Annus Mirabilis - A Guide To The 6th European Lupus Meeting 03-05 March 2005

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Introduction

2005 will be an annus mirabilis for systemic lupus erythematosus (SLE). Several major double blind controlled trials involving hundreds of patients with lupus will be getting started. These trials include a comparison of mycophenolate and cyclophosphamide in lupus nephritis, the use of the humanised anti-CD20 monoclonal antibody, CTLA-4 Ig, anti-BLys and a DNA antibody binding peptide. These potential therapeutic advances have been possible due to basic science research, which has illuminated many facets of the immunopathogenesis of SLE.

At the 6th European Lupus Meeting, held in London at the Royal College of Physicians on 3rd - 5th March 2005, these clinical and basic science aspects of SLE were brought together with detailed reviews of the immunogenetics of lupus, the roles of complement, cytokines, B and T lymphocytes and antibodies in the pathogenesis of the disease, and detailed assessments of the optimal management of lupus in the kidney, brain, lungs, skin and abdomen.

Based upon a selection of the talks given at this meeting the Journal will be publishing a series of reviews designed to provide a comprehensive update on the 'hot topics' in SLE. These reviews however cannot provide the 'full flavour' of the contents of what was a lively and full programme offering parallel clinical and basic research sessions over a three-day period. This article is intended as a companion

piece and introduction to the commissioned updates to provide a fuller assessment of the contents of the meeting. Amore detailed review of the presentations at the meeting will be published in *Lupus* this year.

It is however worth considering why several of the big pharmaceutical companies have taken a major interest in lupus. This interest seems to rest upon the realization that, whilst not common, there are hundreds of thousands of patients with lupus in the United States, Europe and the Far East. A greater understanding of the pathogenesis of lupus has led to the pinpointing of several key molecules which if blocked could provide therapeutic benefit analogous to the results of tumour necrosis factor alpha (TNFα) blockade in patients with rheumatoid arthritis . Several agents that are indeed capable of blocking these key molecules have been developed. Furthermore, the lupus research community has developed the key tools i.e. activity, damage and patient health assessment indices that are needed to make assessment of the effectiveness of these new agents in clinical trials a reality.

Clinical Aspects of Lupus

A session on outcomes in SLE began with recognition of the dramatic improvement in survival rates over the last thirty years. Much of this improvement can be attributed to the development of effective immunosuppressive regimes such as the National Institutes of Health regime for the treatment of lupus nephritis (1). This improvement in survival,

however, coincides with an increase in late morbidity - some of which is related to the drug treatment of the disease. For example, long-term high dose corticosteroid treatment may lead to osteoporosis or avascular necrosis of bone, and cyclophosphamide is associated with infertility (2) and bladder toxicity. Speakers in the session on renal disease provided a timely reminder of the availability of new agents to reduce the development of damage and end stage renal failure in lupus nephritis. Mycophenolate mofetil is one of the best-known examples (3). It is also important to note that other forms of renal pathology, such as thrombo-occlusive disease due to antiphospholipid antibodies, may occur in patients with SLE and may require treatment other than immunosuppression. For cases where treatment of lupus nephritis fails to preserve renal function, living donor renal transplantation was reported to have outcomes no worse in patients with SLE than in patients with other forms of renal failure.

As the early mortality from active disease has fallen, the relative importance of cardiovascular disease as a cause of death in patients with SLE has risen. Patients with SLE are at higher risk of cardiovascular disease than age and sex matched population controls (4). This is partially due to traditional risk factors such as hypertension and smoking but partially due to other factors, which are the subject of intensive research. For example the enzyme paraoxonase normally plays a protective role against oxidation of low density lipoproteins (LDL) and this action may be impaired in patients with SLE and antiphospholipid syndome (APS) (5). How should we manage the increased cardiovascular risk in patients with SLE? A number of non-invasive techniques, such as carotid artery ultrasound and electron beam tomography can be used to detect

atherosclerosis in asymptomatic patients, but should these patients be treated with drugs? Statins, for example, could be introduced at lower levels of cholesterol and/or LDL than in the general population and might also have a beneficial effect on disease activity through their effects on lipid rafts, which are crucial to signalling in lymphocytes.

Central nervous system SLE is often difficult to diagnose and treat, but a session on this topic began with a description of the American College of Rheumatology definition of components of neuropsychiatric SLE (NP-SLE). These definitions will help us to obtain clearer evidence about the best way to treat patients with NP-SLE, though corticosteroids and cyclophosphamide have both been reported to be effective in some patients. Recent work from Betty Diamond's group suggests that some autoantibodies may cross the blood brain barrier to cause direct cerebral toxicity via an interaction with N-methyl-D-Aspartate (NMDA) receptors (6). We do not yet have a good specific biomarker for NP-SLE – though anti-ribosomal P antibodies were previously suggested to fit this purpose, there is no consensus on this subject.

Many patients with SLE suffer symptoms from organs such as skin, lungs and gastro-intestinal tract, which are rarely life-threatening and do not commonly require the use of potent immunosuppressive agents. A session on these manifestations stressed that the majority of patients may benefit from mild agents such as hydroxychloroquine, but that it is important to be alert to the possibility of rare but severe manifestations of SLE such as pulmonary hypertension or mesenteric vasculitis.

The advent of new biologic agents such as anti-CD22, anti-Blys and anti-CD20 was discussed in a plenary session. Encouraging evidence for the use of anti-CD20 in small groups of patients refractory to other treatments has been published (7) and larger trials are likely to follow. Even anti-TNFα has been used in a small group of patients by Josef Smolen's group in Austria (8). Although this agent can cause the production of anti-dsDNA antibodies, and even clinical SLE, in some patients with rheumatoid arthritis, anti-TNFα may be useful in reducing inflammation in patients who already have active SLE i.e. this drug can have opposing effects at different stages of the SLE disease process.

How will we decide which patients should be treated with these new agents? Biomarkers that could predict the onset of severe flares of SLE before the patients became very ill are needed. Anti-dsDNA antibodies have been used for this purpose for many years, though recent work suggests that many of these antibodies are actually anti-nucleosome antibodies. These may develop as a response to the presence of immunogenic material released from apoptotic cells. Measurement of anti-nucleosome antibodies may become a useful assay in the future. Antibodies to complement component C1q (anti-C1q) have not been used as widely as anti-dsDNA antibodies in the management of SLE, perhaps due to lack of specificity for the disease or due to technical factors. Anti-C1q, however, may be especially useful in renal SLE because levels of anti-C1q are particularly high in renal flares and these antibodies appear to sequestrate in inflamed kidneys in lupus nephritis. In murine models, anti-C1q antibodies do not cause nephritis when infused alone but do enhance the pathological effects of anti-glomerular antibodies. Conversely, low levels of

C1q itself are highly specific for SLE but have low sensitivity except in renal flares. 50-80% of patients with renal flares have low C1q. Low C3 and low C4 levels are more sensitive but less specific for SLE than low C1q.

Basic Science and the Pathogenesis of SLE

It was interesting that speakers in many sessions stressed the same themes, though approaching them from different angles. A recurrent theme was the importance of defective clearance of apoptotic cell debris in the pathogenesis of SLE (9). Apoptotic blebs have surface exposed antigens such as chromatin breakdown products and phophatidylserine (PS), which are not exposed in intact cells. PS is especially important because it acts as a recognition signal to mediate interactions between apoptotic cell debris and phagocytes. In patients with SLE, phagocytosis of apoptotic cells is defective and thus potentially immunogenic material can accumulate in lymph nodes and be taken up by dendritic cells. Whereas phagocytosis by macrophages is usually a non-inflammatory event, leading to the release of cytokines such as interleukin-10, uptake by dendritic cells is pro-inflammatory and stimulates an immune response. Dendritic cells, but not macrophages, incubated with apoptotic cells and then injected into mice caused development of high IgG titres and autoantibody formation in those mice, but not development of nephritis.

The autoantibodies produced in response to apoptotic debris in both murine models of SLE and in patients include antibodies to nuclear components such as nucleosomes.

Immune complexes containing these antigens stimulate activation of the Type I interferon (IFN) system, which plays a major role in SLE. Levels of IFNα are high and correlate with disease activity in SLE, microarray experiments show upregulation of IFN induced genes in patients with active SLE and genetic linkage studies in Scandinavian populations showed strong linkage of SLE to two genes involved in the Type I IFN pathway, tyrosine kinase 2 and IFN regulating factor 5 (10) . Furthermore, in a very recent paper, Sophie Koutouzov and colleagues showed that the introduction of IFNα cDNA to pre-autoimmune NZB/W F1 mice on an adenoviral expression vector led to sustained high levels of IFNα in the blood and to earlier development of glomerulonephritis and death (11).

The role of immune complexes in stimulating the autoimmune response in SLE is also important in mediating the effect of complement, which was discussed by a number of speakers. Humans with congenital deficiency of C1q, C2 or C4 have a high risk of developing SLE and C1q knockout mice develop a lupus-like illness with glomerulonephritis (12). Why does deficiency of these early complement components predispose to SLE? Current thinking suggests that these components are important in the clearance of immune complexes and apoptotic cell debris. It is likely that both complement receptors and Fc receptors contribute to clearance of immune complexes and this may contribute to the recognized link between Fcγ receptor polymorphisms and SLE. Kevin Davies and colleagues have shown that clearance of radiolabelled immune complex is abnormal in patients with C2 deficiency but can be corrected by infusing fresh frozen plasma (FFP) as a source of C2 (13). Infusion of FFP only produces a transient

repletion of complement components but wild-type bone marrow transplants into irradiated C1q deficient mice produce sustained repletion of serum C1q. Perhaps bone marrow transplant could be used to treat humans who develop SLE as a consequence of complement deficiency.

Which cells play the major roles in the autoimmune response in SLE? B lymphocytes are clearly important as a source of autoantibodies. Experiments on stored blood from American military personnel show that autoantibodies are present many years before the clinical disease presents (14). B cells can be divided into subsets on the basis of cell surface markers. CD20 is present on most B cells, but not plasma cells, and anti-CD20 has already been used as a therapeutic agent. High surface CD27 levels are a marker of early plasma cells. The frequency of these CD27 high cells rises in patients with SLE and correlates with disease activity (15). Conversely, it is increasingly recognized that a subset of B cells plays a regulatory role in the immune response. In MRL/*lpr/lpr* mice, it has been shown that stimulating these regulatory B cells with anti-CD40 leads them to produce regulatory cytokines. Transfer of the anti-CD40 stimulated cells into recipient MRL/*lpr/lpr* mice delays the development of lupus in those mice.

T lymphocytes are also important. Syamal Datta and colleagues have demonstrated the presence of T cells reactive to histone-derived peptides in both patients with SLE and murine models. Some of these peptides can be used to induce tolerance and delay disease progression in murine models of SLE (16). T cells in patients with lupus may be resistant to anergy due to up-regulation of CD40-ligand and impaired phosphorylation of Cassitus

B lymphoma oncogene b. Up-regulation of CD40-ligand may also enhance the ability of T cells to interact with B cells. T cell activation is partially regulated by the organization of signalling molecules into discrete membrane-associated microdomains called lipid rafts. In patients with SLE, changes in the composition of lipid rafts in T cells may render them more responsive to antigenic signals than T cells from healthy people (17) .

The work of Ward Wakeland and his group on congenic mice has enabled identification of murine loci carrying genes that mediate susceptibility to lupus. Many of these genes are involved in the processes which have been identified as important in the development of lupus using other approaches, as described above. For example, the murine *Sle1* locus contains genes involved in loss of tolerance, the *Sle2* locus genes affect B cell hyperactivity and the *Sle3* locus genes affect regulation of T cells. Some of these genes have orthologues in humans. The murine *Sle1b* locus contains IFN inducible genes, and such genes are also present on human chromosome 1 (region 1q23). Genetic studies in human SLE pedigrees were presented by researchers from America and Scandinavia. The human PDCD1 gene is strongly linked to SLE in Caucasians, and the haplotype most closely related to the disease carries a mutation that reduces binding of the PD1 protein to regulatory proteins and may therefore promote abnormal T cell activation.

Conclusion

There can rarely have been a more exciting time to work in the field of lupus, whether in the laboratory, the clinic or both. Effective collaboration between clinical and basic

science researchers has highlighted the importance of apoptotic cell remnants, immune complexes, interferons and other key aspects of the immune system in the pathogenesis of SLE. Much progress has been made in improving survival but new clinical challenges such as late morbidity and cardiovascular risk have emerged. More targeted therapies are emerging from the laboratory and being tested in large-scale clinical trials starting now.

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