

Review Article

Therapeutic Strategies for SLE Involving Cytokines: Mechanism-Oriented Therapies Especially IFN- γ Targeting Gene Therapy

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Systemic lupus erythematosus (SLE: lupus) is a chronic complicated autoimmune disease and pathogenesis is still unclear. However, key cytokines have been recognized. Interferon (IFN)- γ and also IFN α/β are of particular importance. Depending on the concept that lupus is a helper T(Th)1 disease and that dendritic cells (DCs) determine the direction of lupus, balance shift of Th1/Th2 and immunogenic/tolerogenic DCs is reviewed for therapy. (IFN)- γ - and IFN α/β -targeted (gene) therapies are introduced. These consist of Th1/Th2 balance shift and elimination of IFN- γ and IFN- γ -related cytokines such as (interleukin)IL-12 and IL-18. Other approaches include suppression of immunocompetent cells, normalization of abnormal T-cell function, costimulation blockade, B lymphocyte stimulator (Blys) blockade, and suppression of nephritic kidney inflammation. Moreover, balance shift of IFN- α/β and tumor necrosis factor (TNF)- α together with regulatory T(Treg) cells are briefly introduced. Clinical application will be discussed.

1. Introduction

Systemic lupus erythematosus (SLE or lupus) is a disorder of immune regulation characterized by the breakdown of self-tolerance. The complexities of various humoral and cellular abnormalities under the influence with predisposing genetic, sex hormonal and environmental factors have been reported in pathogenesis of SLE in human and model animals [1, 2]. In general, patients with overt disease will be treated with immunosuppressants (e.g., prednisolone, cyclophosphamide, and tacrolimus) or NSAIDs (nonsteroidal anti-inflammatory drugs). Those immunosuppressive drugs are strongly effective in prevention of the development of lupus nephritis due to reducing the host immune responses. However, less toxic approaches to avoid severe adverse events (infections, infertility, amenorrhea, and metabolic abnormalities) are remained [3, 4]. Thus, there are a lot of trials focusing on improved therapy compared to classic immunosuppressive drugs in experimental basis [5].

Amoura et al. [6] have recently reviewed new bio-therapies that new approaches in human lupus are based

on a better understanding of the autoimmune response as follows. Targets of these new treatments are all steps of the immune response in lupus development. These are (1) “B lymphocyte (BL)” inhibitors such as anti-CD20 monoclonal antibody, anti-CD22 monoclonal antibody, B-lymphocyte stimulator (BlyS) antagonists, tolerogenic peptide [hCDR1: Edratide, which is based on the sequence of the complementary-determining region (CDR)1 of a human anti-DNA monoclonal antibody that bears the major idiotype designated 16/6Id] [7], and LJP 394 (abetimus sodium) which selectively reduces antibodies to dsDNA and their parent B cells via antigen-specific tolerance [1]; (2) “Inhibitors of the costimulation” between antigen-presenting cells and T lymphocyte by monoclonal anti-CD40 ligand antibody or CTLA-4-Ig; (3) “Cytokine antagonists” inhibiting key cytokines of SLE: IL-10 [8], interferon (IFN)- α [9], which are associated with lupus in human [10], IL-6 [11] and tumor necrosis factor (TNF)- α [12]. In addition, the importance of IL-23/IL-17 axis in human lupus and lupus model mice is pointed out [13, 14] and the possibility of IL-17 targeted therapy is recently proposed since its important

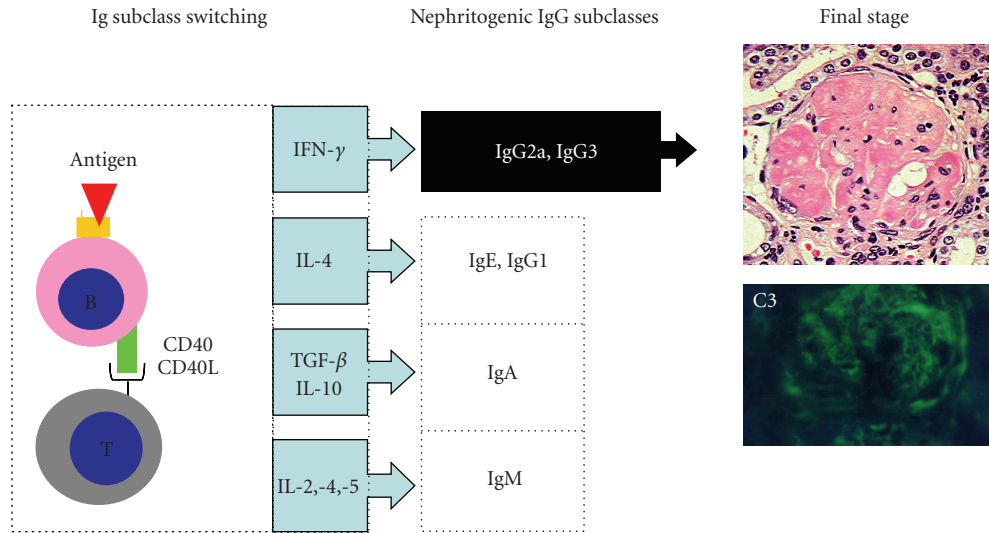


FIGURE 1: Class-switching of immunoglobulin. Cognate contact of T-B interaction and following stimulation of cytokines determine Ig subclass switching in mouse.

role in human SLE [4]. Steinmetz et al. [15] showed for the first time that not only Th1, but also Th17 effector T cells mediate glomerulonephritis in lupus model MRL/lpr mice. They showed that deficiency of the chemokine receptor CXCR3-bearing T cells (highly expressed on Th1 cells) leads to significant morphological and functional improvement of nephritic kidneys. Thus, all of those are expected as an effective new therapy in lupus-like tumor necrosis factor (TNF)- α targeted therapy in clinical application for rheumatoid arthritis (RA) [7, 16]. Obviously, effectiveness is reported by the treatment of anti-IL10 mAb [8], LJP 394 [17], Edratide [7], inhibition of T cell costimulation [18] and anti-IFN α/β [19] in human lupus. On the other hand, Mohrs et al. [20] have reviewed that fusion proteins, peptides, and small molecules rather than therapeutic antibodies which are excellent alternative tools for immune intervention in lupus.

Alternatively, immune regulation forming cytokine networks including intracellular signaling of cytokines is highly complex and the mechanisms of regulation is not as yet fully understood in lupus pathogenesis [21, 22]. Moreover, compared to organ-specific autoimmune diseases, several organs/tissues (e.g., Kidneys, lungs, joints, nervous systems, and serous membranes) are involved in SLE. Also, patients with SLE were often accompanied with secondary Sjögren's syndrome (sSjS) [23]. In each organ involved, pathogenesis is quite different. For example, humoral immunity plays a role in capillary damages in glomeruli, lungs, dermal tissues [24] and other organs whereas cell-mediated immunity develops in interstitium of kidneys, lacrimal, and salivary glands in lupus with sSjS, resulting in damages of those organs [25]. These suggest that therapy focusing on one cytokine (or combination of several cytokines) or one immunocompetent cell seems to be difficult. Thus, the concept is very important by cytokine targeted therapy.

Blocking a single cytokine might be the best to control this clinically heterogeneous disease in lupus. Depending on the concept of balance shift, this review introduces [I] alteration of the balance between Th1(IFN- γ) and Th2 cell (IL-4) activity [28, 29] (Figure 1), but not shift proinflammatory versus immunosuppressive cytokine profiles (IL-1, IL-6, IL-10, TNF- α) and [II] shift from immunogenic (IFN- α/β) to tolerogenic (TNF- α) DCs (Figure 2) in lupus-prone mice. Moreover, informative data are involved in section [I], despite those are different from the concept. The clinical implications including usefulness and unfavorable side effects in those therapies are discussed in each section.

[I] Th1/Th2 Balance Shift

[1] *Lupus is a Th1-Dominant Disease.* Several cytokines and chemokines are involved in the lupus pathogenesis [13, 30] as described above. More than 30 years ago, Hooks et al. [31] found immune interferon (IFN- γ) in the sera of patients with SLE and showed a good correlation between immune IFN- γ titers and disease activity. Moreover, IFN- γ titers correlated positively with antibodies to DNA and negatively with serum levels of third components of complement. After that, however, the dominance of Th1 or Th2 cytokines in SLE patients and lupus model mice has been debated in numerous reports over time until now. Earlier reports proposed a Th2 response (especially IL-4) (or a mixed Th1 and Th2 response) [1, 32–35] whereas recent evidences demonstrated the importance of the Th1 response especially IFN- γ (see [36–41] Figure 1), including IFN- γ inducible IL-12 [42, 43] and IL-18 [44, 45]. Those are involved in the onset and progression of the autoimmune disease in lupus. Moreover, the current consensus is that IL-4 production is not increased in SLE patients by cDNA assay [46]. One might suppose that the autoimmune manifestations of the SLE

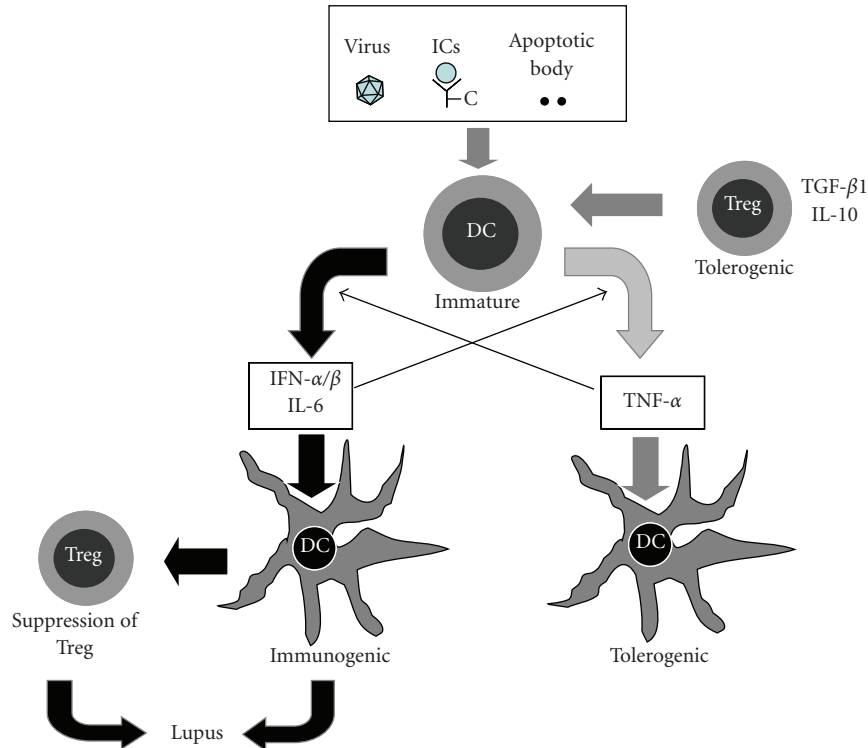


FIGURE 2: Notion of $\text{IFN-}\alpha/\beta$ and $\text{TNF-}\alpha$ axis balance proposed by Banchereau and Pascual [26]. Environmental factors (e.g., viral infections), apoptotic bodies and immune complexes stimulate pDCs through the coengagement of Fc γ R and TLRs trigger the production of $\text{IFN-}\alpha/\beta$ from pDCs and following production of $\text{TNF-}\alpha$ occurs. Also $\text{IFN-}\alpha/\beta$ induces the generation of mature DCs. Banchereau and Pascual hypothesize that increased production of $\text{TNF-}\alpha$ may weaken $\text{IFN-}\alpha/\beta$ production, leading to prevention of lupus. Also tolerogenic peptide activates Treg cells leading to production of $\text{TGF-}\beta$ may inhibit DCs activity, resulting in the suppression of lupus development [27]. These events suppress the lupus development, whereas immunogenic DCs suppress function of Treg cells. Thus Horwitz [22] proposed Treg regulation together with tolerogenic DCs for therapy. Important cytokines are shown in bold arrows letters.

are not based solely on a Th1 type autoimmune response, since Th2 type cytokines such as IL-6, which promotes the terminal differentiation from activated B cells to antibody producing plasma cells, are apparently involved [11, 47]. In addition, proinflammatory cytokines (e.g., IL-1, $\text{TNF-}\alpha$) [12] are also involved other than Th1 and Th2 cytokines. Shimizu et al. [48] reported the evidences that MRL/lpr mice develop diffuse proliferative glomerulonephritis similar to that in human lupus nephritis (WHO class IV) was associated with autoimmune responses dominated by $\text{IFN-}\gamma$, whereas MRL/lpr mice lacking WSX-1 gene, which encodes a subunit of the IL-27R with homology to IL-12R, developed disease resembling human membranous glomerulonephritis (WHO class V) with a predominance of IgG1 in glomerular deposits, accompanied by increased IgG1 and IgE in the sera.

Furthermore, late stages of murine lupus characterized by renal fibrosis (glomerulosclerosis) have been considered a Th2-mediated disease [49].

In fact, many immune responses do not reflect an absolute Th1 or Th2 pattern as described above, but functionally dominated by either Th1 (especially $\text{IFN-}\gamma$) or Th2 (especially IL-4) cytokines in immune disease are pointed out by Paul and Seder [50]. At present, there are general agreements that initiation of events in the early and following

phases of nephritis is due to Th1- and Th1-related cytokines, and therefore, an altered ratio of $\text{IFN-}\gamma/\text{IL-4}$ producing.

Th cell axis could determine the nature, strength, and duration of systemic autoimmune responses in lupus nephritis [1].

[2] $\text{IFN-}\gamma$ Targeted Therapy

(1) *Synthetic Oligodeoxynucleotides (ODN)*. It has been reported that suppressive synthetic oligodeoxynucleotides (ODN) containing TTAGGG motifs, which are found at high frequency in the telomeric region of mammalian chromosomes [52], can downregulate inflammatory responses that are injurious to the host [53, 54]. Sano et al. [55] have reported ODN without CpG motifs, a 6-base DNA motif consisting of an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines, work as adjuvant for the induction of Th2 differentiation in a sequence-independent manner [56].

Dong et al. [57] reported that synthetic ODN delay the onset of glomerulonephritis and prolong survival in lupus-prone B/WF₁ mice, which is one of excellent mouse models for human lupus (Figure 3) with a significant reduction in productions of anti-dsDNA autoantibody, $\text{IFN-}\gamma$, and IL-12.

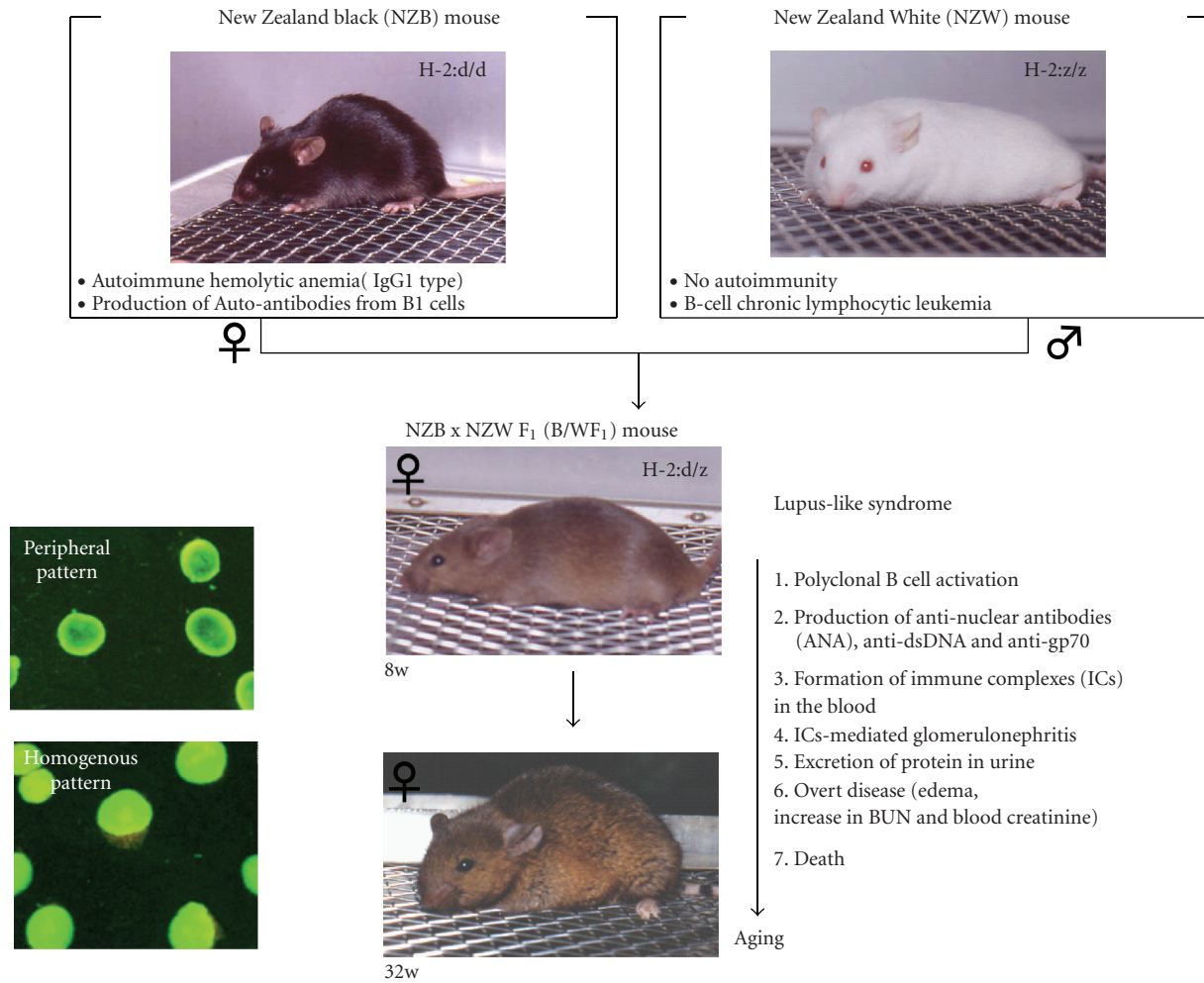


FIGURE 3: Female B/WF₁ mice constitute one of the best-studied animal models of spontaneous systemic lupus erythematosus (SLE) in human. Their characteristic features include polyclonal B-cell activation, production of autoantibodies against autoantigens (e.g., notably dsDNA), which occur when the animals are 5-6 months of age, and development of glomerulonephritis with age which is the major cause of death due to renal failure. Moreover, animals respond to cortisone similar to human patients with SLE [51]. H-2: mouse MHC. ANA: antinuclear antibody.

In addition, production of proinflammatory cytokines, especially IL-6 was blocked by those treatments. Also, repeatedly treated mice with suppressive ODN before or after the onset of proteinuria significantly prolonged lifespan with delaying the onset and progression of glomerulonephritis in B/WF₁ mice. Clinical improvement was accompanied by a significant reduction in anti-dsDNA autoantibody production and by significantly reduced secretion of IFN- γ and IL-12 in vivo. They monitored body weight and general activity as measures of animal well-being by these general criteria, suppressive ODN did not adversely impact animal health (data not shown). They concluded that suppressive ODN may be of benefit in the treatment of chronic systemic autoimmune diseases such as SLE.

Although, the mechanism by which suppressive ODN block ongoing inflammatory immune responses is incompletely understood, suppressive ODN may directly block the signal transduction cascade associated with the production

of IFN- γ and IL-12, thereby influencing the Th1/Th2 balance in vivo [56]. In light of evidence that suppressive ODN slows the development of organ-specific autoimmune Th1 diseases in autoimmune type 1 diabetes (T1D) animal models, suggesting that these agents may be broadly useful in the prevention and treatment of diseases characterized by the overproduction of Th1 and/or inflammatory cytokines. In this report, the sensitivity against pathogens and Th2-dependent allergic diseases is unclear.

(2) *Bioactive Th2 Cytokines (IL-4 and IL-10)*. Suppression of IFN- γ by recombinant (r)IL-4 or rIL-10 may be effective to preventive lupus. However, a large dose and frequent administrations of rIL-4 are necessary to maintain the effective concentration of IL-4 on prevention of T1D in murine models [58, 59], since its half life is very short ($t_{1/2} = 19 \pm 2$ min) in the circulation in mice administrated intravenously by murine rIL-4 [60]. A large dose of rIL-4

or rIL-10 ($t_{1/2}$ = several hours) [61] as the use of Th1/Th2 balance shaft may evoke unexpected side effects in recipients, such as disturbance of host immune states due to temporal large amounts. In addition, the therapy by use of those bioactive cytokines is very expensive. Thus, at present, there is no report using those in lupus model and human lupus.

(3) *Anti-IL10 Antibody*. Llorente et al. [8] showed firstly in their pilot study that elimination of IL-10 (one of Th2 cytokines) by murine monoclonal antibody (mAb) to human patients (20 mg/day intravenous administration of an anti-IL-10 murine mAb (B-N10) for 21 consecutive days) with SLE led to a rapid decrease of disease, which was maintained for 6 months of followup. The study indicates that the use of IL-10 antagonists may be beneficial in the management of refractory SLE. They discussed that this study appears encouraging to justify a larger, randomized, and blinded study using a humanized anti-IL-10 mAb to determine whether this approach allows reduction of corticosteroid treatment including the determination of long-term control of the disease by repeated administration of an anti-IL-10 in patients with poorly controlled SLE.

IL-10, which is considered to be anti-inflammatory, has properties that are somewhat ambivalent, since it can stimulate the immune function of some cell populations and is a potent anti-inflammatory cytokine with strong inhibitory effects on Th1 cells and inflammatory reactions [62]. However, lupus patients with pulmonary involvement have a higher proinflammatory cytokines profile IL-6, IL-8, IL-10, IL-12, IFN- γ , and TNF- α , suggesting the involvement of IL-10 in many facets of development and prognosis of SLE. If that is the case, anti-IL-10 antibody may contribute to suppress lupus by the different mechanisms other than the concept of Th1/Th2 balance shift.

(4) *IL-4 Transgene*. To address the issue of whether IL-4-induced immune deviation can provide protection from ongoing lupus, Santiago et al. [63] reported protective effect on the development of lupus-like glomerulonephritis in the (NZW \times B6.Yaa) F_1 murine model of SLE expressing constitutively an IL-4 transgene (pEP-IL-4) by B cells [64], since this would help establish new strategies for the development of therapeutic approaches in SLE and related human autoimmune diseases.

They showed protection that occurred in association with marked changes in the IgG subclasses rather than in total levels of anti-DNA autoantibodies, with an absence of IgG3 and a decrease of IgG2a subclasses. This was accompanied by a similar modulatory antibody response of the IgG subclass antibody against T-cell-dependent antigen (heat-aggregated human IgG: HGG), but not T-cell-independent antigen (LPS: lipopolysaccharide). The decrease of IgG3 and IgG2a autoantibody production in the transgenic mice is most likely due to the inhibitory effect of IL-4 on the development of the Th1 subset, suggesting downregulation of Th1 autoimmune responses by IL-4, although they did not show values of Th1 and Th2 cytokines. This approach may be applicable as one of strategies for the therapeutic approaches in SLE and related human autoimmune diseases.

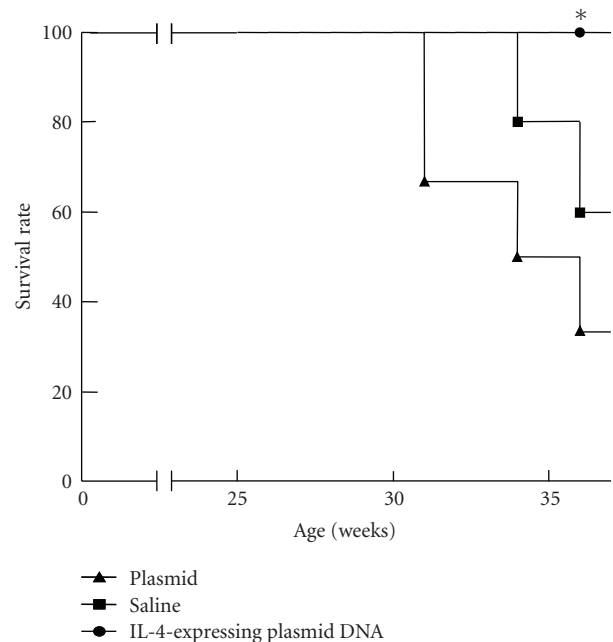


FIGURE 4: Survival rate among three groups. Mice were treated intraperitoneally either with 100 μ g of the IL-4pDNA, 100 μ g of plasmid only or saline only at the age of 4, 6, 8, 12, 16, 20, 24, 28 and 32 weeks. * $P < .001$ (comparison between the IL-4pDNA group and two controls). Provided here is courtesy of NDT 2007, 22, 3131-8: Oxford University Press).

However, pEP-IL-4 transgenic mice are more susceptible to *Leishmania major* infection than nontransgenic mice [65] in which Th1 cells play a crucial role in protection against *L. major* infection [66]. Also, they pointed out that it will be important to determine whether IL-4 transgene expression also leads to protection against SLE in other murine models such as B/WF $_1$ and MRL-lpr/lpr mice, in terms of the issue of whether IL-4-induced immune deviation can provide protection from ongoing disease [67].

(5) *IL-4-Expressing Plasmid DNA*. We have reported that preventive effects of IL-4-expressing plasmid DNA (pIL-4) using the plasmid vector PCGGS (kindly supplied by Dr. Miyazaki), on lupus development in lupus-prone female B/WF $_1$ mice [68]. IL-10-expressing DNA plasmid, which prevent T1D in model mouse [69], was not used, because as described in section [1]-(3), the use of it seems to be complicated in lupus. Successful prevention of pIL-4 in T1D experimental animal model [58, 70] encourages us for application to lupus. B/WF $_1$ mice monthly injected repeatedly with the IL-4p DNA intraperitoneally before and after the active phase drastically delayed the development of glomerulonephritis with deposits of IgG2a and C3 leading to excretion of urine protein, and they showed prolonged survival (Figure 4). Clinical improvement was associated with the reduction in productions of IgG anti-dsDNA autoantibodies with reduction of IFN- γ production and slightly increased IL-4 production from splenic cell, suggesting IL-4pDNA administration may delay lupus onset

and progress by shifting from Th1 to Th2 responses. In addition, CRFK cell line transfected with the IL-4pDNA produced enough concentration of IL-4 (approximately 200 pg/mL) more than 8 days in vitro. Thus, compared to the administration of the bioactive recombinant IL-4, IL-4pDNA may be advantageous and useful for the reduction of Th1-dependent autoimmune diseases including lupus. On the other hand, the plasmid accelerated, to some extent, the lupus development compared to the nontreated control group. It seems likely, since B/WF₁ mice administrated with plasmid containing many CpG motifs [71], which promote lupus [47], may interact and stimulate a variety of immune cells, and then those cells may secrete mainly Th1 (IFN- γ) and Th1-related cytokines (IL-12 and IL-18) including IL-6 [72, 73]. However, values of IL-4 produced by IL-4pDNA may be higher than those of IFN- γ production by CpG ODN stimulation. As a result, IFN- γ production may be inhibited by mutual inhibitory effects of Th1/Th2 cytokines [28]. In addition, CpG ODN may have a possibility to produce anti-DNA antibody formation [73]. However, this possibility may be negligible, since anti-dsDNA production in the IL-4pDNA-treated mice was reduced. Moreover, long-time survived mice did not show any clinical signs such as a dull movement and a loss of hair glittering by renal failure, supporting that idea. Also, there was no development of systemic Th2 response by this treatment like alum adjuvant (aluminium hydroxide gel), which itself induces strong systemic Th2 response (unpublished data). In this experiment which cell type takes up the IL-4pDNA after injection intraperitoneally is unknown. Thus, further study is needed to clarify cell types, including efficiency of transfection, which cells take up the IL-4pDNA and how long the expression of the IL-4pDNA lasts, including how secreted IL-4 protein affects IL-4 receptor since the IL-4 α receptor-chain is a component of both the IL-4 and the IL-13 receptor [20].

Mazda [74] have reported that nonviral gene transfer technologies include naked DNA administration, electrical or particle-mediated transfer of naked DNA such as low immunogenicity, inexpensiveness, and ease in handling, but the common disadvantage is the transfection efficiency. It remains to be determined whether an optimal dose of pIL-4 will have beneficial effects on autoreactive T and B cells without compromising immunity to foreign antigens.

(6) *IL-5 Transgene*. IL-5 preferentially activates B1 cells to produce natural antibodies cross-reactive to self antigens [75]. Wen et al. [76] have reported that B/WF₁ mice congenic for IL-5 transgene (TG-F1) reduced the incidence of lupus nephritis with decreased anti-DNA antibodies and those produced by splenic B cells in vitro. As TG-F1 mice aged, frequencies of peripheral B1 cells progressively increased, and the mice frequently developed B-cell chronic lymphocytic leukemia (B-CLL). They suggest that dysregulated, continuous high expression of IL-5 in SLE-prone mice may directly or indirectly mediate a skewed signaling of proliferation/differentiation of self-antigen-activated B1 cells, leading to suppression of autoimmune disease, but instead to aberrant expansion of B1 cells, giving rise to

B-CLL. Down regulation of Th1 cytokines in IL-5 transgenic mice is unclear.

(7) *IL-12-Expressing Plasmid DNA*. Hagiwara et al. [77] have reported the therapeutic effects of IL-12-encoding plasmid, depending on the concept that lupus is Th2 disease. Thus, they aimed paradigm shift from Th2 to Th1 polarized immune reaction for lupus therapy. MRL/MP-lpr/lpr mice were treated with IL-12-encoding plasmid DNA intramuscularly (every 4 weeks, starting at 4 weeks of age). This intervention significantly inhibited the accumulation of CD4(-)CD8(-) double negative T cells reducing lymphadenopathy and splenomegaly. A significant decrease in serum IgG anti-DNA autoantibody titers with a reduction in the proteinuria and glomerulonephritis. However, serum IFN- γ level was increased. They suggested that the cytokine balance was skewed towards Th1. However, they discussed the mechanism by this therapy is not totally dependent on an IFN- γ -mediated pathway. It may be that this plasmid also induced the production of regulatory cytokines that counter balanced inflammatory cytokines [78].

[2] *Elimination (Neutralization) of Th1 and Th1-Related Cytokines*

(1) *IFN- γ R/Fc-Expressing Plasmid DNA*. Ozmen et al. [79] have reported that mouse soluble IFN- γ receptor (IFN- γ R) prevented the onset of glomerulonephritis in B/WF₁ mice.

Lawson et al. [80] have attempted to eliminate (neutralize) IFN- γ in mouse lupus models using soluble recombinant IFN- γ R. Intramuscular injections of plasmids with cDNA encoding IFN- γ R/Fc can retard lupus development and progression in MRL-Fas(lpr) mice even though when this treatment was initiated at advanced stage. Therapy significantly reduced serum levels of IFN- γ as well as disease manifestations (autoantibodies, lymphoid hyperplasia, glomerulonephritis, and mortality), when treatment was initiated at the predisease stage. On the other hand, there were no changes in mRNA expression levels from splenic cells for IFN- γ , IL-2, IL-4, IL-10, IL-12, and TNF- α with the exception of twofold increase. This therapy makes application of this nonviral gene therapy in humans with lupus and perhaps other autoimmune/inflammatory conditions, since this method seems to be nontoxic and safe with long-term expression. They postulated that a major effect of IFN- γ blockade would be due to downregulation of MHC expression, although it is very difficult to define the exact mechanism(s) by which this cytokine, which has highly pleiotropic properties, promotes autoimmunity. In addition to elimination inflammatory cytokines such as IL-1 and TNF- α other than IFN- γ [80], administration of cDNA encoding soluble TNF- α receptors, or IL-1 receptor antagonist (IL-1ra), protects against lupus [12, 81].

(2) *IL-18 cDNA Vaccination*. Bossù et al. [82] have reported protective effects of IL-18 cDNA vaccination on lupus. Their idea elicited a specific anti-IL-18 antibody response by reducing the activity of endogenous IL-18, which is an inducer of IFN- γ in T lymphocytes and NK cells, in

MRL/Mp-Tnfrsf6lpr (lpr) mice closely resembling human SLE. Young lpr mice were vaccinated against autologous IL-18 by repeated intramuscular injection of a cDNA coding for the murine IL-18 precursor. Vaccinated mice produced autoantibodies in sera to murine IL-18, and they exhibited a significant reduction in spontaneous lymphoproliferation and IFN- γ production as well as less glomerulonephritis and renal damage. Moreover, mortality was significantly delayed in anti-IL-18-vaccinated mice. These studies support the notion that IL-18 plays a major role in the pathogenesis of the autoimmune syndrome of lpr mice and that a reduction in IL-18 activity could be a therapeutic strategy in autoimmune diseases.

However, the exact role of the IL-18 cDNA vaccination in prevention is unclear.

(3) *IL-12- and IL-18-Encoding Plasmids*. Given the facts that injection of recombinant IL-12 or IL-18 proteins in lpr mice worsened the lupus-like disease [83, 84], and elevated IL-12 and IL-18 serum levels were found in SLE patients [85, 86]. Neumann et al. [87] have reported that MRL/Mp-Tnfrsf6(lpr) (lpr) mice with increase in both TNF- α and IFN- γ serum concentrations in age, model for human SLE, were injected intramuscularly with plasmids encoding IL-12 and IL-18, either alone or in combination. Five biweekly injections of combined plasmids starting at 4-5 weeks of age diminished serum levels of TNF- α and reduced an ability of lymphocytes from treated mice to produce IFN- γ in vitro. Injection of both plasmids synergistically attenuated the development of autoimmune syndromes, lymphoproliferation in secondary lymphoid organs, proteinuria and kidney damage, and pneumonitis. Also, IL-12/IL-18 cDNA treatment reduced serum TNF- α concentrations. The constitutively enhanced serum TNF- α level in lpr mice decreased more efficiently by the injection of both cDNAs encoding IL-12 and IL-18 when compared with the single injection.

Lymph node cells from IL-12/IL-18-treated mice produced less IFN- γ after in vitro stimulation, and IL-12/IL-18 cDNA treatment does not affect anti-dsDNA IgG levels. Regarding the possibly reduced IFN- γ generation, the alterations might be due to either the specific elimination of IFN- γ producing T lymphocytes or NK cells from the whole-cell population, or due to their reduced capability to produce IFN- γ , if they persist.

They discussed the cDNA injection procedure presumably inducing anti-IL-12 and anti-IL-18 activities although direct evidences are not obtained. They concluded that IL-12 and IL-18 synergistically affect the pathogenesis of the Th1-dependent autoimmune syndrome of lpr mice and that both IL-12 and IL-18 may be a therapeutic option in the treatment of autoimmune SLE.

[3] *Suppression of Th1, Th2, and Other Immunocompetent Cells*

(1) *TGF- β -Expressing Plasmid DNA or IL-2-Expressing Plasmid DNA*. The therapy focusing on wide suppression of immunocompetent cell has been reported by Raz et al.

[88]. MRL/lpr/lpr mice monthly injected intramuscularly with plasmids cDNA expression vectors encoding for TGF β or IL-2. Injections (between 6 and 26 weeks) significantly elevated the serum levels of TGF- β and IL-2 compared with a control plasmid without insert. TGF- β encoding plasmid had beneficial effects in murine SLE with a prolonged survival of 70% at 26 weeks compared with 40% in the control group, decreased antichromatin and rheumatoid factor antibodies and a 50% decrease in total IgG production. Renal function was improved with reduced blood urine nitrogen (BUN) levels and kidney inflammation. Those beneficial effects occurred in apparent absence of local or systemic side effects. These effects by TGF- β -expressing plasmid DNA may be due to suppressed function of CD4(Th1,Th2), CD8 T,B and macrophages. In contrast, IL-2 cDNA injections appeared harmful with a decreased survival to 20% at 26 weeks (survival rate of nontreated control mice: 40 % at 26 weeks), enhanced total IgG synthesis and autoantibodies production with 4.5-fold increase in antichromatin antibodies. They concluded that somatic gene therapy may provide simple, inexpensive and effective therapies for the long-term control of autoimmune diseases by TGF- β -expressing plasmid DNA.

Huggins et al. [89] also investigated the effects of IL-2 and TGF- β gene therapy in MRL lpr/lpr mice with a different approach. The mice were treated orally with a nonpathogenic strain of Salmonella typhimurium bearing the aroA-aroD-mutations and carrying the murine genes encoding IL-2 and TGF- β . TGF- β gene therapy produced no improvement in pathology and generally had opposite effects to those of IL-2. IL-2 gene therapy restored the defective T-lymphocyte proliferative response to mitogen and suppressed the autoantibody response, nephritis, and growth of lymphoid tumours.

The reasons of these differences are unclear. Mageed and Prud'homme [78] suggested that it is unclear to what extent the cytokines enter lymphoid tissues and/or the circulation, and how immunological effects are mediated. Therefore, these results must be interpreted with caution, and are difficult to compare with those obtained by other methods of delivery. Thus, they claimed these contradictory results highlight the risks inherency in using cytokines as therapeutic molecules.

[4] *Normalization of T Cell Population*

(1) *IL-2/Vaccinia Recombinant Virus*. Gutierrez-Ramos et al. [90] tried lupus therapy in MRL/lpr mice by altering abnormal T-cell population to normal T-cell population, since the intrinsic abnormality of T cells in this mouse strain (defect in IL-2 production appears at 4-6 weeks of age in MRL lpr/lpr and BXS_B mice, and somewhat later in the B/WF₁ mouse and becomes more pronounced with disease progression [78]). Mice-vaccinated intraperitoneally with live vaccinia recombinant viruses expressing the human IL-2 gene showed prolonged survival, decreased autoantibody production and rheumatoid factor titres, marked attenuation of kidney interstitial infiltration and intraglomerular proliferation, as well as clearance of synovial mononuclear infiltrates. Inoculation with the IL-2/vaccinia recombinant virus led, additionally, to

drastic reduction of the CD3⁺ CD4⁻ CD8⁻ double-negative T-cell population, and improved thymic differentiation and restoration of normal values of mature cells in peripheral lymphoid organs.

In contrast, IL-2 treatment enhanced the disease as described in section [3]-(1). These opposite data suggest multifunction of IL-2 in vivo or methodological difference including disease process.

[5] *Costimulation Blockade*

(1) *Anti-CD154 (CD40L) Therapy.* Wang et al. [91] determined the immunologic effects of anti-CD154 (CD40L) therapy in the B/WF₁ mouse (20-week-old and 26-week-old) treated with continuous hamster anti-CD154 intraperitoneally biweekly for 6 months. IgG anti-dsDNA antibodies decreased during therapy and disease onset was delayed, but immune tolerance did not occur. During treatment, there was marked depletion of CD19⁺ cells in the spleen; however, autoreactive IgM-producing B cells could still be detected. In contrast, few IgG anti-dsDNA-secreting B cells were detected. Eight weeks after treatment cessation, the frequency of B cells producing IgG anti-dsDNA antibodies was still decreased in 50% of the mice, and activation and transition of T cells from the naive to the memory compartment were blocked. Anti-CD154 treatment blocked both class switching and somatic mutation by using the marker gene VHBW-16 and induced a variable period of relative unresponsiveness of IgG anti-dsDNA-producing B cells, as shown by decreased expression of the CD69 marker and failure to generate spontaneous IgG anti-dsDNA-producing hybridomas. Treated mice mounted an attenuated IgM response to the hapten oxazolone and produced no IgG antioxazolone antibodies.

Anti-CD154 is a B-cell depleting therapy that affects multiple B-cell subsets. Activation of both B and T cells is prevented during therapy. After treatment cessation, autoreactive B cells progress through a series of activation steps before they become fully competent antibody-producing cells. Wang et al. have stressed that the general immunosuppression induced during treatment will need to be taken into account when using B-cell depleting regimens in humans.

(2) *Adenovirus Vector Expressing CTLA-4/IgG.* Takiguchi et al. [92] reported that gene therapy strategy is to prevent the effector T-cell function by blocking costimulation, because involving Th1 and Th2 cytokines in lupus pathogenesis suggests the difficulty to target specific cytokines. They demonstrated protective effects of adenovirus vector containing CTLA4IgG, which binds to B7 molecules and inhibits B-cell differentiation to plasma cells, gene (Adex1CACTLA4IgG:AdCTLA4IgG) delivery on the development of lupus nephritis, in MRL/lpr mice. It was demonstrated that a single administration of intravenous injection of AdCTLA4IgG into MRL/lpr mice resulted in almost complete amelioration of lupus nephritis. However, side effects such as increased susceptibility to pathogen due to reduced general immune reaction by this treatment are unclear.

Mihara et al. [93] also reported that Ad-CTLA4Ig, which blocks the B7/CD28 pathway, prevents the onset of disease in B/WF₁ mice treated at the age of 20–22 weeks with a single intravenous injection of high dose (2×10^9 pfu). This effect is associated with decreased expansion of both the IgM and IgG autoreactive B-cell population, inhibition of immunoglobulin class switching, and a marked decrease in the numbers of activated CD4⁺ T cells. In contrast, intrinsic B-cell hyperreactivity and the survival of plasma cells in the bone marrow, both of which are less dependent on T-cell help, appear to be unaffected by CTLA4Ig. Administrated Ad-CTLA4Ig existed in the serum for a long time and an immune response to the adenoviral vector was absent. More importantly they showed that CTLA4Ig did not induce a permanent state of tolerance in B/WF₁ mice.

(3) *(AAV8)-CTLA-4/Ig and AAV8-CD40/Ig.* Ye et al. [94] investigated the potency of costimulatory blockade with adeno-associated virus- (AAV)-mediated gene transfer in prevention and reversal of lupus in a murine model. AAV vectors expressing CTLA-4Ig or CD40Ig were injected into intraperitoneally NZB/NZW mice. A single injection of AAV serotype 8 (AAV8)-CTLA-4Ig in neonatal B/WF₁ mice before the onset of lupus effectively delayed and inhibited autoantibody production, proteinuria, and kidney damage and prolonged their lifespan. In addition, coinjection of AAV8-CTLA-4Ig and AAV8-CD40Ig vectors into neonatal mice achieved the synergistic effect and the best efficacy. The preventive effects were attributed to the suppression of CD4⁺ T-cell activation and the transition from naive to memory T cells. Moreover, coinjection of these 2 vectors in adult mice reversed existing autoantibody levels, suppressed the development of proteinuria, and prolonged their lifespan. The therapeutic effects were found to be dependent on the vector dose. Moreover, long-term, high-level costimulatory blockade transgene expression does not completely suppress the host humoral immune response to KLH, a T-cell-dependent antigen. AAV-mediated long-term gene expression did not severely suppress the host humoral response to foreign antigen. They showed that delivery of costimulatory inhibitor transgenes by AAV vectors could prevent and reverse lupus in this murine model, suggesting the potential of AAV-mediated gene transfer as an alternative treatment for lupus, since it did not completely suppress the host humoral response to foreign antigens.

(4) *Adenovirus Vector Expressing PD-L1 Gene with Anti-B7h mAb.* Deliberate delivering of negative costimulation to halt unwanted T- and B-cell activation has been tested by Ding et al. [95], although blocking positive T-cell costimulation has shown effectiveness in preventing the onset of murine lupus as shown in sections [5]-(1) and [5]-(2). They focused on enhancing PD-1 signaling because unlike CTLA-4, PD-1 is more broadly induced on not only T, but also B and myeloid cells after activation [96]. PD-L1 (also called B7-H1) is one of the ligands of this novel immunoinhibitory receptor [97]. Engaging PD-1 by PD-L1 could inhibit the activation of both T and B cells [98, 99]. They constructed an adenovirus containing the full-length mouse PD-L1 gene

(Ad.PD-L1), and tested its efficacy alone and together with an anti-B7h (inducible costimulator ligand, B7 homologous protein (B7h) called as ICOS-L [100]) mAb to block ICOS, a new member of the CD28 family of costimulatory molecules that is induced on T cells after activation, and regulates T-cell-dependent humoral immune responses [101, 102], in preventing lupus nephritis in BXSB mice, which spontaneously develop an autoimmune syndrome characterized by hypergammaglobulinemia, autoantibody production, and development of fatal glomerulonephritis that closely resembles SLE in humans. This combined therapy in mice with intravenous injection dramatically delayed the onset of proteinuria, effectively inhibited IgG autoantibody production, and significantly reduced hypercellularity and deposition of IgG in glomeruli, resulting in almost complete amelioration of lupus nephritis in these animals. Their results indicate the therapeutic potential of simultaneous stimulation of PD-1-mediated pathway and blockade of ICOS-B7h costimulation in the prevention of human lupus nephritis, suggesting the benefits of shifting the balance of positive versus negative costimulation on effector T cells in ongoing autoimmune diseases.

[6] *Blys Blockade*

(1) *Adenovirus Vector Expressing Transmembrane Activator and CAML Interactor Receptor (TACI)*. Liu et al. [103] examined the effect of BlyS blockade in 3 animal models of lupus depending on the evidences that serum BlyS (also known as TALL-1, THANK, zTNF4, and BAFF) is increased in autoimmune diseases, both in animal models and in humans. Antibodies and lupus-like disease manifestations were examined in mice after administration of a single intravenous injection of an adenoviral construct for transmembrane activator and CAML interactor receptor (AdTACI) that produces high serum levels of TACI-Fc fusion protein. In C57BL/6 (B6) lpr/lpr mice (B6.lpr/lpr), which were used to model of autoimmunity in absence of severe disease, treatment of younger mice with AdTACI prevented the development of hypergammaglobulinemia. In contrast, use of AdTACI for BlyS blockade had only transient effects on the levels of IgG in normal B6 mice. AdTACI blocked the development of autoantibodies in younger B6.lpr/lpr mice and reversed the production of autoantibodies in older B6.lpr/lpr mice, and also reduced the numbers of splenic plasma cells. In MRL.lpr/lpr mice, which were used to examine disease manifestations, AdTACI reduced the extent of glomerulonephritis and proteinuria and improved survival, but had little effect on T-cell infiltration and interstitial nephritis. However, in BW/F₁ mice, AdTACI induced neutralizing anti-TACI antibodies and failed to reduce the numbers of B cells. BlyS blockade has little effect on IgG levels in normal mice, but reverses the production of spontaneously produced IgM and IgG autoantibodies in setting of established autoimmunity. Blockade of BlyS ameliorates B-cell-dependent disease manifestations even in the MRL.lpr/lpr model, but its effectiveness on autonomous T-cell aspects of the disease is limited. Moreover, its effectiveness is neutralized by anti-TACI antibodies when it is present.

These results provide a basis for understanding the potential effects of BlyS blockade in human disease.

[7] *Suppression of Nephritic Kidney Inflammation*

(1) *Adenovirus Vector Expressing T β -ExR*. Haviv et al. [104] have reported that systemic adenoviral (Ad) gene therapy for renal disorder in the context of blocking renal fibrosis by AdTbeta-ExR, which encodes a chimeric soluble molecule comprising the entire ectodomain of the human type II TGF- β receptor, genetically fused to the Fc fragment of the human IgG1 (sTbetaRII), and AdCAT β -TR, which encodes only the dominant-negative truncated ectodomain of the human type II TGF- β receptor, intramuscularly in MRL/MpJ^{Tnfrsf6lpr} mice, which are homozygous for the FAS-protein receptor (lpr, lymphoproliferation) spontaneous mutation (Tnfrsf6lpr) and develop systemic autoimmunity and ICs-mediated glomerulonephritis. This therapy focussed on suppression of kidney inflammation using recombinant adenoviral (Ad) vectors holding many advantages, such as infection of a wide range of quiescent renal cells and the potential for organ targeting employing genetic or antibody conjugation strategies TGF type II receptor (AdT-ExR) to block TGF- β , a cytokine playing a key role in fibroproliferative renal disorders and TGF- β , is a key regulator of production, degradation, and accumulation of extracellular matrix.

The onset of the autoimmune disease is at about 8 weeks of age and females die earlier than males, thereby resembling systemic lupus erythematosus-like autoimmune syndromes. sTbetaRII was detected in the glomeruli after remote i.m. injection of AdTbeta-ExR, but not the control AdCAT β -TR, indicating renal deposition of the heterologous soluble fusion protein after its expression in the muscle and secretion into the circulation. AdTbeta-ExR, but not AdCATbeta-TR, could transiently inhibit mesangial expansion, glomerular hypercellularity, proteinuria, and cortical interstitial fibrosis. However, amelioration of renal disease in CXCR3^{-/-} MRL/lpr mice therefore seems to be more a result of an impaired cellular immune response, rather than an impaired humoral immune response.

[II] *Balance Shift from Immunogenic to Tolerogenic Dendritic Cells (DCs)*

[1] *Immunogenic DCs Determine Lupus*. It is well known that viral infections, in general, trigger unabated production of IFN- α/β . SLE patients have long been known to display elevated levels of type I interferon (IFN α/β) in their serum [105], but there are a few attention on the role of IFN α/β compared to other cytokines in lupus pathogenesis. Pascual et al. [105] proposed in 2001 that an excess IFN α/β might break peripheral tolerance through the activation of myeloid dendritic cells (mDCs). And since then, accumulating evidences suggest causal roles of IFN α/β pathway (i.e., human plasma cell differentiation and activation of CD8 T cells) in human SLE.

It has also become evident that antigen-antibody complexes (ICs) containing RNA and DNA activate DCs and B

cells through interaction with Fc γ R and Toll-like receptor (TLRs) 7, 8, and 9, respectively [85]. Thus, lupus-specific autoantigens might act as TLR ligands and contribute to IFN α/β and autoantibody production.

Horwitz [106] proposed in his review that an important goal in the management of human SLE is to restore the DCs together with regulatory T(Treg) cells [107], since once the DC balance has been shifted back to tolerogenic predominance, further stimulation of these tolerogenic DCs with peptide autoantigens should expand or induce new inducible (i)Tregs.

(1) *Restoration of DCs and IFN α/β .* There are two main DC differentiation pathways [108]: mDCs and plasmacytoid DCs (pDCs) [109]. Resident mDCs that encounter bacteria at mucosal surfaces or at sites of tissue damage migrate to the lymph node via afferent lymphatics. Importantly, mDC express receptors, such as TLRs, nucleotide binding oligomerization domain (NOD) proteins, and lectins, to capture these pathogens. Mouse mDCs express TLR7 and TLR9 whereas human mDCs lack TLR7, but express TLR7 upon exposure to IFN- α/β [110]. mDCs can also be activated by immune complexes (ICs) through activating Fc γ RIIIa [111, 112].

Pascual et al. [105] have reviewed that increased IFN- α/β induces the activation of immature mDCs that control peripheral tolerance by deleting autoreactive lymphocytes. IFN- α/β secreted from matured DCs activate and expand autoreactive T cells, both helping autoreactive B-cell development directly, and IFN- α/β also indirectly allows the expansion and survival of CD4⁺ and CD8⁺ T cells as well as the differentiation of B cells into plasma cells. Additionally the capture of apoptotic cells by mDCs and nucleic acid-containing ICs by pDCs and autoreactive B cells broadens the autoimmune reaction, thereby leading to disease manifestations.

pDCs circulate in the blood and lymphoid organs and by viral infection, these cells secrete large amounts of IFN- α/β as well as other cytokines [109]. They also differentiate into cells with DC morphology and function [113]. Both mouse and human pDCs express TLR7 and TLR9 and chromatin-containing and snRNPs-containing ICs are internalized by pDCs via Fc γ RIIa and reach the endosomal compartment where they activate TLR7 and TLR, respectively, leading to secretion of cytokines such as IFN- α/β [26]. (The classic lupus autoantigens snRNPs can also be directly internalized within endosomes where they stimulate TLR7 and TLR8 [114].) Banchereau and Pascual [26] have demonstrated that ICs are formed when autoantibodies are engaged with either (1) chromatin whose DNA can interact with TLR9 inside pDC and B-cell endosomes or (2) ribonucleoproteins whose RNA can interact with TLR7 also inside pDC and B-cell endosomes. TLR signaling in pDC results in the secretion of IFN- α/β and IL-6. The combined triggering of the autoreactive BCR and TLR results in autoreactive B cell proliferation, and IFN- α/β induces their differentiation into plasmablasts, and IL6 induces their differentiation into autoantibody-secreting plasma cells.

Mathian et al. [9] confirmed the critical role of IFN- α/β in SLE pathogenesis. In vivo delivery of IFN- α/β to preautoimmune B/WF₁ mice rapidly results in severe SLE. dsDNA antibodies appear as early as 10 days after initiation of IFN- α treatment, demonstrating a critical role for IFN- α in the selection and expansion of autoreactive clones. Proteinuria and glomerulonephritis-induced death occurred in all treated mice at 9 and 18 weeks, respectively, a time when untreated mice did not show any sign of disease. Banchereau and Pascual [26] reported that the cytokines yield distinct DC subsets that might lead to distinct immunopathologies. Immature DCs exposed to IFN- α/β or TNF- α will yield distinct DC subsets that will uniquely polarize naive T cells through the expression of different cytokines and costimulatory molecules. They proposed the new concept that mutual inhibitory effects of TNF- α /IFN- α/β , which determine the direction of diseases like the mutual inhibitory effects of Th1/Th2, which is proposed by concept of Monneaux and Muller [5]. For example, excess TNF- α production from immature DCs inhibits IFN- α/β production from immature DCs, leading to inhibition of the development of lupus. On the other hand, IFN- α/β polarized immunity lead to lupus, by suppressing the production of TNF- α .

(2) *Prevention by TNF.* Gill et al. [115] found high amounts of soluble TNF- α receptors and IFN- α/β found in SLE patients. Jacob and McDevit [116] observed that soluble bioactive TNF- α prevent lupus in B/WF₁ mice, which bear a genetic deficiency in TNF- α . These evidences suggest that TNF-mediated downregulation of the IFN- α/β pathway in therapy. However, the application of TNF- α remains unclear, since TNF-mediated downregulation of the IFN- α/β pathway is largely unknown.

[2] *Treg Cells and DCs*

(1) *Regulatory T(Treg) Cells.* Horwitz [106] proposed in his review that restoration of the balance between immunogenic and tolerogenic DCs with correction of Treg numbers and function is an important goal in the management of human SLE, although still unsolved issues remain.

Toubi [117] reported that CD4⁺CD25⁺ Treg cells play a role in autoimmune diseases in human. Restoration of regulatory T(Treg) cell numbers and functions in individuals with SLE has the potential to lead to remission of SLE by shifting back to tolerogenic predominance in vivo [106]. This new strategy to normalize Treg function is expected to be ideal therapy not only in SLE but also other autoimmune disease including allergic diseases, which are typical Th2 diseases, although it is not conclusive [118]. Depletion of CD4⁺CD25⁺ cells in B/WF₁ hybrid mice accelerated the onset of glomerulonephritis [119] and sialoadenitis [120].

CD4⁺CD25⁺ natural or thymus-derived CD4⁺CD25⁺Foxp3⁺ cells are decreased in young B/WF₁ and/or SNF₁ mice before they develop glomerulonephritis and adoptive transfer of CD4⁺CD25⁺ cells from young lupus-prone mice have some protective effects on the development of the disease [121].

However, the evidence that Treg defects play a major role in the perpetuation of this disease is only suggestive in human [106]. Also, regulatory/suppressor T cells (Tregs) consisted of heterogeneous populations of CD4 cells, CD8 cells, and even natural killer T cells [122], and approximately 5% of CD4⁺ cells in the mouse are Tregs that express Foxp3, whereas only 1%-2% of the total CD4⁺ T-cell population of CD4⁺ cells express Foxp3 in humans [106, 123]. Thus Horwitz [106] pointed out that the cytokine and gene expression profile of Foxp3⁺ Tregs in SLE in comparison with healthy subjects has yet to be defined. Also, studies of Tregs in SLE are mostly limited to blood lymphocytes and there are controversy evidences that decrease in CD4⁺CD25 high cells in some reports whereas there are no decrease in those cells.

Moreover, IL-2 and TGF- β can convert naïve CD4⁺CD25⁻ T cells to CD25⁺Foxp3⁺ suppressor cells in mice, and Tregs-induced ex vivo with IL-2 and TGF- β have protective lupus-like syndromes in mice [22, 124], but similar conversion in humans is more complex and another problem with measurements of Tregs in humans is that the numbers circulating in the blood may not correlate with the numbers and function of these cells in the tissues, and there is very limited information concerning Foxp3⁺ Treg numbers in lymphoid organs and in tissues of patients with SLE [106].

In both mice and humans, CD4⁺CD25⁺Foxp3⁺ T cells (iTreg) themselves differentiate into Th17 cells in the presence of IL-6 (and in the absence of exogenous TGF- β), indicating that CD4⁺CD25⁺Foxp3⁺ regulatory T cells can function as inducers of Th17 cells and can differentiate into Th17 cells in both mice and human [125, 126]. Zheng et al. [124] found that IL-2 and TGF- β downregulate IL-6 receptor expression and IL-6 signaling. The resistance of iTregs to Th17 conversion suggests that they can function more effectively than nTregs in the central role of IL-2 in combination with TGF- β to maintain immunologic homeostasis in autoimmune diseases [127]. However, this has yet to be demonstrated in human [106]. Moreover, the principal target of Treg activity is probably antigen-presenting DCs rather than T cells [128, 129]. In addition, Tregs may have direct suppressive effects on B cells [130], on natural killer cells [131] in mice. The contact-dependent mechanism of action of CD4⁺Foxp3⁺ Tregs also remains poorly understood [31].

(2) *Interactions between Tolerogenic DCs and Treg Cells.* Min et al. [132] demonstrated that besides controlling the activity of other T cells and B cells, evidence has been obtained that Tregs also induce tolerogenic DCs in transplant tolerance in the C57BL/6 > BALB/c cardiac allograft model using simultaneously targeting T cell and DC function using anti-CD45RB mAb (CD45 is a single chain glycoprotein expressed on hemopoietic cells such as T cells, and members of the CD45 family of transmembrane protein tyrosine phosphatases are critically involved in lymphocyte activation.) and LF 15-0195, a novel analog of the antirejection drug 15-deoxyspergualin, respectively. Moteober, LF inhibits DC and anti-CD45RB mAb inhibits T-cell responses in vitro. They concluded that tolerance induction is associated with

a self-maintaining regulatory loop in which tolerogenic DCs induce the generation of Treg cells from naïve T cells and Treg cells programs the generation of tolerogenic DCs from DCs progenitors.

(3) *Tolerogenic Peptide.* Kang et al. [27] reported that splenic dendritic cells (DCs) captured the subcutaneously injected low-dose peptide (three times with H4 (71–94) peptide (1 μ g/mouse) in every 2 wk.), which is autoepitope for lupus nephritis-inducing Th cells in murine and human lupus and shows cross-reactively recognized by autoimmune Th cells and B cells, rapidly and expressed a tolerogenic phenotype in lupus-prone 12-week-old female SNF₁ hybrids but prenephritic. The DC of the tolerized animal, especially pDC, produced increased amounts of TGF- β , but diminished IL-6 on stimulation via the TLR-9 pathway by nucleosome autoantigen and other ligands; those pDC blocked lupus autoimmune disease by simultaneously inducing autoantigen-specific Treg and suppressing inflammatory Th17 cells that infiltrated the kidneys of untreated lupus mice. Thus, the H4 (71–94) peptide tolerance therapy that preferentially targets pathogenic autoimmune cells could spare lupus patients from chronically receiving toxic agents or global immunosuppressants and maintain remission by restoring autoantigen-specific Treg cells.

2. Future of Balance Shift of Th1 (IFN- γ)/Th2 (IL-4) and Immunogenic DCs (IFN- α/β)/Tolerogenic DCs (TNF- α)

There are two main streams of this review in terms of balance shift from [I] Th1 to Th2 cells and from [II] immunogenic (IFN- α/β) to tolerogenic (TNF- α) DCs, but the former seems to be more realistic.

[I] *Therapy by Th1/Th2 Balance Shift for Clinical Use.* Main issues of this review is balance shift from Th1 to Th2 or alteration of Th1/Th2 ratio by targeting IFN- γ , but not costimulation blockade. For this purpose, gene therapy seems to be suitable compared to bioactive cytokines or antibodies against cytokines.

The number of naked/plasmid DNA (18.3%), adenovirus (24.9%) and retrovirus (21.7%) studies has dramatically increased among all gene therapy in clinical trials (www.wiley.co.uk/genmed/clinical, 2007) [133]. Additionally, the coverage of indications targeted in preclinical and clinical studies using plasmid DNA is broad-covering cancer, infectious and noninfectious diseases in both the prophylactic vaccine setting as well as a therapeutic setting. The nonviral plasmid DNA approach is particularly attractive due to safety, versatility, and ease of preparation [88]. Also, compared to viral vectors, it has the benefit of not inducing immunogenic reactions against components of the vector itself upon administration to patients [134]. This process can be adapted by other academic centers for human or large animal clinical trials with cost-effectiveness [135].

Gene transfer offers numerous advantages for the in vivo delivery of cytokines or their receptors for immunotherapeutic use [136–138]. Actually, there are a lot of trial

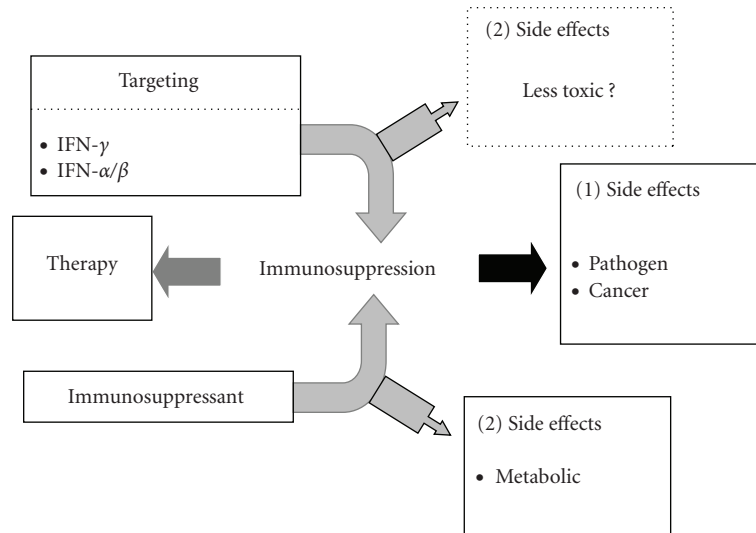


FIGURE 5: Cytokine targeted therapy will be expected to clinical application, but more detailed examination of side effects (e.g., development of infections, cancer, allergic diseases, and autoimmunity) is required. Clinical application to lupus must be based on their safety profile, adverse effects, risks, and advantages. Likewise, in this analysis we offer specific recommendations, based on evidence, for the best, is Th1/TH2 balance shift in vivo by using IL-4 gene therapy.

of gene therapy in other autoimmune diseases (e.g., T1D, rheumatoid arthritis: RA) in model animals and in human with RA [139]. The first human trial for RA began in 1996 [140]. Clinical trials for RA have been initiated (phase II). The first evidence of possible clinical responses to gene transfer was published in 2008 [141]. Plasmid DNA is superior to viral vectors for direct gene transfer into adult mouse skeletal muscle [142].

Still in the experimental phase, gene therapy in lupus promises to correct the aberrant immunological response without the numerous side effects of the currently used immunosuppressant medications in lupus [143] (Figure 5 and Table 1). Some study indicated undesirable side effects such as the impaired response to T-cell-dependent and T-cell-independent antigens, increased susceptibility for infectious diseases. In addition, IL-5 suppresses lupus nephritis, but it promotes B-cell lymphoma later. Moreover, IL-4 and IL-5 may have a possibility to increase susceptibility to Th2 diseases (e.g., allergic rhinitis, asthma). Thus, despite its promise, gene therapy is a young field and a variety of questions must be addressed in lupus [78]. These points are needed to clarify.

[II] *Therapy by Induction of Tolerogenic DCs for Clinical Use.* Bancheureau and Pascual [26] have proposed an extension of the Th1-Th2 (inflammation-allergy) paradigm [110], which integrates autoimmune responses, by two sets of opposite vectors, that is, TNF- α -IFN- α/β and IL-4-IFN- γ . The sum of the cytokines yields an equilibrium point, which allows protective immunity when cytokines are equal. This dynamic system can accommodate the prevalence of either cytokine to a certain extent. However, when one of the cytokines prevails beyond a certain threshold, the equilibrium point moves into a zone of immunopathology, such as autoimmunity,

allergy, or inflammation. Thus, when the TNF prevails, TNF- α -mediated autoimmunity such as arthritis will occur. When the IFN- α/β vector prevails, IFN autoimmunity such as SLE will occur. This might be due to increased bioavailability of one cytokine or decreased bioavailability of the other one. The unabated production of a given cytokine will affect the cells of the immune system and most particularly DCs. Thus, IFN- α/β -stimulated DCs will polarize naive T cells differently than TNF-stimulated DCs.

Also, Bancheureau and Pascual [26] claimed that the basic genetic alterations leading to IFN- α/β overproduction and B cell tolerance breakdown in SLE and establishment of the role of other cells like neutrophils, TFh cells, and Tr1 cells in the pathogenesis of human SLE for new avenues of treatment. Whether, like TNF antagonists, which have brought considerable relief to RA patients, IFN antagonists will bring relief to SLE patient. Blocking of IFN α/β (antagonists, balance shift by TNF) including their side effects and safety is largely unknown.

3. Conclusions

Simple, effective, easy and safe methods with less side effects in vivo may be required for preventive intervention.

- (1) Cytokine-targeted gene therapy can be applied to clinical use as suggested by Piccirillo and Prud'homme [71]. Also this seems to be realistic rather than targeting proinflammatory versus immunosuppressive cytokine profiles. Naked DNA method may be suitable for therapy as shown in Table 1.
- (2) Prud'homme suggested that genes encoding TGF- β 1, IL-4, and IL-10 are most frequently protective in lupus [12, 81], but IL-4 expressing plasmid DNA may

TABLE 1: Several gene therapies in lupus model mice.

	Route	Vector	Model	Effect	mechanism/(evidence) (Reference)
<i>Cytokine</i>					
IL-4	i.p	naked DNA	B/WF ₁	+	Balance shift from Th1 to Th2 [68]
IL-2	i.m.	naked DNA	MRL lpr/lpr	–*	Enhanced autoantibodies production [88]
IL-2	oral	naked DNA	MRL lpr/lpr	+	Restoration of T cell response, and suppression of autoantibody response [89]
IL-2	i.p.	vaccinia virus	MRL lpr/lpr	+	Normalization of T cell population [90]
IL-12	i.m.	naked DNA	MRL lpr/lpr	+	Cytokine balance skewed towards Th1 [77]
TGF- β 1	i.m.	naked DNA	MRL lpr/lpr	+	Suppression of function of CD4 (Th1,Th2), CD8 T, B and macrophages. [88]
TGF- β 1	oral	naked DNA	MRL lpr/lpr	–	No effect [89]
IL-18	i.m.	naked DNA	MRL lpr/lpr	+	Elimination of IL-18 by induction of auto-anti-IL-18 antibody [82]
IL-12/IL-18	i.m.	naked DNA	MRL lpr/lpr	+	Presumably elimination of IL-12/IL-18 induction of anti-IL-12/IL-18 antibodies [87]
<i>Blockade</i>					
IFN- γ R/Fc*	i.m.	naked DNA	MRL lpr/lpr	+	IFN- γ blockade [80]
CTLA-4/IgG	i.m.	adenovirus	MRL lpr/lpr	+	costimulatory blockade [92]
CTLA-4/Ig	i.v.	adenovirus	B/WF ₁	+	costimulatory blockade [93]
CTLA-4/CD40	i.m.	AAV	B/WF ₁	+	costimulatory blockade [94]
PD-L1/anti-B7h	i.m.	adenovirus	BXSB	+	Negative costimulatory blockade [95]
TACI	i.v.	adenovirus	B6.lpr/lpr B/WF ₁ MRL lpr/lpr	– ± ±	Blys blockade [103]
T β -ExR	i.v.	adenovirus	MRL/MpJ ^{Tnfrsf6lpr}	+	TGF- β blockade [104]

*worsen, \pm : less effective, AAV: adeno-associated virus.

be advantageous compared to other two cytokines as discussed in each section. Also, the major activity of IL-18 is the induction of Th1 cells in the presence of IL-12, and IL-12 without IL-18 has an independent pathway to induce Th1 response [144], suggesting complexity of targeting those cytokines. Blocking of costimulation in antigen presenting process and elimination of B cells including IL-6 are effective, but those therapies may severely compromise immune responsiveness in general.

(3) Van Raalte et al. [4] have reported that despite excellent efficacy in terms of immunosuppression, glucocorticoid (GC) therapy is hampered by their notorious metabolic side effect profile. Thus, current trials to segregate GC's anti-inflammatory and metabolic actions, are currently being developed. Actually, they have reported that despite excellent efficacy in terms of immunosuppression, glucocorticoid therapy is hampered by their notorious metabolic side effect profile. Thus, current trials to segregate GC's anti-inflammatory and metabolic actions, are currently being developed. On the other hand, compared to glucocorticoids, cytokine targeted therapy has some merit, since side effects such as increased metabolic action may be less in cytokine therapy. Combination of the use of immunosuppressants and cytokine targeted therapy also seems likely.

(4) Balance shift from immunogenic to tolerogenic DCs by targeting IFN α/β together with normalization of Treg cell function in individuals with SLE has the potential to lead to remission of this autoimmune disease. However, there are a few information about the IFN α/β therapy and complicated interaction of DCs and Treg cells for balance shift. Moreover, the information of side effects is totally lacking. Thus, further studies are needed to accumulate the information for therapy.

(5) Finally, for the clinical application, we have to remind that the data obtained from model mouse are not simply applicable for human with SLE (species difference). For example, Horwitz [106] has stressed to distinguish human lupus from mouse lupus, since in mice the course of the disease is steadily downhill and fatal whereas human disease is cyclic and characterized by exacerbations and remission. In addition, several murine lupus models other than B/WF₁ exist and they can be divided into spontaneous, congenic, and engineered models as follows [145]. The best known spontaneous models arise on New Zealand Black (NZB), New Zealand White (NZW), MRL, BXSB, and SWR backgrounds. Hybrids of some of these background strains, like the NZB/W F₁, NZM2410, and the SWR/NZB F₁ develop ANA, glomerulonephritis, and other features

of human disease. The *lpr* (Fas) or *gld* (FasL) mutations on the MRL background give rise to mice with features of human lupus. However, degree of lymphoproliferation that occurs in these mice is not found in humans. Additionally, MRL-*lpr/lpr* mice spontaneously develop arthritis, and it displays more features of RA than of the arthropathy seen in human SLE. Conversely, humans carrying mutations in the Fas/FasL genes do not develop SLE. The *Yaa* mutation, which accelerates disease on the BXSB background, is due to a translocation of the TLR7 gene into the Y chromosome, explaining the predominantly male predisposition to disease in this particular model [146, 147], which is at striking difference with the 9 : 1 female to male ratio in human SLE.

Acknowledgments

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