Meeting report

Cytokines in systemic lupus erythematosus, London, UK

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Abstract

The meeting consisted of 11 talks that illustrated the complexity of the pathogenetic mechanisms underlying systemic lupus erythematosus and aimed to identify ways in which cytokine modulation might affect those mechanisms. The evidence relating to the involvement of tumour necrosis factor-α, interleukin-10 and BLyS in this disease was discussed in particular detail. A final discussion explored the possible ways in which cytokine modulation might lead to new methods of treating systemic lupus erythematosus in the future.

Keywords: cytokine, systemic lupus erythematosus

Introduction

The aim of the meeting was to provide an overview of the ways in which modulation of cytokines may be important in the pathogenesis and treatment of systemic lupus erythematosus (SLE). It comprised a set of 11 talks from an international group of speakers followed by a vigorous discussion.

Marc Feldmann (Kennedy Institute of Rheumatology, London, UK) and David Isenberg (University College London, UK) welcomed the 50 participants, emphasizing the diversity (clinical and serological) of SLE and the likely complexity of cytokine involvement in its development.

Two major themes emerged from the meeting. First, lupus is a complex disease and many different elements contribute to its pathogenesis. These include factors that are intrinsic to the immune system, such as B or T lymphocyte dysfunction, and factors that are extrinsic to the immune system but linked to it, such as abnormal clearance of apoptotic cells or endothelial activation. All of these factors represent potential targets for cytokine action and hence manipulation, and many different cytokines may be involved. Second, although many cytokines may be involved in the pathogenesis of SLE, research thus far has concentrated on a small group of cytokines, notably tumour necrosis factor (TNF)-α and IL-10. Evidence relating to these cytokines was considered in detail in several of the presentations.

Mechanisms in the pathogenesis of systemic lupus erythematosus

Mark Walport (Imperial College London, UK) described the evidence suggesting that delayed or deficient removal of debris from dying cells may play a role in the development of autoantibodies in SLE [1]. It has been shown that phagocytes from patients with SLE and from lupus prone mice have impaired ability to ingest such material *in vitro*. Material from dying cells such as apoptotic blebs and apoptotic bodies that are not efficiently removed because of this impairment may reach lymphoid tissues and act as antigenic stimuli. The surfaces of apoptotic bodies carry complexes of molecules that are known autoantigens in patients with SLE and related disorders, such as DNA, histones, anionic phospholipids and β_2-glycoprotein I [2]. Such antigens are not generally found on the surfaces of intact nonapoptotic cells. Furthermore, the binding of complement component C1q to these complexes may explain the production of anti-C1q antibodies, which is found in approximately one-third of patients with SLE [3].

Cytokines may be involved in this abnormal clearance of cellular debris. For example, the physiological phagocytosis of apoptotic material is associated with the release of

ds = double-stranded; IL = interleukin; SDF = stromal cell derived factor; SLE = systemic lupus erythematosus; TNF = tumour necrosis factor.

anti-inflammatory cytokines such as transforming growth factor-β, whereas this is likely to be altered under conditions of delayed phagocytosis in SLE.

Sir Ravinder Maini (Kennedy Institute of Rheumatology, London, UK) pointed out that the production of anti-DNA antibodies in some patients treated with anti-TNF-α drugs could be related to this mechanism of impaired waste disposal. Cells bearing surface TNF-α are lysed by the antibody *in vitro*, thus increasing the amount of cellular debris to be removed. However, there is no evidence of this mechanism *in vivo*, although nucleosome antigens resulting from apoptosis are detectable during the normal course of disease in the joints and blood of rheumatoid patients. These would provide the immunogenic drive for antinuclear antibody production. In anti-TNF treated patients the nucleosomal load may be coupled with reduced removal of this debris as a result of reduction in circulating levels of C-reactive protein and serum amyloid protein A.

Michael Ehrenstein (University College London, UK) emphasized the fact that many different mechanisms may lead to the development of SLE. A large number of different and unrelated murine models show clinical and/or histological features akin to human SLE [4]. Although many of these models are deficient in functions related to lymphocytes or clearance of apoptotic cells, there are others in which there is no apparent rationale for the development of a lupus-like disease. There is no consistent cytokine pattern common to all of the models.

A number of these models are characterized by abnormal B-cell function. For example, mice deficient in secreted (but not membrane bound) IgM develop autoantibodies and deposition of immunoglobulin and C3 in their kidneys [5]. These mice exhibit expansion of marginal zone B cells and an increase in the number of B1 cells. The self-renewing B1 compartment is also expanded in lupus-prone NZB/W F1 mice, and these B1 cells can secrete autoantibodies. Proliferation of B1a cells in NZB/W F1 mice is dependent on IL-10 and stromal cell derived factor (SDF)1.

The cytokine BLyS, a member of the TNF family, is important in the development of B lymphocytes. Jane Gross (Zymogenetics Inc., Seattle, WA, USA) reviewed the evidence that BLyS is important in the pathogenesis of SLE. BLyS is elevated in the serum of patients with SLE, and mice that constitutively over-express BLyS develop autoantibodies and glomerulonephritis.

Dorian Haskard (Imperial College London, UK) outlined ways in which the effects of cytokines on the vasculature may contribute to the pathogenesis of SLE. Intravital microscopy shows that transmigration of TNF-α-stimulated leucocytes through chronically activated endothelium is enhanced in lupus prone MRL *lpr*/*lpr* mice as compared to wild-type MRL mice. Similar enhanced leucocyte–endothelial cell interactions may also occur in patients with SLE, in whom circulating levels of TNF-α are sufficient to stimulate the expression of intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin by endothelial cells.

The range of mechanisms that are involved in the pathogenesis of SLE is therefore so diverse that a single cytokine may exert important effects at a number of different levels. TNF-α is the prime example of this phenomenon.

Tumour necrosis factor-α: the Janus cytokine

In NZB/W F1 mice, the administration of TNF-α reduces the severity of the lupus-like illness [6]. This observation has not been repeated in other lupus-prone mouse strains and the effect of TNF-α in NZB/W F1 mice depends on the dose and the age of the mice [7].

Rizgar Mageed (University College London, UK) described a series of experiments designed to clarify the effect of TNF-α in the NZB/W F1 strain. Immunization of young NZB/W F1 mice with phosphatidylcholine/ovalbumin conjugate leads to the production of anti-double-stranded (ds)DNA antibodies. This effect is reduced by administration of recombinant TNF-α and enhanced by anti-TNFα. Histological examination of lymphoid tissues of these mice showed that TNF-α reduces the size of T cell areas, whereas anti-TNF-α promotes T cell function but disrupts B cell migration.

This work led to the hypothesis that different results would be obtained in neonatal mice. It was postulated that, in these mice, anti-TNF-α would enhance T cell function in such a way as to promote tolerance and reduce autoimmunity. However, the results of the experiment did not support the hypothesis. Neonatal mice treated with anti-TNF-α developed increased numbers of T cells, more anti-dsDNA and antinucleosome antibodies, and increased proteinuria in comparison with mice treated with a control antibody.

The concept that TNF-α protects against the development of SLE was also supported by studies conducted in TNF-α-deficient mice, described by Rachel Ettinger (National Institutes of Health, Bethesda, MA, USA). These mice develop antinuclear and anti-DNA antibodies after 15 weeks of age, but do not develop lupus-like illness. However, the actual mechanism of this effect is uncertain because mice that lack both the TNF-55 and TNF-75 receptors do not develop these autoantibodies. Moreover, the effect is highly dependent on genetic background. $TNF^{-/-}$ mice on a B6 background do not develop autoantibodies, whereas those on a mixed B6 × B129 background do. Therefore, it appears that a gene on the 129 background is required to predispose the TNF-deficient mice to autoimmunity. The development of autoantibodies is dependent on T cells and on IL-6,

because autoantibodies do not develop in TNF-$\alpha^{-/-}$ mice that lack either T cells or IL-6.

These experiments in murine models suggest that administration of anti-TNF-α might predispose to the development of SLE in humans. Because anti-TNF-α drugs are now in widespread use in the treatment of rheumatoid arthritis and Crohn's disease, there are clinical data relating to this issue. These data were reviewed by Sir Ravinder Maini.

Anti-dsDNA antibodies occur very rarely in patients with rheumatoid arthritis who have not received anti-TNF-α therapy but were reported in 7% (11/156) of patients who had received such treatment [8]. After a single infusion of infliximab, anti-dsDNA antibodies first develop after a mean of 6.3 weeks and disappear 4–6 weeks later. When repeated infusions are given, the anti-dsDNA antibodies may not disappear until after the last infusion. Anti-dsDNA antibodies have been reported after treatment with either infliximab or etanercept, and the dose of anti-TNF-α given does not affect the likelihood of an anti-DNA response.

The majority of these patients develop IgM but not IgG anti-dsDNA antibodies and do not develop clinical features of SLE. However, clinical SLE can occur following anti-TNF-α treatment, and there are a number of well documented cases [9]. The disease is mild, remits when the drug is stopped, and neither cerebral nor renal involvement has been reported.

Why is the prevalence of clinical SLE after anti-TNF-α treatment so low (0.04–0.2%) when the prevalence of anti-dsDNA antibody production following such treatment is much higher (16%)? Similarly, why do TNF-α knockout mice develop autoantibodies but not a lupus-like illness? One possibility is that TNF-α exerts two opposing effects. The first effect operates at the level of T lymphocytes to suppress autoantibody formation. The second effect operates at the level of the target tissues to promote inflammation. For example, TNF-α is known to activate endothelium, which could lead to transmigration of leucocytes into the tissues.

The concept that TNF-α could have two opposing effects in SLE was aptly summarized by Josef Smolen (University of Vienna, Austria) who dubbed it the Janus cytokine in honour of Janus, the double-faced god of Roman mythology. He pointed out that levels of TNF-α are raised in the serum of patients with SLE [10] (although levels of the soluble inhibitor TNF receptor are also raised) and that it has been detected in renal biopsies of patients with lupus nephritis. These findings suggest that TNF-α blockade might be useful as a treatment for SLE.

Smolen reported his experience with four patients with SLE who had been treated with 5 mg/kg infliximab and concomitant azathioprine. In this open trial, all four patients showed signs of clinical improvement, even though levels of anti-dsDNA rose in two cases.

At this point, therefore, the place of TNF-α blockade in the treatment of SLE is unclear. Although there is a large body of evidence pointing to protective effects of TNF-α against the development of autoimmunity in both humans and mice, there is also evidence that anti-TNF-α could be used as an agent to reduce tissue damage in patients with SLE.

Interleukin-10

In contrast to TNF-α, there is more consensus concerning the role played by IL-10 in SLE. IL-10 levels are consistently high in the serum of patients with this condition, and anti-IL-10 antibodies ameliorate disease in murine models of SLE. In a small clinical trial, 21 daily doses of intravenous monoclonal murine anti-IL-10 antibody led to a clinical improvement in patients with SLE. This was maintained for up to 6 months [11].

Bernard Lauwerys (Universite Catholique de Louvain, Brussels, Belgium) examined the possible mechanism of action of IL-10 in SLE. It seems likely that the balance between IL-10 and IL-12 is important [12]. Supernatants of cultured peripheral blood mononuclear cells derived from patients with SLE inhibit allogeneic T cell reactions *in vitro*, but this effect can be reversed by adding IL-12 or anti-IL-10. Levels of the biologically active form of IL-12 (p-70) are low in the serum of patients with SLE, and the addition of IL-12 inhibits antibody production by SLE peripheral blood mononuclear cells *in vitro*.

What is the source of the raised levels of IL-10 in patients with SLE? B cells are a major source of this cytokine in patients with certain autoimmune conditions, such as SLE, Sjögren's syndrome and rheumatoid arthritis. Dominique Emilie (Institut Paris-Sud sur les Cytokines, Clamart, France) described a possible role played by B1a cells in NZB/W F1 mice. These cells are expanded in this strain under paracrine stimulation by SDF1 secreted from peritoneal mesothelial cells and autocrine stimulation by IL-10 produced by the B1a cells themselves. Treatment of NZB/W F1 mice with either anti-SDF1 or anti-IL-10 reduces proteinuria and prolongs survival. This is associated with a contraction of the B1a cell population in the peritoneum.

Consideration of the role played by IL-10 thus raises three possible avenues for treatment of SLE: anti-IL-10, anti-SDF1 and IL-12. Only the first of these has been the subject of a trial in humans (as described above and by Llorente and co-workers [11]).

Therapy directed against B lymphocytes

A number of lines of evidence implicate B cells in the pathogenesis of SLE, as sources of antibody, cytokines or as

antigen-presenting cells. It is therefore logical to conclude that treatments that target B cells might be useful in SLE.

Michael Ehrenstein described encouraging results obtained with the monoclonal anti-CD20 antibody rituximab in eight patients with SLE [13]. These patients showed improvements in disease activity, measured using the British Isles Lupus Assessment Group index. Improvements in fatigue, arthralgia/arthritis and serositis were especially striking. Because CD20 is present on all B cells from the pre-B-cell stage, rituximab therapy leads to profound B cell depletion, but not all of these patients experienced a fall in anti-dsDNA antibody levels and there were no severe infections. This may be due to the fact that plasma cells do not carry CD20.

Jane Gross described the use of a soluble inhibitor of BLyS function (TACI-Ig), which comprises the TACI receptor fused to an immunoglobulin Fc region. TACI is one of the three cellular receptors for BLyS. Administration of TACI-Ig to mice reduces the numbers of mature B cells and inhibits both T-cell-dependent and -independent B lymphocyte responses. Administration of TACI-Ig to NZB/W F1 mice, either for a short period (between the ages of 22 and 28 weeks) or chronically, reduced B cell numbers, anti-DNA antibody levels and proteinuria, and prolonged survival.

Discussion – where do we go from here?

Peter Lipsky (National Institutes of Health, Bethesda, MA, USA) noted that the position outlined in the day's presentations was similar to that pertaining to cytokines in rheumatoid arthritis 10–15 years ago. There was a certain amount of experimental evidence suggesting that some cytokines were involved in pathogenesis of the disease, and the challenge was to translate that knowledge into the development of new forms of treatment. It was possible to discern a hierarchy of importance for cytokines in rheumatoid arthritis, which eventually led to the development of drugs to target the most important cytokines in the hierarchy, notably TNF-α and IL-1.

Although no such hierarchy is immediately apparent in SLE, we have sufficient evidence to consider certain cytokines as targets in the treatment of this disease. The most notable examples discussed at the meeting were TNF-α, IL-10, IL-12 and BLyS.

Is it likely that government agencies or pharmaceutical companies will fund the large trials necessary to investigate the efficacy of these forms of treatment in SLE? Peter Lipsky stressed the need to develop reliable biomarkers of cytokine function before embarking on such trials, so that we can be sure that any clinical effect of a drug is actually due to its postulated effect on a particular cytokine pathway. David Isenberg pointed out that validated measures of disease activity and damage in SLE already exist, and could be used to assess the response of patients to cytokine-modulating agents.

Josef Smolen addressed the difficulty of organizing large randomized controlled trials of cytokine modulating therapies, especially in a disease such as SLE, which is not common, and in which even those individuals who are affected often do not have sufficiently severe disease to warrant entry into such trials. Perhaps other trial designs might be considered.

Conclusion

The meeting showed the breadth of interest in the role played by different cytokines in SLE. A large amount of work in mice, and a smaller body of evidence on the effects of anticytokine antibodies in humans, suggests possible targets for therapy. A major challenge for the future is to define which of these targets will actually be useful in the management of SLE. In the light of the success in rheumatoid arthritis, this is a topic of high priority.

Competing interests

None declared.

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