads to immune epiphenomena, such as the production of autoantibodies to nucleolar antigens, illustrated by scleroderma; and (v) 'wrong class of immune response', a switch from a Th2 response to a Th1 response, illustrated by type I diabetes and multiple sclerosis (examples

COX = cyclooxygenase; ERK = extracellular signal-regulated MAP kinase; IC_{50} = inhibitory concentration 50%; IFN = interferon; IκBα = inhibitor of κB alpha; IKK2 = IkappaB kinase beta; IL = interleukin; MAP = mitogen-activated protein; MHC = major histocompatibility complex; MKK = MAP kinase kinase; NF = nuclear factor; RA = rheumatoid arthritis; SCID = severe combined immunodeficient; SNP = single nucleotide polymorphism; Th = T helper cell; TNF = tumour necrosis factor.

of a 'correct class of immune response' being IgA responses in the gut, and anterior chamber of the eye driven by transforming growth factor beta and vasoactive intestinal peptide).

Antigen presentation and lymphocyte activation

An important component of this 'danger' theory is the processing of autoantigens by professional antigen-presenting cells and the presentation of specific peptides to T cells. This process is poorly understood in autoimmune disease, but working models propose that post-translational modifications of self antigen might provide one mechanism of generating neoepitopes not encountered by T cells during maturation in the thymus.

Coeliac disease is not strictly an autoimmune disease, since there is an increased precursor frequency of T cells specific for peptides derived from the dietary wheat protein gliadin. But what is interesting about immunodominant gliadin epitopes is that tissue transglutaminase, which is also the target of autoantibodies in patients, plays a role in the deamidation of glutamine residues (F Koning, Leiden, The Netherlands). A modification from glutamine to glutamic acid by transglutaminase requires a specific motif QXPQX, where X may be a hydrophobic residue. Database searches for such motifs reveal hits in gluten, barley and rye (which are not tolerated by coeliac patients), but not in oats (which is tolerated). This raises the possibility that the generation of modified epitopes at sites of inflammation may be tissue specific.

Another good example is the expression of peptidylarginine deiminase type 2 by monocyte-derived macrophages, which leads to the citrullination of potential synovial target antigens such as fibrin and vimentin. These are good examples of neo-epitope targets of the autoimmune B-cell response in rheumatoid arthritis (RA) and are thought to be generated in the synovial joint (E Vossenaar, Nijmegen, The Netherlands).

Members of the tumour necrosis factor (TNF)/TNF-receptor superfamily of ligands and receptors have received much attention recently. The role of RANKL/RANK in osteoclastogenesis and the BLyS/BAFF-R system in B-cell autoimmunity are two such examples.

Insight into the function of another family pair, CD27L(CD70) and CD27, has come from a detailed analysis of CD70 transgenic and CD27 mutant mice (R van Lier, Amsterdam, The Netherlands), the transgenic mouse being the more revealing. While delayed type hypersensitivity responses are depressed in $CD27^{-/-}$ mice, CD70 enhanced delayed type hypersensitivity and cytotoxic T-lymphocyte responses when expressed under the CD19 promoter, as demonstrated by MHC class I tetramer staining. Strikingly, $CD27^{-/-}$ T cells expressed the activation markers $CD44^{hi}$ $CD62L^{lo}$ in the absence of antigenic stimulation *in vitro* and produced more IFN-γ. A decreased life span through infection was reflected in reduced total lymph node and thymic cellularity, suggesting that these T cells, many of which are $Ki67^{+}$, are terminally differentiated and senescent as a consequence of chronic antigenic stimulation. It is now well established that such cells have, as a consequence of their replicative history, shortened telomeres. They do not proliferate well and, historically, this has made senescent T cells difficult to study. This problem has now been resolved in part, since replication is restored, and much of the function is retained by overexpressing hTERT (H Spits, Amsterdam, The Netherlands). This approach does not, however, appear to work in B cells.

Inflammation

The expanding IL-1 superfamily received a good airing at the Workshop. The importance of regulation of IL-1 *in vivo* is now well established, based in part on the phenotype of mice deficient for the naturally occurring IL-1 inhibitor, IL-1ra (G Duff, Sheffield, UK).

The disease phenotypes, which are striking, depend on the background strain. In outbred Swiss albino mice, the predominant phenotype is that of a medium to large vessel vasculitis; survival is decreased to a substantial extent largely through myocardial infarction and aneurysm formation. The same mutation leads to a destructive polyarthritis in Balb/c mice with 100% penetrance, with arterial disease being insignificant, while in C57BL/6 mice there is no vessel inflammation and the penetrance of arthritis is less pronounced.

IL-18 presents itself as a promising therapeutic target in inflammatory arthritis. The effects of anti-IL-18 antibodies, the IL-18 binding protein or the $IL\text{-}18^{-/-}$ genotype on the incidence and severity of collagen-induced arthritis certainly supports this idea (I McInnes, Glasgow, UK). IL-18 is expressed in inflamed synovium in RA, and while *in vitro* the IL-18Rα chain has been shown to be expressed on a subset of Th1 cells, the main target of action appears to be the macrophage. Indeed, IL-18 blockade suppresses TNF production by ~50% in cultures of dissociated synovial cells. Unlike IL-1, however, IL-18 processing from pro-IL-18 appears to be caspase (e.g. IL-1 converting enzyme) independent. Studies using cells from elastase-deficient mice and cathepsin-G-deficient mice suggest that elastase may be important for IL-18 processing, at least in neutrophils.

IL-17 and its role in inflammatory joint disease has received more attention recently. However, it is distinctive in that its expression can be detected in synovial T cells, unlike many T-cell-derived cytokines. The immunobiology of IL-17 is IL-1-like in that it induces TNF and IL-1 expres-

sion, but it also has chemotactic activity for neutrophils. Its expression has been studied in association with the presence of erosions in inflamed joints, and the fact that it upregulates RANKL expression and secretion suggests a possible role in osteoclastogenesis (E Lubberts, Nijmegen, The Netherlands). Consistent with this is the finding that overexpression of IL-17 in collagen-induced arthritis by adenoviral transgenesis promotes cartilage destruction and erosion of bone, effects that may be IL-1 independent. In preliminary studies, the effects of IL-17 blockade seemed less striking than those observed following attenuation of activity of other IL-1 family members.

Diagnostics and prognostics

There seems little doubt that technology has already influenced our capacity to genotype and serotype patients with arthritis and other inflammatory disorders. In the next few years, profiling may become routine. With this technology comes information overload, and physicians will need to be prepared for this. The techniques available to us are expanding, as too are the hardware and software to help us cope. The key lies in pattern recognition, since cohesive patterns of gene regulation determined by cluster analysis, for example, can provide important clues as to cellular processes, signal transduction pathways and gene transactivation by families of transcription factors in the context of disease (C Reich, Boston, MA, USA). To this end, our own prior knowledge must be channelled in ways that will facilitate the process, rather than hinder it.

Exploring the genetic basis of osteoarthritis is a case in point, since the most consistent candidate genes that turn up in association studies include COL2A1, aggrecan and insulin growth factor-1 (E Slagboom, Leiden, The Netherlands). These may seem obvious candidates, but the choice may be biased. Perhaps the biggest surprise over recent years is the significant genetic contribution to the disease, with computations of up to 78% for heritability. The results depend on the phenotype of probands. Nevertheless, the long arm of chromosome 2 has thrown up some potentially exciting genetic intervals as a result of genome-wide scans, with LOD scores ranging from 2.5 to 5.3 (E Slagboom).

Recent data suggest that, across the chromosome, recombination rates are highly variable. This in itself is worthy of more detailed study. The implications are exciting, because they suggest that analysis of haplotypes across large regions of chromosomes may provide a meaningful approach to exploring genetic variation and susceptibility to disease. This was elegantly illustrated with cytokine gene polymorphisms, using as a prototype haplotypes of the IL-1 gene family based on groups of single nucleotide polymorphisms (SNPs) (G Duff, Sheffield, UK). IL-1A, IL-1B and IL-1RN are just three of a cluster of at least 14 genes on chromosome 2 where up to 100 SNPs appear to be inherited in a nonrandom fashion (linkage disequilibrium). For example, haplotyping according to IL-1A, IL-1B and IL-1RN [e.g. IL-1A(–889), IL-1B(–511), IL-1B(+3953), IL-1RN intron 2 variable nucleotide tandem repeats] SNPs reveals one IL-1 genotypic pattern with an odds ratio of 4.0 for cardiovascular disease (comparable with that of smoking).

Combined with cholesterol levels, predictive values are further enhanced. This haplotype pattern also confers threefold to fourfold increases in IL-1β production in response to lipopolysaccharide stimulation. One IL-1B haplotype has been identified in patients with RA. More exciting are the recent pharmacogenotyping analyses of patients treated with IL-1RA, since it turns out that patients carrying the IL-1A*2-containing haplotype are more likely to respond to treatment (63.4%) than non-carriers (26.3%). A similar approach would be valuable for assessing responses to TNF blockade, but there are major economic implications. For example, this approach predicts that physicians and pharmaceutical industries alike face further challenges in the future when confronted with very low frequencies of responder haplotypes within the disease population. Regardless of this, the prospect for genotype-directed therapy, stratified to SNP haplotypes, is an exciting one.

Reverse genomics is a term now being used for autoantibody profiling, a technology where a large number of peptide or protein antigens (200 pg antigen in a 1 nl volume) are 'arrayed' onto poly-L-lysine-coated glass slides, probed with sera from patients and are detected with Cy3-labelled anti-human IgG (W Robinson, PJ Utz, Stanford, CA, USA). This has the major advantage of immediate identification and can also be used for more precise epitope mapping, an approach that has already been validated in mouse models of autoimmunity. For example, the arthritis chip carries proteins such as collagen type II, HCgp-39, vimentin and other citrullinated antigens, and 'mixed connective tissue disease' and 'myelin' chips have also been generated. This large-scale serotypic profiling has huge potential for both diagnostics and prognostics in a broad range of diseases.

Therapeutic targets

The arena of target identification and validation has always been a hot one. For example, there has been considerable progress in the identification of anatomical 'post codes' that direct leukocytes to specific tissues such as the inflamed synovial joint. The laboratory of Pitzalis and colleagues, London, UK, have combined phage display technology and *in vivo* 'panning' techniques using human–SCID chimeras to identify peptide ligands that may selectively target synovial tissue microvascular endothelium. The elegance of this approach is in the *in vivo* validation, since synthetic peptides based on phage

insert sequences can then be tested for their capacity to bind and block leukocyte trafficking to synovial tissue, but not to other organs (L Lee, London, UK). There seem to be specific motifs, but whether these bind one integrin molecule or integrin-like molecule or several distinct surface receptors awaits further investigation.

Signal transduction pathways have for many years seemed attractive candidates as therapeutic targets based on the assumption that they might provide more specificity and selectivity than the current approaches cytokine blockade has to offer. Until the redundancy and pathway overlaps have been comprehensively mapped this will remain an assumption, but much emphasis has been placed on targeting NF-κB, MAP kinase and their upstream activators. There is increasing evidence for NF-κB drive in inflamed RA synovium based on the anti-inflammatory effects of expressing IκBα in dissociated cultures of synoviocytes using adenovirus-based vectors (M Feldmann, London, UK). The effects of NF-κB-inducing kinase in promoting dendritic cell antigen-presenting function would also seem to suggest that targeting this pathway may have effects that are not just confined to the inflammatory response.

Consistent with this model are data demonstrating potent disease-promoting and ameliorating effects of dominant-active IKK2 and dominant-negative IKK2 when injected into the knee in rat adjuvant arthritis (A Manning, St Louis, MO, USA in collaboration with the Firestein Laboratory, San Diego, CA, USA). A putative IKK2 small molecule inhibitor has now been developed (SC-514) that has an IC_{50} value of 10–20 μM following IL-1β stimulation, but does not completely block IκBα degradation at 30 μM. The effects of this agent *in vivo* are awaited with interest, as are the effects on innate immunity.

SP600125, on the contrary, inhibits c-*Jun* N-terminal kinase activity with an IC_{50} value of ~100 nM. Its specificity is suggested by the fact that p38 and ERK activity cannot be inhibited at drug concentrations >30 μM. In assays of T-cell activation and differentiation, SP600125 inhibited anti-CD3/anti-CD28-induced proliferation and inhibited differentiation along the Th1 lineage.

The effects of p38 inhibition are currently under investigation, and several pharmaceutical companies have invested heavily in developing specific inhibitors. Part of the reason for this lies in the potent effects of this MAP kinase on post-transcriptional regulation of inflammatory gene expression. This effect is particularly pronounced for genes that carry reiterative AUUUA repeats in their 3′ untranslated region, such as the COX2 and TNF-α genes (J Saklatvala, London, UK). This has raised the possibility that the p38 pathway may act on RNA binding proteins, leading ultimately to enhanced mRNA stability. This was shown for COX2 mRNA using a Tet^{off} reporter system. The effects can be blocked with the p38 inhibitor SB203580 or promoted with dominant-active mutants of MKK6, which act upstream of p38 (J Saklatvala). More intriguing is the finding that the synthetic corticosteroid dexamethasone is a potent inhibitor of this pathway, leading to destabilisation of inflammatory gene mRNA species. A detailed analysis revealed that dexamethasone at nanomolar concentrations targets p38 directly, perhaps by upregulating the expression and/or activity of a p38 phosphatase. These studies illustrate how detailed molecular analysis of the activities of anti-inflammatory agents that have been tried and tested in the clinic for almost half a century can provide considerable insight into the inflammatory process.

Concluding remarks

Huge progress in the development of new technologies permits us to explore pathways and disease processes on a scale that would not have been possible 5 years ago. One key challenge that must be overcome relates to information overload, and the mechanisms whereby the significant data can be dissected from the background noise. Nevertheless, this meeting has demonstrated unambiguously that such advances are already paying dividends in furthering our understanding of mechanisms of disease pathogenesis. In a convivial atmosphere, we have also experienced first hand that this knowledge is already being translated into the development of exciting new therapeutic targets with a ‘tailor-made’ flavour.

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