Workshop report on some new ideas about the treatment of systemic lupus erythematosus

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Our increased understanding of the pathogenesis of systemic lupus erythematosus is leading to new ideas about its therapy. In this session of the workshop the use of LJP 394 a B cell toleragen and the use of an anti-CD20 monoclonal antibody were discussed in some detail. Their rationale and early clinical results were reviewed; both have shown encouraging clinical and serological benefit. Definitive double-blind clinical trials are still, however, awaited. In addition, the intriguing notion of using a nasal instillation of a histone peptide was described and early work in an experimental model presented. *Lupus (2002) 11, 793–796.

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Given that antibodies to double-stranded DNA (dsDNA) are thought to be causative agents in lupus nephritis, major attempts are being made to block the production or neutralize the effects of these antibodies. Linnick (San Diego) described the development of an antigen-specific approach for the treatment of systemic lupus erythematosus (SLE) that is intended to deplete circulating anti-dsDNA antibodies and tolerate the B cells that produce antibodies to dsDNA. A lead candidate, LJP 394, has been studied in several randomized controlled trials and is currently being evaluated in an international Phase III clinical trial.

LJP 394 provides a tetravalent presentation of B cell epitopes in the form of four 20-mer synthetic, double-stranded oligonucleotide (dsDNA) B cell epitopes on a non-immunogenic organic platform. Importantly, LJP 394 lacks T cell epitopes. To study the efficacy of LJP 394 in vivo, an immunized mouse model using C57B1/6 mice primed with dsDNA-KLH was developed then boosted 3–4 weeks later to elicit an anti-dsDNA antibody (Ab) response. Administration of LJP 394 5 days prior to boosting suppressed circulating anti-dsDNA IgG levels in a dose-dependent manner with a maximum efficacy of > 80% reduction as measured by a Farr assay. The effect of LJP 394 appeared to be specific to the anti-dsDNA Ab as it had no effect on anti-KLH Ab levels. This decrease in Ab levels correlated with a similar dose-dependent reduction (> 80%) in anti-dsDNA specific plaque-forming cells as determined by an ELISPOT assay.

The efficacy of LJP 394 was then tested in male BXSB mice, which have clinical features consistent with human SLE, including spontaneous production of anti-dsDNA antibodies and significant renal inflammation secondary to immune complex deposition. Administration of 300 μg LJP 394 to male BXSB mice twice weekly from 2 to 5 1/2 months of age significantly lowered anti-dsDNA Ab levels relative to vehicle controls with no observed non-specific effects. Diminished anti-dsDNA Ab levels also correlated with decreased anti-dsDNA specific plaque-forming cells in the spleen, as determined by an ELISPOT assay, and increased survival (P < 0.05) in these mice.

Based on the preclinical results, LJP 394 was evaluated in a variety of clinical research trials including several studies designed to study the safety and pharmacodynamic effect of drug treatment on lupus patients with antibodies to dsDNA. These studies included two dose/regimen trials assessing doses of 1–100 mg given weekly for 4 months.

Results suggested that statistically significant reductions in circulating antibodies were observed in patients receiving 10, 50 or 100 mg per week, with
the greatest reductions of about 40–50% seen in patients treated weekly with 100 mg of LJP 394. A surface plasmon resonance-based assay was developed to measure the affinity of a patient’s serum IgG fraction for the oligonucleotide epitope on LJP 394. An apparent dose-dependent reduction in antibody affinity for the LJP 394 epitope was observed for patients treated with 10 or 50 mg LJP 394/week for 4 months when compared with pretreatment affinity levels.

The potential efficacy of LJP 394 treatment to delay time to renal flare in SLE patients with a history of renal disease was then determined in an international, multicentre, randomized, double-blind, placebo-controlled Phase II/III trial. Patients were eligible for study entry if they fulfilled the American College of Rheumatology revised criteria for classification of SLE with a previous episode of SLE renal disease within 4 years prior to study entry, had anti-dsDNA titre of at least 15 IU/ml by Farr assay at study entry and provided voluntary informed consent. Patients were excluded if they had evidence of a renal flare within 3 months of screening or were receiving prednisone or prednisone equivalent > 20 mg/day, azathioprine > 200 mg/day, methotrexate > 25 mg/week and/or cyclophosphamide at any dose within 3 months of screening or had a serum creatinine level > 2.5 mg/dl. Results of any previous renal biopsies were collected, although they were not required for study entry. The protocol was approved by appropriate institutional review boards and ethics review committees.

In this double-blind, placebo-controlled, 76-week study, 230 SLE patients were randomized to receive 16 weekly doses of 100 mg LJP 394 or placebo, followed by alternating 8 week drug holidays and 12 weekly treatments with 50 mg LJP 394 or placebo. An assay measuring the affinity of patients serum IgG fraction to the dsDNA epitope of LJP 394 prior to exposure to drug identified a high-affinity population (189/213; 89% active, 90% placebo). Analyses were performed on the intent-to-treat and high-affinity populations.

Four weekly treatments with LJP 394 reduced circulating anti-dsDNA levels by 25±3% from baseline, while placebo levels were increased by 11±5%, a difference of 36% between LJP 394 and placebo. In the intent-to-treat population, time to renal flare was not significantly different between treatment groups, but LJP 394-treated patients had a longer time to institution of high-dose corticosteroids and/or cyclophosphamide (HDCC; P = 0.033) and required 41% fewer treatments with HDCC (P = 0.026). HDCC was defined as: exposure to cyclophosphamide; an increase in oral, intravenous or intramuscular prednisone (or prednisone equivalent) > 15 mg per day from baseline to a dose of > 20 mg per day for more than 2 days; or any dose exceeding 200 mg in a single day. In the high-affinity population, the LJP 394 group experienced a longer time to renal flare (P = 0.008), 67% fewer renal flares (P = 0.008), longer time to HDCC (P = 0.002) and 62% fewer treatments with HDCC (P = 0.001) compared with high-dose corticosteroids and/or cyclophosphamide (HDCC; P = 0.033), and required 41% fewer treatments with HDCC (P = 0.026). Serious adverse events were observed in 26/114 (23%) LJP 394 patients and 34/116 (29%) placebo patients.

Thus treatment with LJP 394 in patients with high-affinity antibodies to its dsDNA epitopes prolongs the time to renal flare and patients required fewer HDCC treatments compared with placebo. The drug appeared to be well tolerated. LJP 394 is currently being studied in an international phase III randomized controlled trial to determine if treatment with 100 mg/week can delay time to renal flare in patients with SLE and a history of renal disease.

Many authorities now believe the dsDNA is an important antigen in patients with SLE in the context of the nucleosome. Although the antigenic properties of DNA make it an unlikely candidate for immune-based therapy or treatment, histones are proteins with well-defined antigenic and immunogenic properties, able to stimulate both B cells and T cells. Anti-histone immunity arises naturally in lupus disease in many human patients and in particular strains of mice. Norman Staines (London) described work in his laboratory that exploited the potentially powerful immunogenicity of histones in developing a palliative vaccine, based on histones, for lupus. As a rule, protein antigens that enter the body through mucosal tissues (the respiratory and digestive tracts are the best described) induce a form of systemic unresponsiveness that was originally called oral tolerance, and more recently mucosal tolerance. Careful analysis of the tolerance mechanisms in several experimental systems shows that, in most cases, the so-called tolerance is in fact hyporesponsiveness. This capacity resides in a particular arm or arms of the immune response: it arises through an activation of other cells that regulate and modify the action of both T and B effector cells. Thus, delivering antigens trans-mucosally may offer an effective way of immunizing to prevent or control pathological immune reactions. Extensive work on experimental arthritis, and work by many others on other autoimmune disease, has shown that nasal or gastric administration of autoantigen or autoantigenic peptides can both prevent and treat experimental autoimmune conditions in a specific manner. There is limited evidence that inducing
protective mucosal immune responses can similarly treat human arthritic disease.\textsuperscript{10}

Using the (NZB\times SWR)F\textsubscript{1} (SNF1) mouse to study lupus disease, histone peptides were administered nasally to young mice, and the development of their autoimmunity followed. The choice of peptides was based on studies from Syamal Datta’s laboratory in which the histone epitopes against which pathogenic T cells react were mapped.\textsuperscript{11,12} In the current experiments, a synthetic peptide (termed H471), representing residues 71 – 93 of histone H4, was given nasally before or after immunization with the same peptide. These experiments were directed to prevention and amelioration of immune responses associated with disease, respectively. In each case, peptide treatment suppressed the subsequent T cell proliferative response to H471 \textit{in vitro}, using cells taken from animals after they had been immunized intradermally with peptide. The effects were dose-dependent, but in a complex fashion: neither high nor low doses were suppressive when given nasally, the optimum being around 20\textmu g. These findings replicate the complex dose–response effects seen in other diseases where mucosal immunization modifies disease progression.

When animals treated nasally with peptide H471 were immunized with mononucleosomes (the biologically intact version of the nuclear antigen), again systemic T cell responses were also suppressed. In such animals, the overall anti-H471 antibody response was not significantly affected, but analysis of the antibody subclasses showed that IgG1 antibodies were elevated and IgG2a antibodies reduced as a consequence of nasal exposure to H471. The responses to both H471 nucleosome peptide and to DNA were similarly affected, showing that the immune modification (equated with classical mucosal tolerance) involves intermolecular suppression events.

The profiles of cytokines made \textit{in vitro}, assessed by a Ce1ELISA system,\textsuperscript{13} showed that mice treated nasally with H471 peptide, compared with those that received a control OVA peptide (residues 323 – 339), made less IFN-\gamma and IL-2 and more IL-10. Production of IL-4 and IL-2 was generally unaffected. These findings are consistent in part with a deviation of the systemic response to mononucleosomes towards Th2 domination, rather than Th1, but also could be explained by activation of IL-10-producing regulatory cells.

Some experiments have been done on the modification of kidney disease, using repeated nasal instillation of H471 peptide into SNF1 mice. The mean group severity of glomerulonephritis was reduced in mice that received the histone peptide: 1/10 mice treated with H471 had severe nephritis (assessed histologically), compared with 5/10 given the control OVA peptide. The study shows that use of a single histone-derived peptide can modulate the immune response to nucleosome antigenic determinants on both histones and DNA, and can protect against the development of severe glomerulonephritis. This study indicates a route towards a palliative vaccine for the treatment of human lupus disease by mucosal immunization with histone or histone peptide.

Michael Ehrenstein presented some data on the use of treating patients with lupus by B lymphocyte deple- tion using Rituximab. This has been introduced for the treatment of several autoimmune disorders including rheumatoid arthritis and immune thrombocytopenia.\textsuperscript{14} The rationale for B-lymphocyte depletion has been the lowering of autoantibody levels with reduction in the generation of autoantibody producing daughter plasma cells, although the exact mechanisms are uncertain. In brief six patients with systemic lupus erythematosus who had responded inadequately to major immunosuppression with corticosteroids and intravenous cyclophosphamide, azathioprine or mycophenolate were enrolled in a study conducted at the Centre for Rheumatology, Middlesex Hospital, University College London. The patients were treated with two doses of 500 mg Rituximab (between 600 and 700 mg/m\textsuperscript{2} of body surface area in total) and two doses of 750 mg of intravenous cyclophosphamide together with oral prednisolone 30 – 60 mg per day for 5 days starting the day before the Rituximab infusion, given 2 weeks apart. One patient was lost to follow-up after 3 months and had not experienced any significant clinical changes at that period but in retrospect her disease was probably more CNS damage than activity. However the other five patients all improved substantially, with a median BILAG global score level falling from 14 (range 9 – 27) to 8 (range 4 – 13).\textsuperscript{14} At 6 months the BILAG global scores had fallen to a median of 6 (range 3 – 8). Manifestations including fatigue, arthritis, serositis and skin vasculitis responded particularly well to this protocol but in addition two patients with major renal involvement also responded very well, with significant decreases in the urine protein/creatinine ratio from over 250 at baseline to 70 and 60, respectively. The levels of DNA antibodies fell in all of these patients although by varying amounts, and the C3 levels tended to normalize. The DNA antibody levels were not reduced to normal in all cases, however. Dr Ehrenstein felt that the results were very encouraging and provided grounds for establishment of a full double-blind control study.

\textbf{References}


