REVIEW

New methods of treatment in an experimental murine model of systemic lupus erythematosus induced by idiotypic manipulation

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The survival of patients with systemic lupus erythematosus (SLE) has improved tremendously over the past several decades, from a two year survival of 50% in 1939¹ to five and 10 year survival of 90% and 80% respectively in the 1980s.² This improvement is attributed mainly to the availability of dialysis, use of corticosteroids and cytotoxic drugs, and improved antibiotics and antihypertensive agents. However, since the introduction of cytotoxic agents in the treatment of severe SLE, no significant breakthrough in the treatment of lupus patients has been reported. This is probably because the aetiology of the disease is still unknown, and because of the great diversity of the clinical expressions of the disease—which makes it difficult to assess the effect of novel treatments—and the long follow up needed to evaluate changes in prognosis. Moreover, since 80-90% of lupus patients are young women, the use of experimental treatments which might decrease fertility, increase teratogenesis, or have other undesirable side effects is greatly curtailed. Because of these limitations, animal models of SLE could be of considerable value in the evaluation of novel and experimental treatments which that cannot be tested directly on patients. Various animal models of human SLE have been described, which tend to reflect different aspects of the disease. The prototype murine model of spontaneous SLE is the New Zealand black (NZB) mouse,³ principally a model of autoimmune haemolytic anaemia, accompanied by kidney disease and autoantibodies against erythrocytes, ssDNA and dsDNA. A hybrid strain derived from the NZB mouse is one produced by mating this strain with the New Zealand white mouse, the offspring being known as (NZB×NZW)F₁.³ The MRL/lpr mouse strain is a model for an accelerated membranoproliferative glomerulo-nephritis associated with anti-DNA production.⁴ Additional clinical features include lymphoproliferation, synovitis, and vasculitis. Another mouse model for SLE is the B×SB mouse, which is unusual in that the male develops autoimmunity earlier and in a more

severe way than the female. Graft versus host disease (GVHD) is produced in mice by injecting lymphocytes from a parent into an F₁ hybrid differing at one MHC locus from that parent.⁵ Several IgG autoantibodies are made, including anti-dsDNA and anti-histones, and fatal lupus-like nephritis mediated by IgG anti-DNA occurs.

Most of the animal models for SLE described so far—except for GVHD—are genetically determined; hence the ability to manipulate the natural history of the disease or to examine the effect of treatments in different stages of the disease is limited. A few years ago, we introduced a novel method for induction of experimental autoimmune conditions,⁶⁻⁹ including SLE, involving idiotypic immunisation in naive mice. This method is based on Jerne's theory,^{10,11} in which the idiotypic determinant of each autoantibody is complemented by those of another, creating an idiotypic network through which immunoglobulin expression might be controlled. This is manifested by the generation of anti-idiotypic antibodies (Ab) of two functional subsets: those that recognise determinants in the V region and do not involve the combining site for the elicit antigen, and those that represent internal images of the elicit antigen (fig 1). We found that immunisation of naive mice in the footpads with a specific autoantibody (for example, anti-dsDNA, anti-cardiolipin, or anti-proteinase-3 antibodies) emulsified in Freund's adjuvant, followed by a boost injection three weeks later, led to the generation of Ab₂, namely an anti-autoantibody, and later to mouse Ab₃ (anti-anti-autoantibody), which simulates the original autoantibody (human or mouse origin) (fig 1). This ends with naive mice producing specific pathogenic autoantibodies, followed by the emergence of the full blown serological, immunohistochemical, and clinical manifestations of the respective autoimmune disease. In a series of experiments we and others have shown that immunisation of various strains of mice with monoclonal^{9,13} or polyclonal¹⁴ human¹²⁻¹⁵ or mouse¹⁵ anti-DNA antibody, carrying mainly the pathogenic 16/6 Id, ended in the production of a panoply of

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SLE related autoantibodies by the mouse (anti-DNA, anti-Sm, anti-Ro, anti-histones), a phenomenon referred by us as “autoantibody spread”.¹⁶ The serological markers were associated with typical clinical findings of SLE such as increased erythrocyte sedimentation rate (ESR), leucopenia, thrombocytopenia, proteinuria, alopecia, and paralysis, as well as deposition of the mouse anti-DNA antibodies in the glomeruli of the kidneys, skin, and brain^{8,9,12-17} (fig 2). The time interval between immunisation with Ab₁ and generation of mouse Ab₃, and autoimmune manifestations, is probably related to many factors, such as genetic predisposition in certain mice strains, the mode of immunisation, and the pathogenic potential of Ab₁. We found that BALB/c mice immunised with pathogenic anti-DNA antibodies develop mouse Ab₃ 2-3 months after immunisation, and full autoimmune disease after four to six months.

We postulate that the natural analogy of this experimental model in humans resides in the induction of antibacterial antibodies carrying pathogenic idiotypes. Indeed, we have already reported previously on the presence of increased titres of the 16/6 Id in the sera of patients infected with mycobacteria (pulmonary tuberculosis)¹⁸ and klebsiella (pneumonia and urinary tract infections) or other Gram negative bacteria.¹⁹ Thus it is conceivable that infection may trigger autoimmune diseases by inducing antibacterial antibodies carrying the pathogenic idiotypes of autoantibodies (Ab₁). In the presence of adjuvant effect (or superantigen?) attributed to the various bacteria themselves, these antibodies may start—in a subject with the “proper” histocompatibility antigens and hormonal background—the cascade of idiotypic dysregulation shown by us in the experimental models, leading eventually to the generation of Ab₃ (autoantibody spread), which, either by itself or through regulation, may lead to the overt

clinical autoimmune condition (fig 3). Employing this model, we and others have tested the efficacy of several modes of treatments, in different stages of the disease. In this review we summarise the experience with those treatments, with their potential implications to patients with SLE.

Hormonal manipulations

The strongest risk factor for the development of SLE is female gender. It was felt, therefore, that study of sex related factors would offer a clue to the pathogenesis and treatment of SLE. Studies in the NZB/W F₁ murine model for SLE supported a role for female hormones in the modulation of autoantibody production and development of renal disease and death.²⁰ Human studies for the effect of sex hormones in SLE patients are limited, and the results have been conflicting.^{21,22} We studied the effect of sex hormones upon the induction of experimental SLE in BALB/c female and male mice which underwent orchiectomy.²³ It was found that injection of the pathogenic idio type to females, and to orchiectomised male mice, caused a rapid outburst of the disease compared to non-oestrogen-treated mice. Testosterone treated mice developed the usual response to the human anti-DNA antibody, but failed to develop fulminant SLE-like disease. In another study, the effect of tamoxifen, a synthetic non-steroidal anti-oestrogen compound, on the development and course of the disease in lupus mice was examined.²⁴ Although tamoxifen treatment had no effect on the 16/6 Id induced antibody production, it cured the clinical manifestations of the disease. It is noteworthy that delayed tamoxifen treatment also had beneficial therapeutic effects, although it was not as effective as early treatment.²⁴ The results point to the importance of sex hormones in the pathogenesis of SLE, and to the possible beneficial effects of androgen or anti-oestrogen treatment in early stages of the disease. During the last years, not much research has been done in developing new hormonal treatments for SLE patients. Treatment of limited number of patients with the androgen metabolite nandrolone resulted in improvement of disease manifestations in women, but men got worse.²¹ However, in another study this drug was ineffective in SLE patients.²² Until now, only danazol—an attenuated male sex hormone—has been shown to be of some value in treating haematological (thrombocytopenia) and skin manifestations of SLE.^{25,26} We believe that our results might promote further research to establish the potential of hormonal modulation of the disease in lupus patients.

Prolactin, as a sex hormone, has been found to affect the immune response and modify the expression of autoimmunity in animals and humans.²⁷ Bromocriptine, a dopamine agonist, suppresses the secretion of prolactin by the pituitary gland and in human and animal studies has been found to have immunoregulatory properties.^{27,28} Evaluating the effect of bromocriptine treatment on mice with experimental

Figure 1 The idiotypic network according to Jerne's theory: immunisation with the Ag (Ab₁) leads to generation of anti-idiotypic Ab (Ab₂) against the antigen binding site (variable region) of Ab₁. Ab₂ also has idiotypes outside its binding site (parallel Id). The idiotypes of Ab₂ are recognised by the immune system to generate the Ab₃ (anti-anti-Id) which resemble the structure of Ab₁ (A). Alternatively, Ab₂ may be generated against idiotype residing outside the binding site (B), and may function as a regulatory anti-Id, without similarity to the original Ag.

Figure 2 Manifestations of experimental SLE induced in naive mice by immunisation with pathogenic idiotypes of anti-DNA antibodies.

SLE,²⁹ we observed a marked reduction of autoantibody levels accompanied by disappearance of clinical and pathological manifestations of the disease. The effect of bromocriptine seems to be non-specific for SLE, since a similar effect was observed in mice with experimental antiphospholipid syndrome (APS).²⁹ Those results were supported by in vitro non-specific effect of CD8 cells, induced in vivo by bromocriptine, on specific lymph node cell proliferation in the presence of pathogenic and non-pathogenic autoantibodies. We also found that injection of CD8 cells from bromocriptine treated mice with SLE or APS abolished the development of disease in the SLE and APS models. Our findings suggest a possible role for bromocriptine in downregulating autoimmune phenomena through induction of natural non-specific CD8 suppressor cells. The effect of bromocriptine treatment in SLE patients has not been tested in controlled trials, but a few published case reports imply a beneficial effect.³⁰ Recently, it has been reported that SLE patients treated with bromocriptine for six to nine months had a significant decrease in disease activity, associ-

ated with lower titres of anti-dsDNA antibodies.³¹ Our results are in line with those reports, and may suggest a clinical application of bromocriptine treatment in SLE patients.

Immunomodulation

Several immunomodulations have been tried in an attempt to manipulate the course and outcome of mice with experimental SLE induced by idiotype immunisation. These include anti-idiotypic or anti-CD4 antibodies, idiotype specific T suppressor cells, intravenous immunoglobulins, treatment with several cytokines, or an attempt to induce oral tolerance to the pathogenic idiotype.

ANTI-IDIOTYPIC MODULATION

Since the idiotype network is an important mechanism controlling the immune repertoire,¹⁰ and autoimmune diseases may be attributed to disturbance of the network,³² several groups have employed an anti-idiotypic modulation in experimental models. Indeed, successful in vitro and in vivo manipulations of autoantibodies production by anti-idiotypic antibodies have been reported in several animal models for autoimmune diseases.^{33–35} Enhancement of the effect of anti-idiotypic antibodies was also tried by conjugation with cytotoxic agents, one of which was saporin.³⁶ On the basis of those studies, we manipulated mice with experimental SLE with an anti-16/6 Id monoclonal antibody conjugated to saporin.³⁷ We observed a significant reduction in the titres of serum autoantibodies, with diminished clinical manifestations. Those effects were obtained using anti-Id monoclonal antibody, and were even more remarkable when the immunotoxin anti-Id monoclonal antibody-saporin conjugate was employed. These suppressive effects were specific, since this treatment had no effect upon mice with experimental APS.³⁷ The anti-Id effect was mediated through a reduction in specific anti-DNA antibody forming cells, and lasted as long as the anti-Id injections were given. These results add further support to the importance of pathogenic idiotypes in SLE, pointing to the potential effectiveness of anti-idiotypic treatment in SLE patients. Since, however, a growing number of pathogenic idiotypes has already been identified in SLE,³⁸ a combination ("cocktail") of anti-idiotypic antibodies might be necessary for an effective treatment.

Figure 3 Autoimmunity triggered by infection: an ineffective agent induces antibacterial antibody (Ab₁) carrying a pathogenic idiotype. Following the generation of anti-idiotypic antibody (Ab₂) and anti-anti-idiotypic antibody (Ab₃), which is the pathogenic autoantibody, and in the "proper" conditions (for example, HLA antigens, hormonal background, immune dysregulation) autoimmune disease may be induced.

MONOCLONAL ANTIBODIES AGAINST CD4 MOLECULES

The CD4 molecule is a glycoprotein coreceptor of the antigen receptor on T cells, the main functions of which are binding to major histocompatibility complex (MHC) molecules and guiding T cells toward class II MHC recognition.³⁹ Monoclonal antibodies against CD4 molecules can block T cell activation and proliferation and the release of cytokines.⁴⁰ Indeed, immunotherapy with anti-CD4 antibodies has been shown to suppress autoimmunity in several animal models of

autoimmune diseases, and in some human autoimmune conditions.⁴¹⁻⁴² We have found that CD4+ T cells play a central role in the pathogenesis of experimental SLE: injection of CD4+ T cells specific for the 16/6 Id into naive BALB/c mice resulted in the typical serological and clinical manifestations of experimental SLE.⁴³ Following these results, we examined the ability of anti-CD4 antibodies to prevent or to treat experimental SLE.⁴⁴ We treated the mice with rat anti-CD4 monoclonal antibody, either before disease induction or when the mice had already developed serological but not clinical signs of the disease. Mice which were treated with anti-CD4 monoclonal antibody developed significantly lower levels of autoantibodies, and lacked the clinical manifestations of SLE. It seems therefore that anti-CD4 treatment is particularly effective in early stages of the disease, when only the serological markers of the disease are present.

SPECIFIC T SUPPRESSOR TO THE PATHOGENIC AUTOANTIBODY

T Suppressor (Ts) activity is an important immunoregulatory mechanisms, controlling autoantibody production in B lymphocytes. Several publications have reported decreased number and activity of Ts cells in mouse models for SLE,⁴⁵ and SLE patients.⁴⁶ If the production of autoantibodies in SLE is related to downregulation of Ts cells, then reconstitution of Ts cell number and activity, especially the Ts specific to the pathogenic autoantibody, may lead to amelioration of disease manifestations. Indeed, previously a few studies have confirmed this assumption.⁴⁷⁻⁴⁸ To study the role of T cells and pathogenic idiotypes in SLE, we have established Ts cells specific for anti-DNA idotype 16/6. These Ts cells were generated from BALB/c enriched T cells exposed *in vitro* to the pathogenic 16/6 Id.⁴⁹ We found that weekly intravenous injections of 16/6 Id specific Ts cells, given to SLE mice at different stages of the disease, prevented the clinical and serological manifestations. It should be emphasised, however, that this treatment failed once the disease was well established. The results of this study support the notion of the role of pathogenic idiotypes in murine SLE, and the role Ts cells may take in induction as well as in modulation of autoimmune conditions.

HUMAN IMMUNOGLOBULIN GIVEN INTRAVENOUSLY

Normal human immunoglobulin given intravenously (IVIG) has been reported to be effective in treating several autoimmune diseases.⁵⁰ Several recently published case reports imply that IVIG may have beneficial effect upon various expressions of SLE.⁵¹⁻⁵⁴ The advantages of IVIG treatment are considerable because of its infrequent side effects and lack of immunosuppression. The precise mechanism of action of IVIG in autoimmunity is not yet clear, but much of its immunomodulation is attributed to

manipulation of the idiotypic network.⁵⁰ We evaluated the effect of IVIG treatment on immunological and clinical findings in mice with experimental SLE⁵⁵ which were treated with IVIG (whole molecule, F(ab)₂ or Fc fragments). SLE mice, treated with IVIG or its F(ab)₂ fragments—but not the Fc fragment—had a complete clinical, serological, and pathological remission which lasted as long as the treatment was given. Inhibition studies pointed to the presence of anti-idiotypic activity to anti-dsDNA antibodies in the IVIG preparation.⁵⁵ This implies that the therapeutic effect of the IVIG treatment in our model might be mediated through manipulation of the idiotypic network and neutralisation of pathogenic autoantibodies. The findings are in line with previous studies that show the presence of anti-idiotypic activity in IVIG preparations to several autoantibodies associated with autoimmune diseases.⁵⁶⁻⁵⁸ This may raise the possibility of analysing patients' serum with the specific IVIG batch before treatment, in analogy to bacterial sensitivities to various antibiotics. Our results further strengthen the role of IVIG treatment in SLE, and may promote the handling of controlled clinical trials in this expensive, yet apparently effective, treatment.

SYNTHETIC IMMUNOMODULATOR AS101

The CD4+ cells are now recognised as having two subsets—Th1 and Th2—based on their cytokine secretion. Th1 clones produce interleukin-2 (IL-2), interferon (IFN- γ), and tumour necrosis factor (TNF), and are the principle effectors of delayed type hypersensitivity reactions. Th2 cells produce IL-4, IL-5, IL-10, and IL-13, stimulate IgE and IgG1 antibody production, and inhibit macrophage function.⁵⁹ Prevalent Th2 response seems to be involved in the immunopathogenesis of SLE, since a major abnormality of the immune system in SLE is the T cell dependent B cell hyperactivity, and impaired cell mediated immunity.⁶⁰ Moreover, a Th2-type immune response has been clearly shown to be pathogenic in experimental SLE induced by allogeneic stimulation or chemicals.⁶¹ There have also been reports of a decrease in the production of IL-2 in autoimmune mice and humans.⁶²⁻⁶³ In preliminary results, we noticed that the production of IL-2 is reduced in mice with experimental SLE. This led us to consider treatment of those mice with the synthetic immunomodulator AS101, which has been found to have a potential therapeutic effect on various immune mediated conditions, attributed to its ability to increase the production of IL-2 and colony stimulating factor *in vivo* and *in vitro*.⁶⁴ We found that splenocytes from the SLE mice had a significant decrease in the ability to secrete IL-2. Treatment of the mice with AS101 restored the ability of the splenocytes to secrete IL-2 to normal levels. The treatment, however, had no effect on clinical or serological indices of SLE.⁶⁵ In another study, mice were treated with IFN- γ before disease induction. It was found that those mice

Table 1 The effect of various experimental treatments on mice with experimental SLE

Treatment	Rationale for treatment	Study protocol	Effect on experimental SLE
Sex hormones ^{23,24}	Sex hormones may play a role in the pathogenesis of SLE, since females are much more prone to develop SLE	The hormones were implanted subcutaneously, to achieve constant levels. One week later, the mice were immunised with the 16/6 Id	Testosterone and tamoxifen attenuated, while oestrogen aggravated SLE expression
Bromocriptine (BRC) ²⁹	Prolactin (PRL) enhances the expression of autoimmunity in humans and animals, BRC suppresses PRL secretion	Immunised mice were treated with IP BRC for 6 weeks, starting 2 months after disease induction (ie, early disease stage)	Complete serological, histological, and clinical remission
Cytokines ⁶⁵	Th2 cells are important in the immunopathogenesis of experimental SLE. Role of Th1 cells unclear	Immunised mice were treated with AS101 (increases the production of IL-2), IP for 7 weeks, or with INF- γ , before disease induction	No effect of IL-2. INF- γ caused rapid outburst of the disease, with raised IL-4 and IL-6
Anti-idiotypic antibodies ³⁷	Autoimmune diseases may result from dysregulated idiotype network	Immunised mice were treated, 2 months after disease induction, with anti-16/6 Id Sb by daily IP injections for 1 month	Attenuation of disease manifestations, mediated through reduction of anti-DNA Ab forming cells
Id-specific Ts cells ⁴⁹	Production of autoantibodies in SLE may be related to downregulation of Ts cells	SLE mice were treated, at different stages of the disease, with weekly IV injections of 16/6 Id-specific Ts cells, for 8 weeks	Early treatment prevented disease manifestations, treatment at late disease stage was less effective
Anti-CD4 antibodies ⁴⁷	Anti-CD4 Abs can block T cell activation, proliferation and release of cytokines, and suppress autoimmunity	SLE mice were treated with rat anti-CD4 mAb, either before and during disease induction, or 2 months after disease induction	Prevention of clinical manifestations, especially in early stages of the disease
IVIG ³⁵	IVIG treatment was found effective in several autoimmune diseases, including case reports on SLE	SLE mice were treated with IVIG (whole molecule, F(ab) ₂ or Fc fragments), starting 2 months after disease induction, for 6 weeks	Complete remission, probably mediated by anti-idiotypic activity
Oral tolerance	Systemic tolerance can be achieved by feeding with pathogenic proteins	Mice were given different doses of the 16/6 Id (10–1000 μ g) by oral intubation, 3 times every 3 days, before immunisation with the pathogenic Id	Different doses of oral 16/6 Id did not change disease expression

developed earlier and more severe disease, accompanied by raised concentrations of IL-4 and IL-6. These results point to the importance of Th1 as well as Th2 in the immunopathogenesis of SLE in our model.

SYSTEMIC TOLERANCE

Systemic tolerance to various antigens can be achieved by feeding with pathogenic proteins.⁶⁶ It has been found that oral administration of autoantigens suppresses disease development in animal models of autoimmunity.⁶⁷ Nevertheless, immune responsiveness can also be enhanced by antigens presented to, and absorbed through, the intestine.⁶⁸ Strains of lupus mice (B $\times$ SB, MRL/lpr, and NZB) differed in their capacity to become orally tolerised after feeding with bovine γ globulin and casein.^{69,70} The mechanisms of tolerance in lupus-prone mice seem to be affected by multiple factors not present in normal strains of mice, and the reasons for such a difference

between lupus-prone and normal mice remain unclear.⁷¹ In an attempt to establish antigen specific unresponsiveness in the experimental SLE model, mice were given different doses of anti-DNA (16/6 Id+) antibody, by oral intubation, after which they were immunised with the 16/6 Id. A significant increment in the level of anti-16/6 Id and anti-DNA antibody titres was detected in the sera of the 16/6 Id orally treated mice in comparison with the control groups, while the clinical indices of the disease remained the same. Hence, it was found that oral feeding of 16/6 Id did not induce tolerance, and might even induce priming of the disease.

Summary

In this article we have presented our experiences and those of others with various experimental and novel treatments in an experimental model of murine SLE, induced by immunisation with pathogenic anti-DNA antibody (fig 4). Many of the treatments (summarised in the table) were highly effective in ameliorating clinical, serological, and histological manifestations of the disease. According to our results, it seems that hormonal treatments—such as testosterone metabolites, anti-oestrogens, or bromocriptine—as well as immunomodulation with IVIG or anti-CD4 antibodies, hold the most promising potential for application in lupus patients. We believe, therefore, that these types of treatment should receive high priority in human trials. It should be emphasised, however, that the timing of treatment may be critical, since several treatments were effective when used before or during the induction of the disease. This limitation may pose difficulty for human application, since the aetiology of SLE is still obscure and is probably multifactorial³⁸; therefore it is not yet possible to identify patients at risk of developing SLE. Nevertheless, those treatments which proved to be effective might be used early in the course of the disease in

Figure 4 Effect of various experimental treatments on mice with experimental SLE (see also the table).

lupus patients and hence influence the outcome of the disease, or may even induce partial or complete remission.

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