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Induction of mucosal tolerance in SLE: A sniff or a sip away from ameliorating lupus?

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by aberrant immune responses against intracellularly derived self antigens. Treatment for SLE relies on the use of aggressive immunosuppressants and steroids that are nonspecific and can cause serious adverse effects. The observation that a systemic immune tolerance to self antigens or generation of regulatory T cells may follow mucosal (nasal or oral) exposure to self proteins or monoclonal antibody against CD3 respectively suggests that induction of mucosal tolerance offers the basis of a side effect free therapy that could re-establish the ability to distinguish self from non-self and restore peripheral tolerance in individuals susceptible to developing autoimmune diseases. Here I review studies on mucosal tolerance in autoimmune diseases and discuss the therapeutic potential of inducing tolerance for the treatment of SLE.

Introduction

The primary and most worrying problem with all existing treatments for autoimmune diseases is specificity. The nonspecific nature of the treatments compromises normal immune surveillance and hampers protective immunity. As a result patients are often vulnerable to opportunistic infectious agents that can cause serious complications. This problem associated with treatment is most challenging in systemic lupus erythematosus (SLE). Lupus, as it is sometimes referred to, is a chronic autoimmune syndrome that is characterized by a destruction of tissues and organs such as joints, kidneys, heart, lungs, brain, skin, and blood vessels by the very immune system that is designed to protect them. Disease pathogenesis is a result of a cognate interaction between T and B cells that recognize intracellularly derived self antigens [1]. Autoreactive T and B cells mediate inflammation and/or direct tissue damage by secreting inflammatory cytokines and anti-nuclear autoantibodies respectively [2]. The fact that many vital organs may be targeted in lupus has led to the use of powerful immune suppressive or modulating drugs in disease treatment. Thus there is a real sense of urgency for development of new therapies that can be given over long periods without causing global immune malfunction to treat lupus.

The observation that a systemic immune hyporesponsiveness or tolerance to a protein may follow mucosal (nasal or oral) exposure to the protein has led to a surge of excitement in the immunology community devoted to finding an effective treatment for autoimmune diseases. As induction of mucosal tolerance to self-antigens associated with autoimmune diseases could

re-establish the ability to distinguish self from non-self in individuals susceptible to developing autoimmune diseases, it offers the basis of a side effect free therapy that could potentially replace current nonspecific immunosuppressive drugs [3]. Virtually all manifestations of specific immune responsiveness tested can be suppressed by different regimens of mucosal antigen administration. This includes *in vivo* responses such as formation of Ig of different isotypes, [4,5] delayed hypersensitivity reactions [6,7], and changes in the rate of antigen clearance from the circulation [8], as well as *in vitro* assays such as specific plaque forming cells [9,10], lymphocyte proliferation [10–13], and cytokine production except for IL-10 and TGF-β [14–21].

The immunological mechanisms of mucosal tolerance

Three independent mechanisms behind mucosal tolerance have been put forward: firstly, ignorance of the antigen by the immune system (anergy); secondly, deletion of T cells that respond to the inhaled or ingested antigen; thirdly, generation of regulatory T cells that control and/or down modulate the inflammatory response against the antigen. Since identification of these mechanisms, evidence has been accumulating that suggest the three forms of tolerance are not mutually exclusive and there are considerable overlaps. One finding among others that could link these apparently distinct mechanisms is the secretion of the regulatory cytokine TGF-β that can be induced by treating T cells with anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) antibody, despite the fact that CTLA-4 was first described as being involved in the induction of 'anergy' *in vivo* [22,23]. Other studies also describe them as anergic [24–26]. The primary factor that determines which form of tolerance develops following mucosal administration of antigen is the dose of antigen given. Low doses of antigen favors the generation of regulatory T cell-driven tolerance whereas high doses of antigen favor deletion or anergy-driven tolerance [7,27].

Regulatory T cells and mucosal tolerance-associated cytokine network

Regulatory T cells were first described in the 1970s when they were considered to be mainly CD8+ and were referred to as suppressor T cells. More recently there has been a surge of research activity aimed at elucidating the phenotype(s) and function(s) of regulatory T cells in various areas of immunology. The naturally occurring, thymus derived regulatory T cells that are positive for CD4 and CD25 surface expression have generated the highest level of interest amongst immunologists. CD4+CD25+ regulatory T cells play a major role in the maintenance of self-tolerance and the control of various autoimmune diseases [28,29]. They are also involved in the regulation of T cell homeostasis [30,31] and in the modulation of immune responses to allergens [32], cancer cells [33,34], and pathogens [35,36]. Initially reported by Nishizuka [37,38] and further explored by Sakaguchi [39–41] in studies on animals thymectomized as neonates showed that autoimmune pathology, characterized by gastritis, oophoritis and orquitis arise as a result of the ablation of a subpopulation of thymic T cells. These cells are able to restore immunoregulatory function in disease mice upon adoptive transfer. The expression of a high affinity receptor for IL-2 on the cell surface (CD25) is required for the function of these regulatory T cells, although it is not clear whether IL-2 acts as a peripheral differentiation factor or an expansion factor [42,43] or it is directly involved in their function [44,45]. Recently other markers such as glucocorticoid-induced tumor necrosis factor (TNF) receptor [46,47] and the nuclear transcription factor foxhead/winged helix 3 (foxp3) have been found in CD4+CD25+ regulatory T cells [48–50]. Conversion of naïve T cells to CD4+CD25+ regulatory T cells can be achieved by stimulation via the T cell receptor and ligand-activated transcription factor aryl hydrocarbon receptor (AHR) which directly interacts with target sequences on the foxp3 gene and upregulates its expression [51]. CD4+CD25+ regulatory T cells are anergic, have suppressive properties that work in a cell contact dependent fashion. Further studies have demonstrated that foxp3 physically interacts with AML1 (acute myeloid leukaemia 1)/Runx1

(Runt-related transcription factor 1), a transcription factor crucially required for normal haematopoiesis including thymic T-cell development, activates IL-2 and IFN-γ gene expression in conventional CD4+ T cells through binding to their respective promoters, and this interaction suppresses IL-2 and IFN-γ production, upregulates regulatory T cell-associated molecules and results in suppressive activity [52]. A number of studies in lupus prone animals [53,54] and SLE patients [55–58] have demonstrated a significant reduction in number and function of these naturally occurring foxp3+CD4+CD25+ regulatory T cells and adoptive transfer of *ex vivo* expanded regulatory T cells reduced the incidence of glomerulonephritis and prolonged survival in mice with established lupus [59]. Induction of nasal tolerance using a histone peptide expressing a dominant T cell epitope in histone H4 protein of the nucleosome [60] was shown to have a positive effect on the number of the CD4+CD25+ regulatory T cells [53]. Furthermore, suppression of disease was demonstrated following injection of the peptide in mice and disease protection was associated with an upregulation of CD4+CD25+ regulatory T cells that produced high levels of TGF-β and exerted suppression in a partially cell contact dependent fashion [61,62]. Upregulation of regulatory T cell activity and TGF-β production leading to suppression of autoantibody production and disease development in lupus prone mice can also be achieved by injection of consensus peptides based on V(H) regions [63,64] or CDR1 regions of anti-dsDNA autoantibody [65,66].

As an important part of maintenance of peripheral tolerance other types of regulatory T cells (Th3, Tr1 and CD4+CD25−LAP+ cells) can be triggered by nasal or oral administration of antigen. These cells mediate their suppressive function by secreting anti-inflammatory cytokines IL-10 and/or TGF-β. They are referred to as ‘acquired’ regulatory T cells as opposed to the naturally occurring or ‘innate’ thymus derived CD4+CD25+ regulatory T cells [67]. The relationship between the two classes of regulatory T cells is largely unknown however, in studies of colitis induced in mice by adoptive transfer of $CD45RB^{high}$ T cells it was shown that disease could be suppressed by $CD4+CD25+CD45RB^{low}$ T cells with regulatory properties that resembled both the adoptive and innate population in that suppression was mediated via secretion of IL-10 and TGF-β [68,69]. Moreover, TGF-β has been shown to contribute to the development and expansion of CD4+CD25+ regulatory T cells [70–72].

Initially the regulatory activity of CD4+ T cells was associated with an upregulation of Th2 type cytokines IL-10 and IL-4 and a suppression of Th1 type cytokines IL-2 and IFN-γ [14, 73–78]. Suppression of Th1 type immune responses is still a widely used indication of induction of mucosal tolerance [79–81] and Th2 regulatory T cells generated by mucosal (nasal or oral) administration of antigen have been shown to suppress experimental allergic encephalomyelitis, EAE [82] and diabetes [83] in mice. IL-10 is a Th2 cytokine with potent anti-inflammatory properties. A variety of cell types produce IL-10 but it is produced at exceptionally high levels by CD4+ regulatory T cells (Tr1 cells) [69]. Production of high levels of IL-10 by regulatory T cells is more often seen with nasal tolerance rather then oral tolerance induction. In fact neutralization of IL-10 does not abrogate oral tolerance induction nor block established tolerance *in vivo* [84]. Our recent studies on mucosal administration of anti-CD3 monoclonal antibody in mice with EAE and mice that develop lupus spontaneously demonstrated that delivery of the antibody orally induced CD4+CD25−LAP+ regulatory T cells that suppressed EAE and lupus (Wu *et al* unpublished) in a TGF-β dependent fashion without the need for IL-10 [21]. However, nasal administration of anti-CD3 suppressed lupus before and after disease onset by inducing CD4+CD25−LAP+ regulatory T cells that suppressed the function of CD4+ICOS+CXCR5+ follicular helper T cells thereby inhibiting helper T and B cell interaction leading to downregulation of plasma cell formation and autoantibody production. *In vitro* suppression was IL-10 dependent and both IL-10 and TGF-β are required for *in vivo* suppression by the LAP+ regulatory T cells (Wu *et al* in press). In an elegant study by Akbari *et al* it was shown that dendritic cells (DCs) are at the heart of immunological tolerance and pulmonary DCs isolated after nasal administration of allergen

produced large quantities of IL-10 which was required for nasal tolerance induction [85]. On the contrary, DCs isolated from mesenteric lymph nodes of the gut following antigen feeding express increasing amounts of TGF-β and enhanced production of TGF-β by CD4+ cells, a phenotype consistent with Th3 regulatory T cells [86]. Thus it appears that the mucosal immune system has a unique immunological milieu that is based on two tolerance inducing cytokines, IL-10 and TGF-β and the milieu acts, in part, via the DCs to induce different phenotypes of regulatory T cells (Diagram 1).

As mucosal tolerance has been usually defined in terms of Th1 responses, anything that suppress Th1 and/or enhance regulatory T cell induction and Th2 responses would enhance mucosal tolerance. Th3 cells appear to use IL-4 and TGF-β for growth and differentiation. It has been shown that oral administration of IL-4 and antigen enhanced oral tolerance induction to the antigen [87]. In the arthritis model, administration of TGF-β intraperitoneally enhanced the induction of oral tolerance to collagen II even after the onset of disease [88]. Large doses of IFN-γ given intraperitoneally abrogate oral tolerance induction [89] and anti-IL-12 enhanced oral tolerance and upregulated TGF-β production [90]. In addition, subcutaneous administration of IL-12 prevents the induction of oral tolerance [91]. Oral IFN-γ and IFN-t synergize with the induction of oral tolerance in mice fed low doses of myelin basic protein (MBP) [92–94]. Nasal administration of cytokines also enhances tolerance induction and regulatory T cell differentiation. Nasal but not subcutaneous administration of IL-10 suppressed clinical signs of EAE in Lewis rats and prevented the development and relapse of protracted-relapsing EAE in rats [95]. Co-administration of IL-10 and antigen reduced proliferative responses and IFN-γ production, increased IL-10 production by T cells and enhanced protection from EAE compared to antigen alone [96]. Nasal administration of minute amounts of IFN-γ and acetylcholine receptor (AChR) reversed tolerance to AchR and inhibited protection from autoimmune myasthenia gravis (EAMG) by AchR administration alone in rats [97]. Suppression of IFN-γ production in response to systemic challenge is almost a universal finding in mucosally induced tolerance. However, some reports have shown that induction of tolerance is preceded by priming of antigen-specific IFN-γ producing cells [74,90,98–101]. Furthermore, studies that support a regulatory role of T cells bearing γδ TCRs in mucosal tolerance [102–104] have shown that suppression of IgE antibody production following nasal administration of proteins appears to be mediated by IFN-γ [105]. In addition, there are reports that oral tolerance cannot be induced in IFN-γ-deficient mice [106] or in adoptively transferred TCR transgenic T cells on the IFN-γ-deficient background [107]. Studies in children with peanut allergies showed that tolerance to peanuts was associated with a Th1-skewed response to peanuts and Th2 responses were only observed in allergic children. Peanut reactive T cell clones from orally tolerized children produce high levels of IFN-γ and TNF-α, suggesting that oral tolerance to Th2 type of inflammatory responses, such as in allergic reactions, may be accomplished by immune deviation toward Th1 responses [108]. Thus the role of Th1 cytokines, particularly IFN-γ, in the induction and mechanisms of mucosal tolerance needs to be further investigated.

Several factors were determinant in the growing interest on the role of CD4+ T cells in mucosal tolerance. Namely, removal of CD4+ T cells at the time of oral administration of Ovalbumin (OVA), prevented the induction of tolerance to a subsequent challenge with OVA [109,110] and CD4 T cell deficient animals failed to become tolerized to contact sensitizing agents [111]. Apart from this growing interest in CD4+ T cells however, the original reports on oral tolerance have suggested that CD8+ T cells might be the suppressor cells involved in its induction [6,9,76,112,113]. Later studies showed that feeding of Lewis rats with MBP induced TGF-β producing CD8+ regulatory T cells that suppressed EAE upon adoptive transfer [114]. Further evidence that CD8+ T cells may participate in oral tolerance comes from studies showing that a population of CD8+ regulatory T cells that produce IL-4 or IL-10 can be primed by antigen feeding, even when CD8+ CTLs are tolerized [103,115]. One of the questions regarding the relationship between CD8+ regulatory T cells and mucosal tolerance is how such cells could

recognize mucosally administered exogenous antigen. A conceivable mechanism is 'cross-presentation'. Several reports have demonstrated that soluble molecules presented by APCs, specifically DCs, can leak into the major histocompatibility complex (MHC) class I pathway and be presented to CD8+ T cells [116,117]. Alternatively, priming of CD8+ T cells with regulatory properties in the gut can occur via presentation of fed antigens by mucosal DCs in the context of a MHC Ib molecule Qa-1 that is able to recognize self antigens and bacterial heat shock proteins [118,119]. The role of CD8+ T cells has also been examined using genetically engineered CD8 deficient mice [120–122] and in mice treated with anti-CD8 antibodies [109, 110,123]. In all the studies, oral tolerance was induced normally suggesting that there is no absolute requirement for CD8+ T cell in the induction or maintenance of systemic tolerance. Nevertheless, whether these cells contribute to individual aspects of the tolerant state or play discrete roles in different tissues, such as the mucosa itself, remains to be elucidated. In addition to CD8+ regulatory T cells, there have been three reports that suggest NK T cells can transfer tolerance following feeding of haptenized colonic proteins [124,125] or allo-antigens [126]. However, other workers have shown normal oral tolerance in mice lacking NK T cells due to a genetic deficiency in Jα 281 component of the invariant TCR found on most of these cells [127]. Taken together, these results indicate that as well as CD4+ T cells, other T cells may have a role in the regulatory events triggered by oral tolerance, but they do not seem to be essential for them. Thus induction of mucosal tolerance can trigger different types of regulatory T cells (Diagram 1), suggesting that the mucosal route is a robust way to induce or restore peripheral tolerance and may benefit individuals with autoimmune diseases.

Therapeutic applications of mucosal tolerance in autoimmune diseases and its potential in the treatment of SLE

Several human trails of oral tolerization have been carried out in patients with multiple sclerosis (MS), rheumatoid arthritis (RA), diabetes and uveitis. In all studies no systemic toxicity or exacerbation of disease was observed, although clinical efficacy resulting in an approved drug has yet to be achieved. A mucosal tolerance approach to treating patients with SLE has yet to be carried out. However, promising results have been demonstrated in lupus prone animals.

MS

Feeding of MS patients with bovine myelin demonstrated that MBP- and proteolipid protein (PLP)-specific proliferative responses were affected and TGF-β secreting Th3-type cells were present in peripheral blood of treated patients but not in untreated patients [128,129]. However, a 515 patient, placebo-controlled, double-blind phase III trail of single-dose bovine myelin in relapsing-remitting MS did not show differences between placebo and treated groups in the number of relapses with a large placebo effect [129]. Trials in MS with the MBP analog glatiramer acetate (GA), which is currently given by subcutaneous injection to MS patients, have shown promising results and induced regulatory T cells that mediate bystander suppression [130,131]. However, a phase III trial of oral GA given daily at 5 and 50 mg verses placebo found no clinical or immunologic effects and magnetic resonance imaging did not show improvement of affected areas in the brain. Phase II trial with 300 and 600 mg oral GA are currently in progress.

RA

A double-blind, placebo controlled phase II trial was carried out in 280 RA patients. Oral doses of liquid bovine type II collagen (CII) ranging from 0.025 to 10 mg demonstrated statistically significant positive effects in groups treated with the lowest dose [132]. While oral CII at higher doses did not lead to significant clinical improvement although there was a higher prevalence of responders following oral CII compared to placebo. In another placebo-controlled trial of bovine collagen significant effects were seen in those receiving 0.5 mg but not in groups

receiving 0.05 or 5 mg [133]. These findings were consistent with findings in animal studies [89,134]. Five double-blind phase II randomized studies of oral CII (Colloral) have been carried out. A total of 805 patients were treated with Colloral and 296 treated with placebo. A dose refinement study tested Colloral at 5, 20 and 60 μg. Weighted averages for the Paulus 20 and Paulus 50 responses were calculated for the 60 μg dose and placebo. A significant effect favoring 60 μg was observed for both the Paulus 20 and the Paulus 50 responses. Safety analysis demonstrated that Colloral was remarkably safe with no side effects. The magnitude of the clinical responses to Colloral appears to be on the same level as non-steroidal anti-inflammatory drugs for the majority of patients. However, there was a subgroup of patients who appeared to have a more significant response to the medication [132,135]. On the basis of these data, a 760-patient phase III trial was performed comparing 60 μg of Colloral to placebo. However, no differences were observed. There was a large placebo effect in the control group. Clinical trials may be required to determine whether withholding no-steroidal anti-inflammatory drugs and prednisone will enhance the induction of oral tolerance in RA patients [136].

Oral CII was also tested in an open-label pilot study in juvenile RA with significant positive results and no toxicity observed [137]. The absence of toxicity is an important feature for the clinical use of oral tolerization, especially in children for whom the long-term effects of immunosuppressive drugs is unknown. Oral CII in juvenile RA was associated with clinical improvement and decreased CII-specific IFN-γ and increased TGF-β [138].

Diabetes

Several trials of mucosal administration of recombinant human insulin as a therapy for type I diabetes are underway or already completed in Europe and the US. In France a double blind study is comparing oral insulin therapy and parenteral insulin therapy versus placebo in patients during the remission phase. 131 autoantibody positive diabetic patients aged 7–40 years were given 2.5 or 7.5 mg oral insulin daily or placebo for 1 year, in addition to subcutaneous insulin therapy. Findings in follow up showed that oral administration of insulin at these doses did not prevent the deterioration of beta-cell function or diminished titers of antibodies to insulin, glutamic acid decarboxylase or islet antigen 2 [139]. In Italy a multi-center double blind study is evaluating the effect of oral insulin verses placebo in diabetic patients who are treated with intensive insulin therapy by measuring cytokine and autoantibody responses to insulin. After 12 months of treatment there was a significantly higher level of TGF-β with reduced IFN-γ production in patients who received oral insulin compared to those who received placebo. Serum levels of IgG1 and IgG3 anti-insulin antibodies were also significantly lower in the patients treated with oral insulin. However, no clinical effect was observed and it was concluded that poor timing of initiation of treatment was responsible for the lack of positive effect on disease [140]. In a double-blind, placebo controlled safety study in Finland (Type I Diabetes Prediction and Prevention Study, DIPP) insulin was given nasally to children who are at risk of developing diabetes. Insulin given nasally was well tolerated with low risk of hypoglycemia and no adverse effects were detected [141].

In the US a multi-center double-blind study is evaluating oral insulin therapy versus placebo in adults and children with recent-onset disease. No adverse effect was detected, and patients diagnosed after 20 years of age who were fed 1 mg insulin showed preserved β-cell function compared to patients who received placebo [142]. In another double-blind, placebo controlled study oral insulin did not delay or prevent type I diabetes in nondiabetic relatives at risk for diabetes. However, subjects who received insulin orally had significantly lower autoantibodies against insulin compared to subjects who received placebo [143].

Uveitis

A pilot study in two patients, one with pars planitis and the other with Behcet's disease, feeding of the retinal S-Ag resulted in these patients' immunosuppressive medication being decreased and/or stopped. The trial yielded valuable information on dosage and expected immune responses and led to a larger randomized, masked study looking at the effect of feeding retinal antigens to uveitis patients [144].

SLE – taming the wolf by restoring self-tolerance?

New advances in the treatment of SLE have been documented in recent years. Most notably, a chimeric human-murine monoclonal antibody directed against CD20 (Rituximab) on B cells and their precursors but not against plasma cells, which do not have this surface marker was tested. The use of Rituximab has been widely used in the management of lymphoma, with a decent record of safety, and is well tolerated. Leandro [145] and Anolik [146] and their colleagues undertook the first studies of Rituximab in SLE and since then there have been many small open-label trials. The overwhelming consensus is that Rituximab has the potential to produce long remissions after only two to four infusions. However, results of a recent placebo controlled phase II/III clinical trial in SLE patients demonstrated no beneficial effect of Rituximab. This could be due to the formation of anti-chimeric antibody that hampered the efficacy of Rituximab [147]. There are various protocols in use that combine Rituximab with intravenous cyclophosphamide and methylprednisolone, and we do not know whether maintenance of immunosuppressant is needed after Rituximab to prevent B cell re-accumulation and possible subsequent disease flares. There are several trails in progress to address these issues [148–151]. The precise mechanism(s) of action of Rituximab remain unclear. In addition, humanized monoclonal anti-B-cell antibodies are in clinical trials and Dorner and colleagues' findings [152] suggest that infusion of Epratuzumab, a fully human anti-CD22 monoclonal antibody, is safe in patients with lupus and reduces disease effectively in the short term. We await data on the long term clinical effects of Epratuzumab. Furthermore, in a phase II placebo controlled clinical trial, infusion of anti-B-lymphocyte stimulator (BLys) human monoclonal antibody (Belimumab) which neutralizes soluble BLys used in plasma cell formation in SLE patients showed a significant reduction in CD20+ B cells and plasma cells over a 76 week follow-up period with no increase in adverse events including infections [153].

So far there have been no clinical trials in SLE patients to evaluate the effect of mucosal administration of self-antigens or monoclonal antibodies on disease. In fact, unlike other autoimmune diseases, studies on mucosal tolerance in animal models of SLE have been relatively limited. Initial studies in animals suggested that oral tolerance is defective in the lupus prone (NZB×NZW)F1 (BWF1) mouse [154]. In a recent study oral administration of OVA failed to inhibit the secondary IgG response after systemic immunization, again suggesting defective oral tolerance in BWF1 mice prone to developing lupus [155]. As a novel approach to disease therapy we have carried out the first studies of nasal tolerance using a critical lupus autoantigen in mice that spontaneously develop a lupus like disease [19]. In the early studies we used histone peptide H471 (derived from histone H4, position 71–93) expressing a dominant T cell epitope in the mononucleosome [60] to induce tolerance to itself and to the whole protein by nasal instillation in (NZB×SWR)F1 (SNF1) mice. We deliberately chose to deliver the antigen via the nasal cavity rather than orally because the nasopharyngeal environment which lacks the high acidity and proteolytic enzymes of the gut, is less degrading to proteins, and particularly peptides. Also, the respiratory mucosa is more accessible for small amounts of antigen than the gut mucosa. This is advantageous when expensive peptides or purified proteins are used as tolerogens.

Young pre-nephritic female SNF1mice were nasally dosed with the H471 peptide dissolved in PBS for 5 consecutive days (4 μg per day) (day –12 to –8). On day 0, each mouse received 100

μg H471 or mononucleosome emulsified in complete Freund's adjuvant (CFA) intradermally and on day 10, T cell responses to H471 were tested *in vitro*. To examine the effect on disease progression and severity, similar young pre-nephritic female SNF1 mice were nasally dosed with H471 or irrelevant control peptide at 2-wk intervals until 32 weeks of age at which point disease related pathology and immune responses were examined. Nasal administration of H471 in mice markedly reduced subsequent T cell proliferative response to the peptide [19]. The mechanism behind the observed T cell hyporesponsiveness was T cell anergy as increasing the *in vitro* concentration of antigen or adding exogenous IL-2 could reverse the anergic state of the T cells. Subsequent studies on nasal tolerance showed that T cell anergy induced by nasal administration of H471 peptide in lupus prone NZB mice was a result of antigen presentation by immature B cells that lack CD80 and CD86 expression [156].

One of the major obstacles in developing treatment for systemic autoimmune syndromes such as lupus has been to design a therapy that allows the suppression or elimination of autoimmunity against multiple self-antigens and/or tissues. Thus it was encouraging to learn that T cells from mice nasally tolerized to H471 also demonstrated significantly reduced proliferative response to mononucleosomes [19]. Suppression of immune responses to mononucleosomes may prevent or delay the development of autoreactivity against other antigens such as DNA and histones. This was demonstrated in histopathological studies in SNF1 mice that were nasally treated with the H471 peptide over a long period of time (3 months) showing a significantly lower incidence of severe glomerulonephritis compared to mice treated with control peptide. The significant improvement in the severity of disease pathology as a result of nasal treatment of mice with histone peptide was associated with downregulation of autoantibody production [19].

One potential drawback with a peptide-based mucosal therapy in SLE is that not all patients are sensitized to the peptide thus restricting its applicability. Therefore we recently carried out studies in two different strains of lupus prone mice, SNF1 and BWF1, with a CD3-specific monoclonal antibody that has been previously shown to generate inducible regulatory T cells and suppress EAE [21]. Nasal or oral administration of anti-CD3 monoclonal antibody attenuated lupus development and arrested on-going disease. Nasal anti-CD3 induced a CD4+CD25−LAP+ regulatory T cell that secreted high levels of IL-10 and suppressed disease *in vivo* via IL-10 and TFG-β dependent mechanisms whereas suppression of lupus by oral anti-CD3 was associated with an increase in TGF-β secreting CD4+CD25−LAP+ regulatory T cell. Animals treated with nasal anti-CD3 had diminished antibody reactivity in autoantigen microarrays and ELISA, and significantly less glomerulonephritis. Disease suppression was associated with a significant downregulation of IL-17+CD4+ICOS+CXCR5+ follicular helper T cell function and IL-21 expression (Wu *et al* in press). In contrast to animals with lupus where IL-10 ameliorates disease, several studies have demonstrated that IL-10 is markedly upregulated in SLE patients and its levels correlate with disease activity [157,158]. This suggests that IL-10 plays a role in the pathogenesis of SLE. In a small open-labeled study in SLE patients infusion of anti-IL-10 monoclonal antibody was beneficiary in 5 out of 6 patients [159]. However, treatment with anti-IL-10 antibody did not lead to a decrease in circulating anti-DNA antibodies and the generalized improvement in patients treated with anti-IL-10 was due to an autoantibody independent mechanism [159]. The high level of IL-10 in SLE patients could come from damaged tissues as a mechanism to suppress inflammation. Thus the role of IL-10 in the pathogenesis of SLE is unclear. We hypothesize that production of IL-10 by CD4+CD25−LAP+ regulatory T cells in lymphoid tissues results in suppression of helper T cell function thereby inhibiting B cell activation and autoantibody production (diagram 2).

In animal models of mucosal tolerance in which the subjects are typically tolerized before disease induction, there is usually a significant reduction in the quality and measure of pathology. This paradigm is, however, not applicable to clinical situations involving lupus

because it is impossible to make a diagnosis before the patient has already developed disease. Thus, it is particularly encouraging to learn that nasal or oral anti-CD3 in 7 months old female SNF1 and BWF1 mice with persistent proteinuria markedly reduced disease severity and progression and significantly improved survival compared to mice treated with isotype control antibody. We observed no mitogenic effect of nasal or oral anti-CD3 antibody in mice and no evidence of cytokine release syndrome (wasted appearance, ruffled fur) even after 30 nasal administrations. We performed our experiments with an F(ab')2 antibody to eliminate any potential side effects related to the Fc portion of the molecule that might occur after multiple administrations of the antibody. No anti-F(ab')2 antibody response was seen in mice treated nasally or orally with the antibody (Wu *et al* in press). Thus nasal or oral administration of CD3-specific antibody would appear to be clinically applicable for chronic therapy, with few expected side effects such as cytokine release syndromes and anti-globulin responses. CD3-specific antibody would seem to be safe for human nasal or oral administration, given the long experience with intravenous CD3-specific antibody in humans, though nasal or oral CD3-specific antibody has never been tested in humans.

Concluding remarks

A specific and side effect-free treatment for SLE remains elusive. Novel treatment strategies that are disease specific and can reduce the possibility of compromising normal protective immunity and immune surveillance have been the focus of many immunologists. Despite the limited volume of research, data suggest that mucosal-based therapy holds good potential in the treatment of SLE. However, there are many important areas that need to be investigated and understood before the theory turns to reality. The unknown areas include factors that determine the efficacy of treatment and the immunological mechanism(s) behind disease suppression. There has not been a new drug approved to treat SLE for over 40 years. In light of a new millennium we hope that a relief for lupus patients is no more than a sniff or a sip away.

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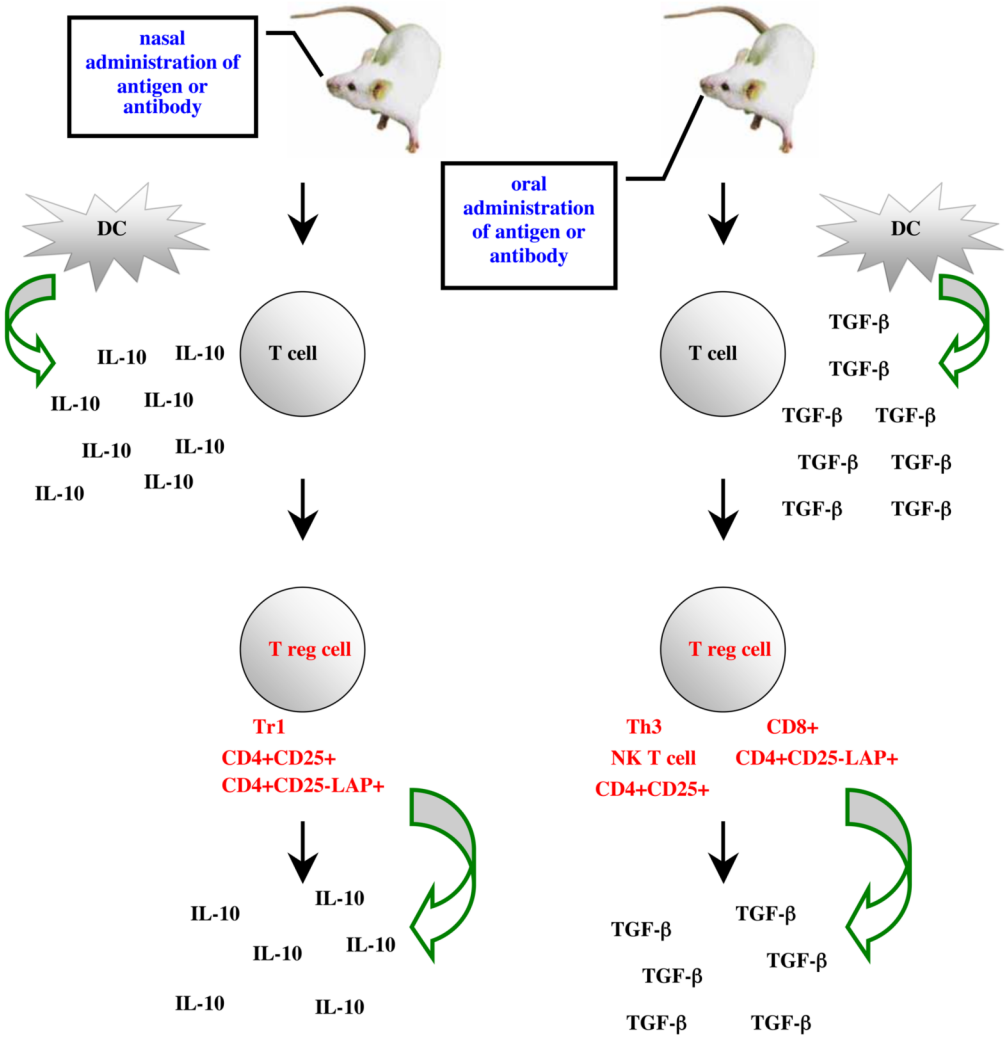

Diagram 1.
Regulatory T cells and cytokines associated with mucosal tolerance induction.

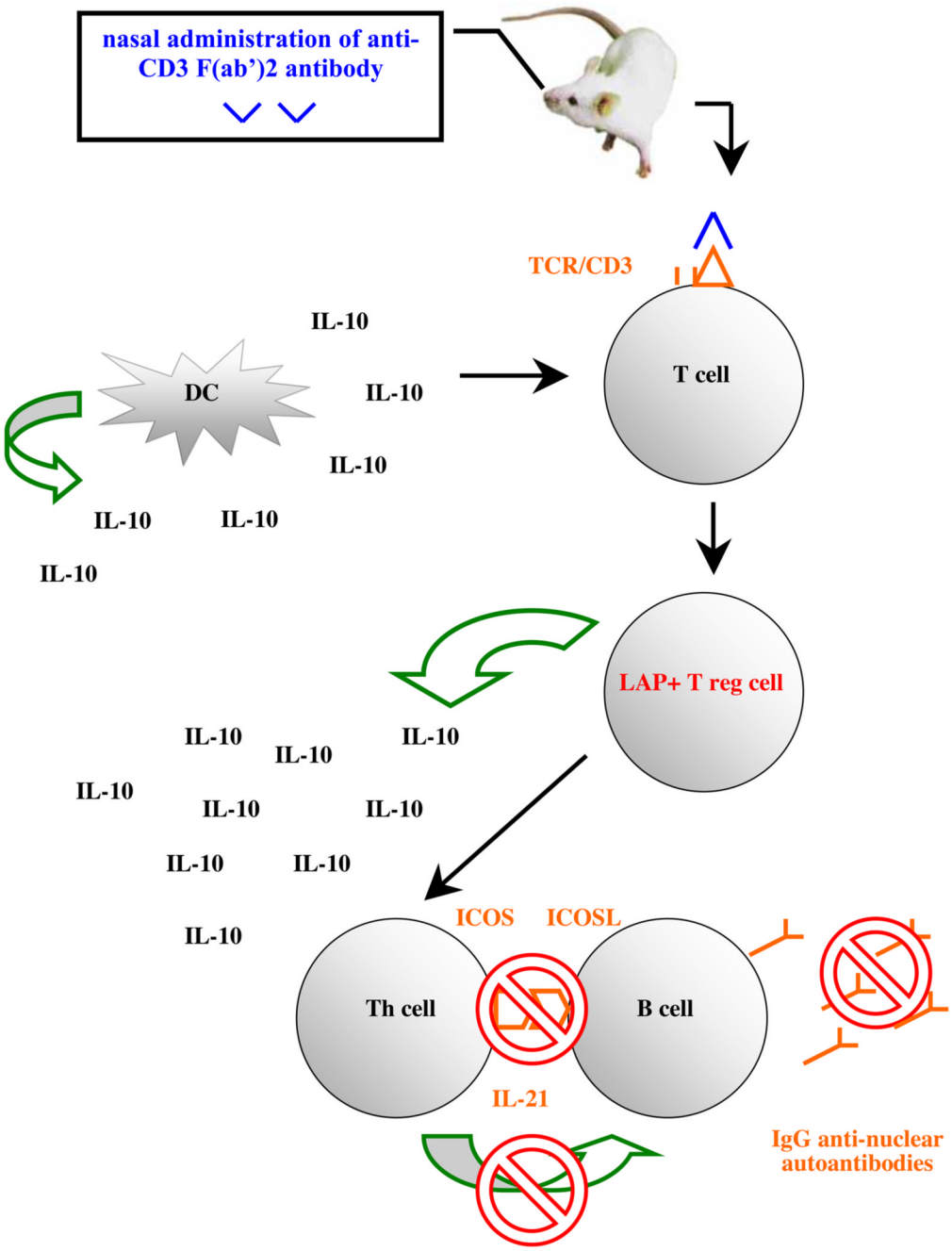

Diagram 2.
IL-10 secreting LAP+ regulatory T cells suppress the function of follicular helper T cells leading to disruption of Th-B cell cognate interaction, suppression of autoantibody production and disease pathology.