Meeting report

4th meeting of the EU research network EUROME: From the identification of genes and cellular networks in murine models of arthritis to novel therapeutic intervention strategies in rheumatoid arthritis, London, UK, 9 March 2004

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Received: 6 May 2004 Accepted: 19 May 2004 Published: 18 June 2004

Arthritis Res Ther 2004, **6**:155-158 (DOI 10.1186/ar1200)

Abstract

Rheumatoid arthritis (RA) is a common human disease with a prevalence of about 1% in most parts of the world. At the time of symptom onset it is difficult to predict the severity of subsequent disease course. After 2 years joint erosions are seen in most patients, and most patients become clinically disabled within 20 years. A recent meeting at the Kennedy Institute of Rheumatology (Imperial College, London) brought together representatives from several European centres of excellence, to discuss research funded by the EU Framework 5 Quality of Life Programme. This research network combines gene and protein expression profiling with different animal models of RA to identify cells, genes and pathways contributing to arthritis initiation, progression and chronicity. The studies discussed highlight the reality that collaboration between different research groups is the basis of groundbreaking research and, it is hoped, eventual new therapies for RA.

Keywords: arthritis, genomics, therapy

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown aetiology and is one of the most common causes of disability in the Western world. The research network, EUROME (http://www.eurome.de/), supported by the EU Framework 5 Quality of Life and Management of Living Resources Programme, represents European Centers of Excellence (based in Germany, Greece, the UK, Sweden, Finland and Switzerland) applying state of the art functional genomics technologies such as genome, transcriptome (microarrays), and proteome analysis, to the study of animal models of RA. This programme promotes collaborative research between different centres across Europe, each bringing its own strengths and expertise, from the development of anti-tumour necrosis factor-α (anti-TNF-α) therapy to cutting-edge expression profiling and proteomics.

The 4th meeting of the EUROME participants was held at the Kennedy Institute of Rheumatology at Imperial College, London, on 9 March 2004.

Genetic approaches to therapy for rheumatoid arthritis

Analysis of gene expression using gene or protein chips and SNP analysis is a novel means to understanding the role of different proteins and pathways in different stages of arthritis. Dr Saleh Ibrahim (University of Rostock, Germany) reported on the Rostock group's progress in identifying new genes and pathways contributing to the pathogenesis of murine collagen-induced arthritis (CIA) [1]. The group has previously described the gene expression profile at the peak of disease in DBA1/J mice [2], and more recently has established the gene expression profile of different disease-related tissues such

BMDC = bone marrow-derived dendritic cell; CIA = collagen-induced arthritis; DC = dendritic cell; NF = nuclear factor; RA = rheumatoid arthritis; Th = T helper; TNF = tumour necrosis factor; TNF-R = tumour necrosis factor receptor; VEGF = vascular endothelial growth factor.

as lymph nodes and joints at various stages of disease in susceptible DBA/1J and resistant FVB/N strains. In parallel, a genome screen was performed of the F_2 progeny mice of a cross between the strains to identify additional quantitative trait loci for CIA. Two quantitative trait loci identified in previous studies were confirmed, namely severity-controlling Cia2 and controlling onset trait Cia4, both on chromosome 2. In addition, five new quantitative trait loci were identified, one for collagen II-specific IgG2a levels on chromosome 5, two controlling collagen II-specific IgG1 response on chromosomes 10 and 13, one for CD4/CD8 ratio on chromosome 2, and one for cell proliferation on chromosome 16. The group also described the first example of an epistatic interaction involving mitochondrial and nuclear genomes in CIA. In the same cross a locus on chromosome 7 was found to interact with the mitochondrial genome and control diverse arthritis-related traits such as disease severity, cell death, CD4/CD8 ratio, ATP/ADP ratio and the production of reactive oxygen species.

Professor Rikard Holmdahl (Lund University, Sweden) described the identification of the *Ncf1* genetic polymorphism controlling arthritis severity. The Lund group uses pristane-induced arthritis in the rat as a model of RA. A joint-specific disease with many similarities to human RA develops after a single injection of pristane subcutaneously [3]. This contrasts with murine pristane-induced arthritis, where a systemic granulomatous disease, including arthritis, occurs after repeated injections of pristane intraperitoneally. To identify the genes controlling this disease the group has made crosses between susceptible and resistant rat strains. Several loci that control the onset of arthritis, the severity and chronicity of the disease, and autoantibody production have been identified and been confirmed in congenic strains. One gene, *Ncf1*, has been identified that controls arthritis severity [4]. The *Ncf1* gene unexpectedly controlled T cell activation through the release of reactive oxygen species.

Dr Vassilis Aidinis (BSRC Fleming, Athens, Greece) focused on the role of synovial fibroblasts in RA, using differential gene expression analysis, DNA microarrays and subtractive hybridizations coupled with large-scale sequencing [5]. Two spontaneous animal models were used, namely transgenic mice overexpressing human TNF (Tg197 hTNF$^{+/-}$) and knock-in mice overexpressing murine TNF (mTNFΔARE$^{+/-}$). Deregulated genes were replaced by their corresponding Gene Ontology terms, to look for deregulated functions rather than genes. Statistical analyses indicated that cytoskeleton organization becomes deregulated, in addition to the known major functional changes (collagen metabolism, immune and stress response). This hypothesis was validated both *in vitro* and *in vivo*, in that arthritic fibroblasts exhibited F-actin stress fibres that were most probably due to the increased adhesion to the substratum (extracellular matrix). More importantly, knocking out the expression of gelsolin, an actin-binding protein with filament-severing properties found downregulated in RA, resulted in mild exacerbation of the arthritic phenotype. Furthermore, bone marrow grafting experiments were performed into lethally irradiated hosts. Wild-type, mTNFΔARE$^{+/-}$, Tg197 hTNF$^{+/-}$, TNF/TNF receptor (TNF-R)$^{-/-}$ and mTNFΔARE$^{+/-}$/TNF-R$^{-/-}$ mice were used as recipients and/or donors of bone marrow cells. The results indicated that there is redundancy in pathogenic TNF sources (bone marrow cells in the TNFΔARE model or stromal-radioresistant cells in the Tg197 hTNF$^{+/-}$ model) that suffice for the induction of arthritis. In contrast, in all cases examined, the indispensable receptor for the arthritic process is TNF-R1 in recipient mice.

The research within EUROME also focuses on the identification of possible therapeutic targets in RA, using different animal models of disease. Dr Ewa Paleolog (Imperial College, London, UK) described the effect of angiogenesis blockade in murine CIA. Angiogenesis represents an attractive target for therapy in RA, in that increased synovial vessel density is a feature of RA and several angiogenic factors are expressed in RA, including vascular endothelial growth factor (VEGF). The London group has investigated the effect of angiogenesis blockade in murine arthritis, using CIA in genetically susceptible DBA/1 mice. With the use of an adenoviral gene delivery system expressing soluble VEGF receptor type I, disease severity and paw swelling were significantly suppressed. Furthermore, blockade of VEGF resulted in reduced joint levels of the vascular marker von Willebrand factor, indicating that VEGF inhibition was associated with reduced synovial vascular density. Finally, soluble VEGF receptor type I reduced synovial inflammation and bone destruction in CIA [6]. To study the mechanism of action of VEGF blockade in CIA, endothelial cells were infected with NF-κB–luciferase reporter adenovirus, because many genes involved in proliferation and apoptosis are regulated by NF-κB. Significant activation of NF-κB was observed in response to VEGF. When the endogenous NF-κB inhibitor IκBα was overexpressed in endothelial cells, VEGF-mediated NF-κB activation, as well as expression of anti-apoptotic proteins Bcl-2 and members of the inhibitor of apoptosis family (cIAP-1, XIAP and survivin, which directly bind to and inhibit caspases), was strikingly reduced.

Dr Brigitte Mueller-Hilke (University of Rostock, Germany) presented studies aiming at a cellular immunotherapy in murine arthritis. Dendritic cells (DCs) have a central role in the initiation and regulation of immune responses. Several mechanisms have been suggested to regulate the differentiation of immature DCs into distinct populations supporting the polarization of naive CD4$^+$ T cells into

either T helper (Th) 1 or Th2 effector cells. The goals of this arm of EUROME are to identify genes and pathways involved in this differentiation of DCs and to set up an *ex vivo–in vivo* cell therapy whereby *in vitro* differentiated DCs supporting Th2-type responses will be transferred into CIA mice to ameliorate the autoimmune process. On the basis of the previous finding of a differential impact of Th1 and Th2 cells on the function of bone marrow-derived DCs (BMDCs), transcriptional changes induced in BMDCs by Th effector cells were investigated. By using oligonucleotide microarrays the group showed that BMDCs co-cultured with either Th1 or Th2 cells display different gene expression patterns. A total of 115 differentially expressed genes were identified, which might be involved in the regulation of Th cell polarization and the shaping of the immune response.

Dr Harald Illges (Biotechnology Institute Thurgau, Switzerland) described studies on the K/BxN murine model of arthritis, in which autoantibodies directed against glucose-6-phosphate isomerase are responsible for pathology and can reproducibly transfer the disease into naive animals. Experimental work with this model has established roles for B-cell-secreted autoantigenic immune complexes in activating alternative complement, its subsequent association with C5aR and FcgRIII-mediated cell activation resulting in innate cell mediator activation and the production of inflammatory cytokine interleukin-1 and TNF-α, leading to joint destruction. In recent studies, mice depleted of macrophages by clodronate liposome treatment were found to be completely resistant to arthritis induced by K/BxN sera. Reconstituting clodronate liposome-treated mice with macrophages from naive animals could reverse this resistance – deficiencies in Wiskott–Aldrich syndrome protein and CD40, both of which are implicated in macrophage activation, chemotaxis and phagocytosis, are not essential in sera-induced arthritis.

Professor Seppo Meri (Department of Bacteriology and Immunology, University of Helsinki, Finland) discussed arthritis as a parainfectious or postinfectious complication to a microbial infection, with a focus on the complement system. In addition to direct activity in antimicrobial defence, the complement system has an important role in the clearance of cell and tissue remnants after damage caused by infection, ischaemia, apoptosis or physical injury. A failure in this activity predisposes the host to several modified antigens and antigen-modifying factors that could induce post-translational changes in proteins. Arthritis that is associated with an infection by *Borrelia burgdorferi* ('Lyme arthritis') very closely mimics RA and is even associated with the same HLA-DR4 class II histocompatibility antigens. The fact that *B. burgdorferi* can cause a chronic infection is based on the ability of the bacterium to escape complement-mediated opsonophagocytosis by binding the complement inhibitor factor H (and in some cases, also the factor H-like protein 1) to its surface. Binding is mediated by two types of plasmid-encoded protein, class I (20 kDa proteins) and class II (27.5–35 kDa proteins). Outer surface protein E was described as the first example of class I proteins. It constitutes a family of homologous proteins, of which several different types, each encoded in a different but homologous cp32 plasmid, exist on a single species of *B. burgdorferi* [7]. A second example of microbe-induced arthritis is reactive arthritis. It follows an infection caused by a Gram-negative enterobacterium (*Yersinia*, *Salmonella*, *Shigella* or *Campylobacter* sp.) or *Chlamydia trachomatis*. Some of the enterobacteria that initiate reactive arthritis possess proteases, such as PgtE in *Salmonella enterica*, that cleave the complement components C3b and C4b and many other host proteins. Because the proteases can be active inside cells, these could generate *de novo* antigenic peptides inside microbe-infected cells and elicit an immune response that leads to arthritis.

Finally, Dr Thomas Svensson (Arexis AB, Mölndal, Sweden) discussed the development and implementation by Arexis of a database application that facilitates experimental genetic research. The application is based on an Oracle database engine, and functionalities include the management of experimental objects, for example patients or animals, and their corresponding phenotypes of interest, as well as collected genotypes. The database application also offers comprehensive sorting and formatting of data to prepare for statistical analysis by stand-alone software.

Conclusion

No single animal model of RA, whether it be the conventional CIA mouse model, K/BxN transgenics or mice overexpressing TNF, is likely to allow the identification of cells, genes and pathways contributing to RA. Nevertheless, as this meeting highlighted, animal model studies can yield valuable data about new susceptibility genes. By making use of adenovirus-based and cell-based transfers, the feasibility of novel therapeutic interventions will be capable of determination in future.

Competing interests

None declared.

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