Review of Presentations at the 6th European Lupus Meeting 03-05 March 2005

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Summary

The 6th European Lupus Meeting was held at the Royal College of Physicians of London and was attended by 450 delegates. The conference brought together leading speakers from Europe and North America who reviewed current knowledge and exciting new developments in both clinical and basic science aspects of systemic lupus erythematosus. This review summarises the major points covered in each session.

The conference began with an evening of entertainment, including a live performance by the singer Dido who also spoke about her experiences as a relative of a patient with lupus. Her appearance had been a well-kept secret until the opening ceremony and was complemented by Mitchell Dalton's guitar solo and classical music from the Florilegium quartet. The evening's proceedings started with Sir Peter Lachmann's historical, geographical and scientific tour of lupus. It finished with an iconoclastic poem by Anisur Rahman ("The Appropriate Management of SLE on the Planet Mars" – currently in press in *Rheumatology*) and a 60's style guitar band styled "Lupus Dave and the Davettes" featuring David Isenberg on lead vocals (don't give up your day job yet David!).

The meeting was a sell-out with 450 delegates. It was structured as parallel clinical and basic science sessions throughout apart from one plenary session. This review will describe the main points outlined in each session.

Clinical Sessions

Lupus and the kidney.

Peter Schur pointed out that there were only some twenty-five randomised controlled trials in the literature of lupus nephritis (1) and even in those that have been performed there are serious problems of definitions of disease features and outcomes and problems of quality control, particularly with regard to renal histological findings. **Jo Adu** set out the thesis that tubulo-interstitial damage, which determines the extent of long-term renal impairment, is the sequela of glomerular injury, hypertension and

hypertrophy, leading to proteinuria and ischaemia, both of which cause progressive interstitial fibrosis and tubular atrophy. In support of this hypothesis, macrophages are found in high density in areas of tubular damage where the monocyte chemoattractant protein MCP-1 is also highly expressed. Urinary MCP-1 levels correlate inversely with renal survival and a renal damage index correlates better with outcome than the WHO class of lupus nephritis (2). Therapies that reduce proteinuria (e.g. ACE inhibitors) and macrophage infiltration are likely to improve renal outcome. **Harry Moutsopoulos** reviewed antiphospholipid syndrome (APS) nephropathy in which patients typically are hypertensive, and may have chronic renal failure, mild proteinuria and intermittent haematuria (3). The renal arterial pathology comprises both fibrotic and thrombo-occlusive features, which can also be seen in other conditions such as hypertension, renal artery stenosis, scleroderma etc. In SLE these features are seen most commonly in patients with the APS but are also common in patients with antiphospholipid antibodies (aPL) alone (although a third of these will progress to APS) and occasionally in those without aPL. Presence of thrombo-occlusive features is unrelated to WHO class and raises the question of whether these patients should be on prophylactic aspirin or warfarin. Finally **Jo Berden** finished the session with a review of end-stage renal failure in SLE (predictors of which are greater renal impairment, nephrotic syndrome, persistent disease activity, hypertension, WHO class III or IV, male sex, ethnicity) and of dialysis (no preference for haemodialysis or peritoneal dialysis, no excess mortality, beneficial effects of dialysis on disease activity) and transplantation. His unit has an extensive pre-transplant assessment programme screening for cardiovascular disease, the APS, osteoporosis and autoreactive lymphocytes. Living donor transplantation has the best outcome and there is no worsening of outcome in SLE versus non-SLE patients. For

immunosuppression they use a variety of agents for induction (Cyclosporin A, tacrolimus, mycophenolate mofetil (MMF), prednisolone) but favour MMF or azathioprine plus one other of the induction agents for maintenance therapy as these have less adverse effects on blood pressure or lipid/diabetic profiles. The recurrence rate of lupus nephritis post transplantation is low.

Lupus and Atherosclerosis

Ian Bruce reviewed the literature demonstrating that SLE patients develop earlier atherosclerotic cardiovascular disease than the general population and this is the major cause of excess late mortality in patients with SLE (4). Corticosteroid use may contribute to this and patients with the APS are at much higher risk. Even accounting for conventional risk factors, which themselves may be commoner in SLE patients, there is still an unexplained excess risk of 5-15 times the general population for cardiovascular events. He finished by asking whether statins and hydroxychloroquine will reduce this risk – clinical trials are needed. **Jose Delgado-Alves** described the pathway by which oxidation of low density lipoproteins (LDL) activates monocytes that develop into foam cells leading to atherosclerosis and thrombosis. This process is moderated by high density lipoproteins (HDLs) and the enzyme paraoxonase. He set out the evidence to support the hypothesis that, in patients with SLE, anti-HDL antibodies and/or aPL interfere with these protective mechanisms (5). **Susan Manzi** described some of the non-invasive techniques that have been used to quantify the increased frequency of atherosclerotic lesions in SLE patients including thallium/dual isotope scans, electron beam CT (which can pick up calcification of the coronary arteries), intracoronary ultrasound (which can identify 20-25% stenosis compared

with the 45% stenosis required before they can be identified by conventional angiography), endothelial function tests and carotid ultrasound to measure plaque thickness (6). Carotid ultrasound in particular may be a useful predictor of future events and for the assessment of progression of atherosclerosis. Finally **David D'Cruz** went through approaches to the reduction of conventional risk factors. He pointed out that angiotensin receptor blockers do not prevent myocardial infarctions and may not be interchangeable with ACE inhibitors. Hormone replacement therapy should be avoided in anticardiolipin antibody (aCL) positive patients. He raised cautions over the use of COX-2 specific anti-inflammatories in patients with SLE and posed the question whether all SLE patients should be taking low dose aspirin. He finished by emphasising the importance of good oral hygiene to reduce periodontal disease, which has been linked with an increased cardiovascular risk.

Themed Abstract Session (Lupus. Vol 14(3):216-218)

This session started with two presentations on the epidemiology of SLE, one worldwide (**Danchenko** et al, abstract OP6) and one on the effects of ethnicity in the UK (**Patel** et al, abstract OP7). **McHenry** et al (abstract OP8) presented data on the increased prevalence of cervical smear abnormalities in patients with SLE. **Katz** et al (abstract OP9) presented data that patients with SLE have particular difficulty maintaining 'discretionary', mainly leisure activities such as entertainment, sports, hobbies, travel etc. and that this correlated with depression scores. The same group (**Yelin** et al, abstract OP10) then presented data showing that SLE patients had more work stops and starts than controls but overall those in employment were able to work less than controls during the study period.

Lupus and the Skin, Lungs and Abdomen

Using the comprehensive database of clinical information available in her unit at Johns Hopkins, **Michelle Petri** described lupus chest disease. Pleurisy is commoner in African-Americans than in other ethnic groups and can often be treated with anti-malarials and /or NSAIDs. More severe cases may require short courses of corticosteroids but pleurectomy and pleurodesis are rarely needed. Rare manifestations of SLE include lupus pneumonitis, pulmonary haemorrhage (seen in 2% of the Hopkins cohort), shrinking lung syndrome (<1%) and diffuse interstitial lung disease (in 3%). Pulmonary hypertension in SLE is usually mild but may be increasing in prevalence as the population of patients with SLE becomes older.

Peter Maddison described the spectrum of lupus skin disease from chronic cutaneous forms to skin involvement in acute systemic disease. It is important to stress the clear link between exposure to sunlight and cutaneous SLE and to advise patients to take adequate measures to protect themselves from the sun. A multi-disciplinary approach involving rheumatologists, dermatologists and nurses, incorporating a strong emphasis on self-management is often preferable.

Anisur Rahman explained that, while abdominal symptoms are commonly reported by patients with SLE, these symptoms are not usually due to the disease itself. Mouth ulcers are frequently painless and estimates of their prevalence vary. Patients with SLE and an acute abdomen may have mesenteric vasculitis. The rarity of this complication means that evidence about it comes from retrospective studies, which are difficult to compare. Ultrasound scan and angiography are often uninformative but computed tomography may be helpful. However, in many cases a laparotomy may be

needed to make the diagnosis. Some authors report a high mortality, especially from perforation, but other report good outcomes with courses of intravenous corticosteroids.

Outcomes in Lupus

Murray Urowitz presented data on the huge improvement in survival rates for SLE over the past 30 years. This has led to an increase in late morbidity, at least in part due to corticosteroid use. Avascular necrosis is one important form of late morbidity related to corticosteroids. Neurocognitive dysfunction, atherosclerotic cardiovascular disease and osteoporosis are also common forms of late morbidity. **Ann Clarke** presented data from the Tri-nation Study on resource utilisation by patients with SLE in the USA, UK and Canada which was generally higher in the USA than in the other countries (7). **Clarissa Pilkington** concluded the session with a review of paediatric SLE. Key features are that lupus in males is relatively more common (1:4) in children, and it often evolves from other conditions such as idiopathic thrombocytopenic purpura. Fatigue, haematological and central nervous system (CNS) involvement are common. Chronic active hepatitis is also more frequent as is the macrophage activation syndrome (seen only rarely in adults). Patients often lose long periods of school time with substantial consequences. Teenage compliance and the management of the whole family are important paediatric issues.

Central Nervous System Lupus

John Hanly started by describing the work of the *ad hoc* American College of Rheumatology (ACR) committee on agreeing the definitions and components of neuropsychiatric (NP) lupus (8). In terms of markers, aCL are associated with a fall in cognitive function over a five-year period. It is unclear whether anti-ribosomal P antibodies are relevant to NP lupus. Measurements of blood-brain barrier function are impaired in a third of patients. Markers that may prove to be useful include CSF cytokine levels. Matrix metalloproteinase 9 is increased in CSF of patients with NP-SLE and may correlate with MRI findings and markers of neuronal damage. **Betty Diamond** described her work on cognitive impairment induced by anti-N-methyl-D-aspartate (NMDA) receptor antibody in mice. This followed an observation that a monoclonal anti-dsDNA antibody cross-reacted with an NMDA peptide. Injection of such an antibody with lipopolysaccharide (LPS) (to induce permeability of the blood-brain barrier which is critical for the antibody to cause cerebral damage) caused cognitive impairment via preferential binding to the hippocampus i.e. an antibody mediated non-inflammatory model of cerebral lupus (9). **John Axford** described imaging techniques available for SLE including MR spectrometry and Diffusion Tensor Imaging. **Dafna Gladman** concluded the session with a review of therapy for CNS lupus ranging from symptomatic treatment to immunosuppression with cyclophosphamide. In some patients cognitive dysfunction can improve with corticosteroids and if the APS is present it is important to treat it by anticoagulation.

Themed Abstract Session (Lupus. Vol 14(3):220-221)

Ruiz-Iratorza et al (abstract OP16) presented a retrospective study suggesting that hydroxychloroquine may enhance survival in SLE. **Fernandez** et al (abstract OP17) presented data from the Lumina study on HRT use in SLE patients suggesting no

clinically relevant adverse events and no differences among different ethnic groups. **Guzman** et al (abstract OP18) summarized their experience of Rituximab therapy and **Willeke** et al (abstract OP19) described their groups' experience of improvement in 11 patients treated with immunoadsorption therapy. Finally **Brunner** et al (abstract OP20) presented findings that the medication cost of treating childhood SLE is 18,000 US dollars per annum compared to 4,000 US dollars per annum for adults with SLE.

Plenary Session: Translational Research to Treatment

Graham Hughes started this session on new therapies with an overview of therapy including treatment of cutaneous lupus with combinations of hydroxychloroquine and mepacrine or low-dose thalidomide, and severe systemic lupus with pulsed cyclophosphamide, MMF or rituximab. Epratuzamab, a humanised anti-CD22 monoclonal antibody, is in development and intravenous immunoglobulin (IVIg), stem-cell transplantation and plasmapheresis may all have a role in some patients. **Josef Smolen** reviewed his experience of using anti-tumour necrosis factor alpha (TNFα) therapy in six patients with SLE. **Michael Ehrenstein** described the potential role of statins as an immunomodulator in SLE with particular reference to the restoration of normal lipid raft responses following T cell activation (10). **Betty Diamond** presented data on the use of single dose CTLA-4 Ig interference on co-stimulatory activity on inducing remission in a safety study of 26 patients treated so far. **Joan Merrill** firstly summarised the basic science of BlyS and related molecules and their receptors involved in macrophage – B cell interactions. Over-expression of BlyS is found in murine models of autoimmunity and in some patients with SLE. At least one anti-BlyS monoclonal antibody is in development. Secondly she described

the use of anti-double stranded DNA (dsDNA) antibody binding peptides in murine lupus. A phase-1 study of one such peptide (TV4710, Teva Pharmaceuticals) has recently been completed. Finally **David Isenberg** described the University College London Hospitals experience with Rituximab using a regime of IV methylprednisolone 750mg and Rituximab 1g on day 1 and IV cyclophosphamide 750mg on day 2. Patients have their full blood count checked on day 10 and the process repeated on days 14/15. Patients stopped their previous treatment. Infection has not been a major problem. Anti-dsDNA antibody levels fall markedly in responders and B cells return at a mean of 4 months. Relapse only occurs when the autoantibodies return. Improvement is seen across all components of the BILAG index (11).

Biomarkers

The four speakers in this session discussed the importance and clinical utility of different antibodies, which have been suggested as markers of activity in SLE.

David Pisetsky discussed antibodies to dsDNA, which are the most widely used biomarkers in SLE. High levels of anti-dsDNA antibodies are almost specific to SLE and these levels correlate with disease activity in many (but not all) patients. These antibodies may be pathogenic by a number of different mechanisms including deposition of circulating immune complexes (IC), binding to an antigen (such as nucleosomes) deposited from the blood, direct binding to tissue antigens (such as laminin or alpha-actinin) and penetration of living cells. Bacterial DNA is immunogenic in healthy people whereas mammalian DNA is not. It is possible that the ability of patients with SLE to make antibodies against mammalian DNA arises

from stimulation by antigens containing DNA, for example chromatin breakdown products from mammalian cells.

The possibility that "anti-dsDNA" antibodies in SLE may actually be anti-nucleosome antibodies was mentioned by Dr Pisetsky, and discussed in greater detail by **Sylviane Muller**. It is important to distinguish anti-histone antibodies, which are not specific or sensitive for SLE, from anti-nucleosome antibodies, which are more specific – though this depends on the method used to prepare the nucleosomes. Anti-nucleosome antibodies appear earlier than anti-dsDNA in some murine models and can be induced in healthy mice by the injection of syngeneic apoptotic thymocytes. These facts support the increasingly widely accepted hypothesis that abnormal clearance of apoptotic cell debris is one of the earliest events in the pathogenesis of SLE. Epitopes on this apoptotic material act as immunogens for both T cells and B cells. The idea that peptides derived from histones could therefore be used as tolerogens to treat SLE has been tested by Datta's group, who injected such a peptide into SNF1 mice and prolonged survival (12). Thus, a better understanding of the anti-nucleosome response in SLE may be crucial to the management of the disease in future.

Cees Kallenberg discussed antibodies to complement component C1q (anti-C1q). Although these are not specific for SLE, they may have a predilection for cases of renal SLE. Two groups published studies suggesting that high anti-C1q are more closely associated with renal flares than with flares in other systems. An autopsy study showed that anti-C1q antibodies seem to be present at higher levels in the kidneys of patients with lupus nephritis than in their blood, suggesting that they are sequestrated in the inflamed kidney. Anti-C1q may play a number of roles in lupus

nephritis. Although injection of monoclonal anti-C1q antibodies alone did not lead to nephritis in healthy mice, these antibodies did enhance the renal damage caused by rabbit anti-mouse nephrotoxic antibodies. Anti-C1q antibodies may also interact with surface C1q on apoptotic cell debris, promoting clearance of these cells by a pro-inflammatory route. However, the anti-C1q assay is not used in clinical practice as commonly as the anti-dsDNA assay, perhaps due to technical factors.

Anti-ribosomal P antibodies have been a source of controversy for some years. Data relating to these antibodies were reviewed by **Ellen Ginzler**. Although the prevalence of anti-ribosomal P antibodies in patients with SLE is not more than 20% in most studies, it is higher (36-38%) in Chinese patients. Although some have argued that high levels of these antibodies correlate with the presence of NP lupus, most studies are small and the results are inconsistent. There is therefore no consensus to support the measurement of anti-ribosomal P as a biomarker in routine clinical practice.

Basic Science Sessions

Lupus – the Link to Apoptosis

A striking association is found between distinct autoantibody profiles and clinically distinct autoimmune diseases despite the fact that the best defined autoantigens in various systemic autoimmune diseases are generally ubiquitously expressed in all cells. This conundrum of specificity was addressed by **Anthony Rosen**. His group have examined disease-specific autoantigen expression in normal and myositis muscle (13). They found that myositis specific antigen expression (e.g. Jo-1) was increased in

myositis muscle only. Conversely, the expression of non-myositis autoantigens & non-autoantigens was not increased in myositis muscle. Furthermore, myositis specific autoantigen expression was highest in regenerating muscle cells which were prominent in the myositis patients. Thus, Dr Rosen thought that healthy muscle is unlikely to be the site of initial immunologic injury and perhaps specific antigen expression patterns in regenerating muscle cells shape the phenotype-specific immune response (13).

Significant challenges exist to studying auto-antigen expression in lupus patients in the same way. In particular, which tissue should be studied and how best to access the relevant tissue at the relevant time? The search for the most relevant animal model led Dr Rosen and colleagues to study chronic graft versus host disease (GVHD) in DBF1 mice. In this disease model they found some similarities with SLE; antibodies to poly-ADP ribose polymerase (PARP) – found in approximately 20% of patients with SLE - frequently occurred in the parent-F1 GVHD mice. Furthermore, female mice had much higher titres of autoantibodies and had worse disease compared to male mice. The titres of PARP antibodies were increased in male mice by administration of apoptotic splenocytes, but not live cells, after induction of GVHD. Thus, Dr Rosen's conclusion was that development of autoimmunity in GVHD mice (as in patients with autoimmune rheumatoid disease) requires simultaneous satisfaction of a complex group of circumstances, which include presentation of appropriate autoantigen.

The importance of defects in clearance of apoptotic cells in patients with SLE was reviewed by **Martin Herrmann**. He described how apoptosis is usually a non-inflammatory event with apoptotic cells stimulating antigen presenting cells (APCs)

to increase the release of interleukin (IL) 10 and produce less TNFα. During apoptosis chromatin is degraded before the cells lose their membrane integrity. The cells are rapidly engulfed and digested by phagocytes, especially macrophages. Tingible body macrophages (TBMs) are found in germinal centres (GC) of secondary lymphoid organs. These TBMs efficiently remove apoptotic cells thus avoiding contact of nuclear remnants with the immune system. This in turn prevents production and affinity maturation of anti-nucleosome/anti-dsDNA antibodies.

These mechanisms are defective in patients with SLE. In particular, work performed by Dr Herrmann's group has shown that in a subgroup of patients with SLE, apoptotic cells accumulated in lymph node GCs and the number of TBMs was significantly reduced. Consequently, nuclear autoantigens bind to follicular dendritic cells (DCs) and may thus provide survival signals for autoreactive B cells (14).

Furthermore, Dr Herrmann described how treatment of apoptotic cells with Annexin V (AxV) significantly increases the humoral response against these cells (15), by masking exposure of phosphatidylserine (PS) - a recognition signal for macrophages - on the outer cytoplasmic membrane early in apoptosis. In addition, AxV delayed the clearance of apoptotic cells by macrophages but not DCs. Thus exposure of PS serves as an anti-inflammatory and immunosuppressive signal which is opposed by AxV and may be important in the generation of autoimmunity.

The ability of DCs and macrophages exposed to various activation stimuli as well as meals of apoptotic and necrotic cells to break tolerance in normal mice was discussed by **Keith Elkon** (16). His group has found that mice injected with DCs incubated with

apoptotic cells or necrotic cells developed high levels of IgG autoantibodies including anti-dsDNA and aCL. Similar results were obtained using DCs cultured in medium alone. Mice injected with DCs which had undergone enhanced maturation with LPS developed significantly higher levels of total IgG, IgG anti-dsDNA and IgG aCL. In contrast, macrophages treated in the same way failed to induce generation of IgG antibodies. Despite high serum levels of IgG anti-dsDNA antibodies, as well as prominent renal deposition of IgG in the kidneys, mice injected with DCs did not develop overt nephritis. This lack of clinical disease was thought to be explained by a marked skewing of autoantibodies towards the IgG1 isotype as opposed to the more pathogenic IgG_2a isotype. Thus, Dr Elkon concluded that mature DCs are able to break tolerance and induce autoantibodies in normal mice but other susceptibility factors are required to induce long lasting autoimmunity and clinical expression of disease (16).

In the context of autoimmune disease, DC uptake of apoptotic cells in the presence of a maturation signal breaks tolerance by the coordinate engagement of Toll-like receptors (TLR), CD40 or activating Fcγ receptors. Dr Elkon reviewed evidence that immune complexes consisting of DNA and anti-dsDNA antibodies can activate DCs to produce high levels of inflammatory cytokines, such as interferon (IFN)α, involved in the pathogenesis of SLE. This process occurs relatively independently of TLR4, TLR9 (17) or MyD88 (18) signalling pathways. Furthermore, the entry of mammalian DNA into DCs is a potent inducer of type 1 IFN through a non TLR 9 route.

In the final part of his talk Dr Elkon addressed the question of how apoptotic cells suppress immune responses. He presented data demonstrating that cell-cell contact

with apoptotic cells is sufficient to induce profound inhibition of IL-12 production by activated macrophages, through interaction of PS on apoptotic cells with its receptor on macrophages. He also reported the identification of a novel zinc finger nuclear factor, named GC binding protein, induced by phagocytosis of apoptotic cells by macrophages or by treatment with PS, which selectively inhibits IL-12 gene transcription (19).

Interferons, cytokines and lupus

This session dealt with four of the cytokines which have been studied most extensively in SLE and which may offer the best hopes for therapeutic intervention.

Lars Ronnblom discussed IFNα. Patients with SLE have high levels of IFNα, which correlate with disease activity. Cells expressing IFNα have been demonstrated in both inflamed and non-inflamed skin of patients with cutaneous SLE. Microarray studies to profile gene expression show that peripheral blood cells of patients with SLE tend to show upregulation of IFN induced genes. Dr Ronnblom outlined the theory that IC containing nuclear material from dying cells bind to IFN-producing cells via the FcγRIIa receptor. This triggers the production of IFNα, which itself promotes B cell maturation and antibody production, creating a positive feedback loop. The potential importance of IFNα in SLE was underlined by studies showing that knockout of interferon genes ameliorates disease in murine models of lupus.

Genetic studies in Scandinavia looked for association of SLE with the presence of 44 single nucleotide polymorphisms (SNPs) in 13 genes (20). There were strong

associations with two genes involved in the interferon system – tyrosine kinase 2 and IFN regulating factor 5. It must be noted, however, that both genes are also involved in other cytokine pathways and there are more than 200 other genes related to the Type I IFN pathway. Any conclusions about the roles played by these genes in the pathogenesis of SLE remain speculative.

Rizgar Mageed discussed TNFα, which has a complex mode of action in SLE. Studies in NZB/W F1 mice showed that this cytokine ameliorates lupus-like disease in that model, and anti-TNFα drugs given to patients with rheumatoid arthritis can cause production of anti-dsDNA antibodies. In established SLE, however, there is evidence that TNFα is involved in causing tissue inflammation, and some groups advocate using anti-TNFα to treat patients with SLE. Professor Mageed's group have studied the effect of TNFα and anti- TNFα on adult and neonatal NZB/W F1 mice. In the adult mice, as expected, administration of TNFα reduced the size of germinal centres and reduced levels of anti-dsDNA antibodies. In neonatal mice, the original hypothesis that TNFα might promote autoimmunity by interfering with thymic development of T cell tolerance was disproved. In fact, anti-TNFα given to neonatal mice leads to increased B cell proliferation, no effect on T cell proliferation at four weeks and increased levels of anti-dsDNA and anti-nucleosome antibodies. Anti-TNFα therefore seems to promote autoimmunity in both adult and neonatal NZB/W F1 mice.

Professor Mageed also described early results from studies using B cells from patients with SLE and raised the intriguing possibility of using targeted gene therapy to modulate TNFα function in lupus-affected tissues such as the kidney.

Dominique Emilie discussed IL -6 and IL-10. IL-6 levels tend to be high in patients with SLE and are related to disease activity. Since activated B cells produce IL-6 and have IL-6 receptors, a positive feedback loop may operate. In NZB/W F1 mice, blocking IL-6 or the IL-6 receptor delays the onset of nephritis.

IL-10 levels are also raised and correlate with disease activity in patients with SLE. IL-10 acts as a polyclonal activator of B cells. When peripheral blood mononuclear cells (PBMC) from patients with SLE are injected into severe combined immunodeficiency (SCID) mice, the amount of human IgG produced by those cells is reduced by the administration of anti-IL-10 to the recipient mice. Levels of autoantibodies produced by the human PBMC are particularly sensitive to anti-IL-10. A pilot study in human SLE showed some reduction in disease activity following administration of a murine monoclonal anti-IL-10 antibody (21). Attempts to develop a humanised anti-IL-10 for further trials seem likely to follow.

In NZB/W F1 mice, survival of peritoneal B lymphocytes is promoted by the chemokine SDF-1, and this effect is enhanced by IL-10. Administration of anti-SDF-1 to NZB/W F1 mice reverses established nephritis. In recent experiments, Koutouzov and colleagues used an adenoviral vector to administer murine IFNα cDNA to pre-autoimmune NZB/WF1 mice (22). The recipient mice developed sustained high levels of serum IFNα. These mice developed earlier glomerulonephritis with proteinuria and died earlier than control mice that received vector containing no cDNA or no vector at all. The effect was strain-specific such that BALB/c mice treated in the same way did not develop any similar disease.

Complement and Lupus

Inherited deficiencies in the complement system can predispose to the development of SLE. **Gunnar Sturfelt** described the hierarchical relationship of complement deficiency and SLE within the classical pathway as C1q/C1r/C1s > C4 > C2. In a cohort of 43 patients in Sweden with homozygous C2 deficiency, invasive infection is the most frequently observed manifestation (23/40), followed by cardiovascular disease (myocardial infarctions in 10 patients and stroke in six), followed by SLE in 11 patients (23). None of these patients have antibodies to dsDNA, despite one patient developing lupus nephritis. Anti-C1q antibodies are present in 56% and 44% have aCL despite none developing the APS. *In vitro*, serum deficient in C1q or C2 results in reduced clearance of apoptotic cells, which is normalised after replenishing the deficient complement, suggesting a possible mechanism contributing to the eventual loss of tolerance to these autoantigens.

The possibility of using levels of complement components as biomarkers in SLE was discussed. Low C1q as a biomarker of SLE has a very high specificity of 96% but the sensitivity is low at 20%, compared to low C3 or low C4, which both have higher sensitivities of 64% (24). Low levels of C1q are present in 50-80% of renal SLE flares, and tend to precede drops in C3 and C4 levels. Low C1q can predict histopathological outcome of lupus nephritis, as evidenced by a strong association with WHO Class III-IV. It was stressed that measurement of complement activation products may be valuable in terms of monitoring disease activity with serial

measurements, rather than as a diagnostic tool. Urine C3d levels are increased in active lupus nephritis but this test has low specificity for SLE.

The 'waste-disposal' hypothesis put forward by Mark Walport and colleagues proposes that defects in the clearance of dying apoptotic cells and / or IC may provide the source of persistent autoantigen driving the inflammatory process. **Kevin Davies** summarised evidence linking the role of defective mechanisms of IC clearance, complement and Fc receptors to the pathogenesis of SLE. In healthy people IC are opsonised by bound C3b allowing them to bind to erythrocyte-CR1 receptors clustered on the surface of red blood cells. The erythrocytes transport the IC to the hepatic phagocyte where they are removed via Fc binding. Erythocyte-CR1 is reduced in patients with SLE. Experiments using radiolabelled IC in the normal population have shown these IC are cleared into the liver and spleen equally. In patients with SLE however, 80% are cleared by the liver and 20% by the spleen. Furthermore, clearance into the liver is more rapid as is subsequent release of the complexes implying defective retention. IC clearance studied in one patient with C2 deficiency showed similar abnormal results in both the kinetics and localisation of IC clearance, with no uptake of IC in the spleen. These abnormalities were reversed with C2 repletion. These experiments, among others, suggest that soluble IC cleared by Fc-receptors are processed by the liver. This processing is defective with poor retention of these complexes, suggesting a possible link with Fc-R polymorphisms observed in SLE. Splenic uptake of IC however is complement-dependent.

A dynamic flow system has been used to show that uptake of IC by mononuclear phagocytes is more efficient when the complexes are bound to erythrocytes in the

fluid phase under physiological flow. Transfer may involve an adhesive interaction between erythrocytes bearing the IC and receptors of both Fc and complement on the phagocyte surface. FcγRIIa polymorphism influences binding of IgG_2 containing IC, with a reduction of FcγRII demonstrated on phagocytes from patients with lupus (25).

In keeping with the 'waste-disposal' theme, **Marina Botto** discussed the possible role of C1q in the defective clearance of apoptotic cells. Clinically C1q deficiency is closely associated with SLE. In one cohort studied 39 out of 42 patients with inherited C1q deficiency suffered from SLE. C1q knockout mice develop glomerulonephritis with the presence of glomerular apoptotic bodies. Experiments using both IgM and C1q deficient mice showed that uptake of apoptotic thymocytes by macrophages *in vitro* was normal only when normal levels of both C1q and IgM were present, suggesting that C1q binding to apoptotic bodies is mediated by IgM.

Anti-C1q antibodies are strongly associated with the presence of lupus nephritis and accumulate in the nephritic kidney. Murine anti-C1q antibodies infused into mice naïve to these antibodies have no pathological effect. However if these mice are pre-injected with antibodies to the glomerular basement membrane, anti-C1q antibodies cause significant albuminuria and enhanced histological renal damage. It thus seems that anti-C1q antibodies bind C1q in the kidney but only induce disease in the context of glomerular IC deposition (26). Finally work investigating the possibility of manipulating levels of C1q *in vivo* was discussed. Patients with inherited C1q deficiency and SLE infused with fresh frozen plasma develop normal levels of C1q that lasts for only 48 hours, requiring re-treatment every two to three weeks. Irradiated C1q deficient mice reconstituted with wild-type bone marrow develop

sustained presence of serum C1q detected up to 60 weeks post bone marrow transplantation. Could bone marrow transplantation be a possible therapeutic strategy in the treatment of SLE associated with genetic deficiency of the classical pathway components?

Cell signalling defects

Activation of B cells in autoimmunity may not always lead to a pathogenic outcome. Accumulating evidence for the existence of a subset of B cells that have a suppressive/regulatory effect on the expansion/differentiation of autoreactive T cells was reviewed by **Claudia Mauri**. Previous work has shown that B cells treated with agonistic anti-CD40 inhibit Th1 cell differentiation and prevent murine collagen induced arthritis by the production of IL-10 (27).

To test whether regulatory B cells can be generated in other autoimmune diseases such as SLE, Dr Mauri described work carried out by her group in lupus prone MRL/*lpr*/*lpr* mice. Purified B cells from these mice at 9-10 weeks of age were transferred to recipient mice and did not delay the onset of proteinuria or confer any enhanced survival. However, stimulation of the B cells with anti-CD40 prior to transfer delayed the onset of proteinuria and autoantibody production. This phenomenon was associated with a switch to IgG_1 antibody production from the pathogenic IgG_2a subtype, similar to that induced by mature DCs described by Dr Elkon.

The phenotype of these regulatory B cells was studied by analysing various markers which characterise subsets of B cells at different stages of maturation. Immature B cells leave the bone marrow and enter the spleen where they differentiate into transitional type 2 (T2) B cells that reside within the splenic follicle. Activation of specific signalling events in T2 B cells leads to their further differentiation into follicular mature or marginal zone (MZ) B cells (28). Dr Mauri found T2/MZ like B cells, generated upon anti-CD40 stimulation, to be the major producers of anti-inflammatory regulatory cytokines. Furthermore, transfer of T2/MZ like B cells delayed the progression of spontaneous lupus-like disease in MRL/*lpr*/*lpr* mice as shown by reduced levels of proteinuria, IgG anti-DNA antibodies and nephritis. In addition, unlike other subsets of B cells *in vivo*, T2/MZ B cells induced a switch from IgG_2a to IgG_1 antibodies and *in vitro* efficiently suppressed the proliferation of activated T cells and Th1 differentiation.

Elizabeth Jury described how the organisation of signalling molecules into discrete membrane associated microdomains, called lipid rafts, regulates T cell activation pathways. In resting T cells, lipid rafts - detected by Cholera toxin B binding to glycosphingolipid (GM1) – form a low proportion of the plasma membrane. T cell activation however, induces an increase in the proportion of the plasma membrane adopting a lipid raft conformation, measured by up-regulation of raft-associated GM1, and concentrates signalling molecules such as lymphocyte specific protein tyrosine kinase (LCK) to the immunological synapse.

Studies performed by Dr Jury have shown that LCK expression is significantly reduced in both raft and non-raft fractions in T cells isolated from patients with SLE.

Furthermore, this reduction is associated with disease activity and parallels an increase in LCK ubiquitination independent of T cell activation (29). T cells freshly isolated from patients with SLE also express higher levels of raft associated GM1 with an increased expression of co-localised CD45 and activated LCK compared to controls (30). Thus, these T cells are "primed" for activation and respond more readily to antigenic triggers than T cells from normal controls.

Intriguingly, the well described immunomodulatory effects of statins may occur due to their ability to reverse lipid raft abnormalities in lupus T cells. Dr Jury explained how atorvastatin interferes with T cell activation by: - depleting membrane cholesterol; disrupting the integrity of lipid rafts; altering the membrane distribution of raft-associated signalling molecules; restoring LCK expression in SLE T cells; and reducing LCK activation in SLE T cells. Thus, statins prevent the formation of a stable immunological synapse and T cell activation in addition to their cholesterol lowering properties (10).

Syamal Datta described peptide therapy in lupus prone mice. Nucleosome derived peptide epitopes were administered subcutaneously in very low doses to these mice. These peptides induced tolerance by generating long-lasting regulatory T cells that suppressed the production of pathogenic autoantibodies and prolonged the life of the mice by delaying the onset of nephritis. Thus, the beneficial effects of the peptides outlasted their short half-life and generated long lasting regulatory T cells, without causing allergic/anaphylactic reactions or generalised immunosuppression (12).

Dr Datta reviewed a number of intrinsic T cell mechanisms that contribute to the breakdown of tolerance in lupus. Up-regulation of CD40 ligand (CD40L) is found in T cells of lupus patients and occurs with suboptimal TCR stimulation. This CD40L up-regulation is associated with impaired phosphorylation of Cassitus B lymphoma oncogene (Cbl) b, an enzyme with several important functions: - down-regulation of critical kinases such as LCK; facilitation of endocytosis of T cell receptors (TCRs) after signalling; and targeting downstream signalling proteins. Consequently lupus T cells with impaired Cbl-b function show persistent activation of the mitogen activated protein kinase extracellular regulated kinase which renders them resistant to anergy. Impaired function and expression of Cbl-b could also explain alterations in receptor clustering and lipid raft aggregation in the immunological synapse of lupus T cells, described by Dr Jury. Thus, increased expression of CD40L mediates abnormally prolonged costimulatory signals to autoantibody producing B cells and may allow APCs to present apoptotic autoantigens in an immunogenic fashion in SLE (31).

In addition Dr Datta described a novel role of COX-2. A sustained up regulation of COX-2 and the anti-apoptotic molecule cellular Fas-associated death domain-like IL-1β converting enzyme inhibitory protein (c-FLIP) has been found in T cells of patients with SLE compared to healthy controls. Recent work has shown that certain COX-2 inhibitors such as celecoxib and niflumic acid induced marked apoptosis of anergy-resistant lupus T cells by down-regulating c-FLIP. The same COX-2 inhibitors also blocked nucleosome-specific T-cell recognition and autoantibody-inducing help in lupus prone mice (31). This raises the question of whether these drugs may be used as a maintenance therapy to prevent relapse in young patients with lupus who are in remission and do not have any contra-indications to COX-2 therapy.

Genetic abnormalities

Ward Wakeland summarised complex work using different animal models to dissect out genetic pathways that modulate susceptibility to systemic autoimmunity. The NZM2410 lupus prone mouse crossed with a B6 lupus resistant strain has been used to produce three congenic strains each carrying lupus susceptibility genes called *Sle1*, *Sle2* and *Sle3* respectively. *Sle1* mediates loss of immune tolerance, *Sle2* B cell hyperactivity and *Sle3* mediates dysregulation of T cells. However none of these mice develop a lupus related clinical syndrome. Reassembly of congenic intervals B6.*Sle1*, B6.*Sle2* and B6.*Sle3* producing bi and tri congenic strains indicate that *Sle1* is key for the development of fatal lupus. The combination of *Sle1* with *Sle2* or *Sle3* results in fatal systemic autoimmunity with variably penetrant glomerulonephritis. In contrast the combinations of *Sle2* and *Sle3* failed to mediate fatal disease (32). Fine mapping studies of the *Sle1* gene revealed a cluster of four loci (*Sle1a*-*Sle1d*) within the B6.*Sle1* congenic interval. *Sle1b* is the most relevant with regards to pathogenicity. Genomic characterisation of the *Sle1b* locus identifies a highly polymorphic cluster of SLAM/CD2 family genes in the middle of the *Sle1b* critical interval (33). Studies were presented demonstrating that the SLAM/CD2 family polymorphisms modulate the cytokine secretion profile of $CD4^+$ T cells, resulting in decreased IL-4 production and preferential IFN-γ production.

The *Sle3, Sle5* and *Yaa* alleles mediate disruption of the immune system. Producing bi congenic intervals of these strains with ANA positive, non-pathogenic B6.*Sle1* strains leads to fatal autoimmune disease. Combining the bi congenic B6.*Sle1/yaa* with the

autoimmune protective locus *Sles1* however, results in a subsequent switch form of severe fatal autoimmunity to a non-autoimmune phenotype. The work presented underlines the diversity of regulatory genes in the immune system, with unfortunate combinations leading to imbalances in immune regulation.

Marta Alarcon-Riquelme discussed evidence linking polymorphisms in the *PDCD1* gene to lupus susceptibility and presented work identifying possible functional mechanisms. The human *PDCD1* gene has been shown to be located in the chromosomal region 2q37, a region that has been linked to SLE in multiplex Nordic SLE families. Sequencing the complete gene identified a number of SNPs and haplotypes constructed that were seen to preferentially transmit through to SLE-affected individuals. The haplotype PD-1.3A was found in studies of ~1300 SLE Caucasian patients versus ~7000 controls to be associated with disease at a $P<0.0000001$ (odds ratio=1.7) (34).

The PD1.3A haplotype may function to increase susceptibility in SLE as PD-1 is thought to control peripheral tolerance. PD1.3 is located in an intron which represents the binding site of the transcription factor RUNX/AML-1. The RUNX family of regulators (RUNX 1, 2 and 3) generally act as regulators of immunity and inflammation and can repress each other. RUNX1 and RUNX3 regulate $CD8^+$ T cell development in mice. RUNX1 binds to 'wild-type' PD1.3G but not to its allele PD1.3A. PD1 is expressed only in activated T cells in the PD1.3G haplotype but in both activated and non-activated cells in the PD1.3A haplotype, most likely due to consequent poor binding of regulatory proteins. This haplotype could therefore promote abnormal T cell function and hence promote autoimmunity.

Tim Vyse gave a broader overview of SLE genetics and pointed out the difficulties associated with extrapolation of murine genetic observations to SLE disease in man. In mouse models such as the NZB/WF_1 or MRL/*lpr/lpr* strains, the genes targeted induce disease with much less of an environmental effect. One must also consider that the mouse population is inbred with limited genetic variation, whereas the human population continues to undergo massive expansion. There are clearly common linkage regions in murine and human autoimmune disease, but orthologous regions are hard to establish. Detailed study of the region containing the high susceptibility murine *Sle1b* locus identifies the presence of IFN inducible genes *Ifi202* and *Ifi204*. Analysis of the region 1q23 in the human chromosome reveals the presence of human *Ifi* genes encoding the transcriptional regulators MNDA, IFI-X, IFI-16 and AIM2. These are members of the HIN-200 family of proteins, expressed in haematopoietic and epithelial cells and up-regulated by type I and type II IFN.

John Harley attempted to address how clinical variation in SLE may inform genetic studies. Data from a number of meta-analyses was presented stating the odds ratio for the association of SLE with variants of mannose-binding lectin gene (OR=1.4), TNFα genes (OR=4.6), PTPN22 (OR=1.6) and CR1 (OR=1.5). The Oklahoma Lupus Genetics Multiplex Pedigree Collection has an impressive total number of 416 pedigrees. Linkage to FcγRIIA is seen in 31 of the 127 African-American pedigrees. Mapping reveals a SNP in FcγRIIA causing a single point mutation arginine to histidine at position 131 (OR=1.35, p0.03) and a SNP at FcγRIIIA causing a phenylalanine to valine mutation at position 176 (OR=1.63, p0.002).

A possible relationship of SLE with Klinefelter's syndrome was discussed. Out of a total of 213 men with SLE studied five were found to have Klinefelter's syndrome through karyotype analysis. The expected prevalence of Klinefelter's is 17:10,000 live births. Thus the actual observed rate of the extra X chromosome in men with SLE is in the region of 10 times that which one would expect, similar to the ratio of females to males with SLE. It would seem that the Y chromosome is irrelevant and the occurrence of SLE in patients with Turner's syndrome (XO), though reported (one published case (35)) is extremely rare.

In the final part of Dr Harley's talk linkages to SLE by stratification using clinical features was discussed. Patients with anti-dsDNA antibodies exhibit linkages to 19p13. A recent study by Sigurdsson et al (20) has identified two SNPs found in exploring 13 IFN responsive genes separately in Swedish, Icelandic and Finnish patients with SLE. SNPs were identified in the tyrosine kinase 2 (*TYK2*) gene at 19p12.2 that displayed strong signals in joint analyses of linkage and association. TYK2 binds to the type I IFN receptor complex.

Antigens and antibodies in lupus

Bevra Hahn described the panoply of autoantigens which may be involved in SLE. Some of these are foreign antigens such as Epstein-Barr virus. Children with SLE are 50 times more likely to be seropositive for EBV exposure than healthy children. Self-antigens, however, have been more extensively studied in SLE. Many of these are proteins or nucleic acid/protein complexes found on apoptotic blebs and other

subcellular particles released after apoptosis. Arbuckle's study on stored blood from American military personnel showed that autoantibodies to these antigens may be present in the blood of patients with SLE years before they develop clinical features of the disease (36). Different autoantibodies may develop as the disease progresses and this may arise from epitope spreading, a process in which exposure to one autoantigen gradually leads to the production of antibodies to a range of related antigens. For example, an initial anti-nucleosome response may be followed by production of anti-dsDNA and anti-histone as well as anti-nucleosome antibodies.

Some autoantigens may be important as targets in the disease process. These include alpha-actinin, identified by two separate groups as being a possible target in renal SLE. Antibodies that cross-react with DNA and NMDA bind the mouse hippocampus and may be involved in cerebral SLE. Antibodies which bind beta-2-glycoprotein I, prothrombin or thrombin are important in the APS.

Autoantibodies may themselves act as autoantigens. Peptides from the VH regions of some human anti-dsDNA antibodies can stimulate T lymphocytes from patients with SLE to produce IFN-γ. T lymphocytes from healthy people are less likely to be stimulated by these peptides.

Mark Shlomchik addressed the issue of how autoreactive B cells are activated in autoimmunity with reference to an elegant model using mice transgenic for a murine allotype-specific rheumatoid factor. In normal mice, the transgenic B cells are anergised, whereas on a lupus prone background the transgene is expressed. Expression is higher in mice that have the target allotype. In these mice, the

autoreactive, rheumatoid factor positive B cells lie outside the germinal centres, and may escape from normal tolerance mechanisms. These autoreactive cells are stimulated when allotype positive anti-chromatin antibodies are administered to the transgenic mice. This response may be mediated by TLR9 reacting to chromatin/anti-chromatin complexes. However, although knocking out the TLR9 gene in MRL/*lpr*/*lpr* mice (by cross-breeding with TLR9 knockout mice) reduces production of anti-dsDNA antibodies, these mice still develop glomerulonephritis.

Thomas Dorner looked at the issue of B cell subsets in patients with SLE. His group, and others, have identified CD27 high early plasma cells as a population whose levels are raised in SLE and correlate with disease activity (37). Some of these cells express high levels of surface HLA-DR antigen and can migrate towards the bone marrow. The ICOS-ICOS ligand system may be important in the development of B cells in SLE. ICOS deficiency in humans leads to low B cell numbers and low IgG levels. In patients with SLE, T cell ICOS expression is high and ICOS-ligand on B cells is down-regulated, leading to the speculation that this pattern is due to recent T-B cell interactions, which may be important in disease pathogenesis.

Conclusion

The 6th European Lupus Meeting was generally agreed to be a great success, with a broad range of subjects being discussed in detail. Interested readers who were unable to attend the conference itself will find more information in the references listed below. The 7th European Lupus Meeting will be held in Amsterdam.

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