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Cellular and Molecular Mechanisms of Regulation of Autoantibody Production in Lupus

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Abstract

The hyperactive interaction between helper T cells and autoimmune B cells in individuals predisposed to systemic lupus erythematosus (SLE) can be interrupted by induction of regulatory and suppressor T cells. Using two strategies—high dose tolerance to an immunoglobulin-derived peptide, and minigene vaccination with DNA encoding T cell epitopes presented by MHC class I molecules—our group has induced at least three types of regulatory/suppressive T cells. They include CD8⁺ T cells that suppress helper T cells by cytokine secretion, CD8⁺ T suppressors that kill B cells making anti-DNA antibodies, and peptide-binding CD4⁺CD25⁺ regulatory T cells that suppress B cells by direct cell contact. Each of these lymphocyte subsets suppresses anti-DNA antibody production and delays the onset of nephritis in BWF1 lupus-prone mice. Patients with SLE have amino acid sequences similar to those from murine anti-DNA antibodies used in these studies, and at similar locations in the VH regions of anti-DNA immunoglobulins. Therefore, strategies described here might ultimately be useful in therapy of the human disease.

Keywords

systemic lupus erythematosus; CD8⁺ T cells; regulatory T cells; suppressor T cells

INTRODUCTION

Because systemic lupus erythematosus (SLE) depends on the production of auto-antibodies by B cells driven by helper T cells—a process that occurs in a milieu of permissive cytokines, chemokines, and sex hormones influencing interactions between cell surface receptors and their ligands—interruptions of any of these circuits are likely to alter disease. Our laboratory has focused on interrupting helper T cell–B cell interactions in the (NZB/NZW)F1 (BWF1) murine lupus model via induction of immune tolerance to immunoglobulin-based peptides, which calls up regulatory and suppressive T cells.

CONTROL MECHANISMS THAT PREVENT AUTOREACTIVITY IN HEALTHY INDIVIDUALS

In recent years there has been great interest in the role of regulatory/suppressive T cells in preventing and controlling autoimmunity.¹ There are several natural mechanisms for controlling autoreactivity. Many T cells with high avidity for self-antigens are deleted in the thymus (negative selection). T cells with relatively low avidity for self are likely to enter the peripheral immune system (positive selection), where they are closely regulated by peripheral tolerance mechanisms in healthy individuals. Although such cells have the potential upon contact with self or cross-reactive antigens to expand, activate, and cause autoreactivity ultimately leading to autoimmune disease, several inactivating mechanisms exist to prevent this. The mechanisms of peripheral tolerance include (a) activation-induced death, which occurs in T and B lymphocytes that receive very strong activating signals, capable of activating Fas/FasL interactions or tumor necrosis factor receptor (TNFR)-family death receptors; (b) anergy—a state in which T and B cells cannot secrete cytokines or immunoglobulins after activation, particularly if they receive signals only through their T cell receptor (TCR) or B cell receptor (BCR) without second signals through CD40/CD40 ligand (CD40L) pathways or CD28/CD80:CD86; (c) cytokine production that suppresses autoimmunity, including IL-4, IL-10, and transforming growth factor (TGF); and (d) active suppression by antigen-specific T cells.^{1,2} Our work has focused on this last mechanism, the induction of T cells that actively suppress autoimmunity.

T CELLS INVOLVED IN SUPPRESSION

T cells that can suppress T and/or B cells include NK T cells, CD4⁺CD25⁺ regulatory T cells, and CD8⁺ suppressors (Table 1). Many CD8⁺ T cells suppress responses in a largely antigen-nonspecific manner, but recent data have suggested that some subsets can operate via mechanisms that involve antigen recognition.^{1,3} The induction of CD8⁺ suppressor/effectors from precursor cells requires interaction with certain CD4⁺ T cell subsets, which in certain cases can be activated T cells expressing Qa-1 (an MHC class Ib molecule that can present autologous or exogenous peptides to CD8⁺ T cells).^{1,4,5} In turn, the target cells downregulated by CD8⁺ suppressors probably include CD4⁺Qa-1⁺ antigen-specific CD4 recognized by the TCR of the CD8⁺ suppressors.⁶ There are several different types and/or mechanisms of action of CD8⁺ T cell suppressor/effectors (Ts). These include cytotoxic T cells that require cell–cell contact, cells that secrete cytokines (TGF- β , interferon [INF]- γ) that induce apoptosis or death in their targets, and Ts cells that interact with antigen-presenting cells (APCs) to render them “tolerogenic,” that is, capable of producing anergy in CD4⁺ helper T cells and of inducing CD4⁺CD25[−] T regulatory cells.^{3,6–8} There are examples of two types of CD8⁺ Ts cells protecting against SLE-like disease in NZB/NZW F1 (BWF1) lupus-prone mice, as discussed below. Further-more, recent experiments in our laboratory have shown that as BWF1 mice age, their peripheral CD8⁺ T cells become increasingly defective.⁹ CD8⁺ T cell populations in these mice do not expand as dramatically as do CD4⁺ cells, and only small proportions of CD8⁺ T cells express activation and memory surface markers. In contrast, such markers are found on the majority of BWF1 CD4⁺ T cells. When activated, significantly higher proportions of BWF1 CD8⁺ T cells from older mice undergo apoptosis compared with CD8⁺ T cells from young, healthy BWF1 mice or other nonlupus murine strains. Therefore, abnormal elimination of activated CD8⁺ suppressor cells probably contributes to the failure of BWF1 mice to regulate autoantibody production and to their predisposition to SLE.

Murine CD4⁺ T cells contain not only helper cells and suppressor-inducers, but also direct suppressors.^{1,10–14} These cells, now termed “regulatory T cells” (Tr cells) were first discovered by Sakaguchi *et al.* in thymectomized mice that developed organ-specific autoimmunity.¹⁰ Tr cells are CD4⁺CD25⁺CTLA4⁺ and depend on the DNA-binding Foxp3

gene to exert suppressive activity.^{13,14} They are educated in the thymus and then migrate to the periphery. The target of these Tr cells in some experimental systems is other CD4⁺ T cells (CD4⁺CD25⁻), which cannot proliferate in the presence of Tr; data from our laboratory (discussed below) suggests that some Tr cells also directly suppress B cells. Their mechanism of action is complex and differs in different systems. In some experimental systems (particularly colitis and diabetes) suppression depends at least in part on the CTLA4 surface molecule;^{14–16} the “suppressive” cytokines IL-10 and TGF- β may also be involved.¹⁶ In other systems, including the BWF1 mouse, interactions between the target cells and membrane-bound TGF- β or glucocorticoid-induced TNFR-related gene (GITR) on Tr cells have been implicated.^{17,18}

POTENTIAL IMPORTANCE OF REGULATORY/SUPPRESSIVE T CELLS IN HUMAN SLE

Although Tr and CD8⁺ Ts cells were originally defined in mice, and much of the information discussed above was derived from studies in mice, similar cells exist in the peripheral lymphoid compartments and circulation of healthy humans, as well as in abnormal human tissues (inflamed synovium, tumor masses, virally infected tissues).^{19–22} In patients with SLE, one study reported that the numbers of circulating CD4⁺CD25⁺ T cells during periods of disease activity are significantly lower than in normal individuals.²¹ Another group of investigators has shown that during periods of disease activity, CD8⁺ T cells in the circulation of SLE patients have impaired ability to suppress autologous CD4⁺ T cell proliferation; this proliferation returns toward normal when disease is controlled.^{22,23} Impairing the function of IFN- γ and IL-6 impairs the suppressive effects of SLE CD8⁺ Ts cells, and IL-10 is required to generate Ts cells from CD8⁺CD28⁻ precursors. Because Tr and Ts cells can control autoimmunity, we performed several interventions in lupus-prone mice to establish the *in vivo* utility of this approach to treatment.

INDUCTION OF REGULATORY/SUPPRESSIVE T CELLS TO CONTROL LUPUS-LIKE DISEASE

Regulatory/suppressor CD4⁺ and CD8⁺ T cells become defective in unmanipulated BWF1 mice as they age, probably permitting sustained helper T/B interactions that drive disease.⁹ We developed strategies to induce Tr and Ts cells. To induce both, we administered intravenously high doses of a tolerogenic peptide, pCONSENSUS (pCONS), to young, healthy BWF1 females. Spleen cells were harvested one week later; different populations were studied for their ability to influence autoantibody production and clinical lupus-like nephritis. The pCONS peptide is an artificial 15-mer peptide based on amino acid sequences in the first hypervariable/second constant region of the V_H region of a BWF1 monoclonal IgG antibody to DNA. Similar sequences are found in the same regions of human anti-DNA monoclonal antibodies (mAbs) from SLE patients.²⁴ We have previously shown that monthly administration of pCONS, either to young mice or mice with established nephritis, prolongs survival by significantly delaying or suppressing autoantibodies and nephritis.²⁵

CD4⁺ Tr cells are induced by this tolerance regimen. As shown in FIGURE 1, these Tr cells suppress anti-DNA antibody production.¹⁷ In these experiments, CD4⁺CD25⁺ T cells isolated from spleens of BWF1 mice one week following injection of pCONS were divided into peptide-binding and nonbinding populations (using labeled soluble dimers of I-Ed containing pCONS to identify binders). The suppression was confined to the peptide-binding CD4⁺CD25⁺ cells, and they required cell-cell contact to function. Their targets were B cells. Tr cells obtained *ex vivo* from tolerized mice prevented B cells from unmanipulated BWF1 mice from secreting immunoglobulin (including anti-DNA antibodies) *in vitro*. This regulation was interrupted by

adding to B + Tr cell culture antibodies to membrane-bound TGF- β (LAP-TGF- β) or antibodies to GITR. In contrast, CTLA4 immunoglobulin did not impair the regulation. As expected, the pCONS-binding Tr cells expressed Foxp3.

We have also characterized the CD8⁺ Ts cells found in spleen cells one week after a single injection of 1 mg of pCONS (Ref. 26 and manuscript in preparation). These cells suppress anti-DNA antibody production and nephritis *in vivo*, and anti-DNA antibody production *in vitro* (Fig. 2). They do not depend on cell-cell contact, but suppress primarily by production of TGF- β . Preliminary work suggests that the suppressive capacity of these CD8⁺ Ts cells also involves expression of Foxp3. These Ts cells are similar to those induced by repeatedly immunizing normal, non-lupus-prone mice with heavy chains or V_H peptides from BWF1 anti-DNA autoantibodies.²⁷ In immunized normal mice, anti-DNA antibodies can be induced by such immunizations, and proteinuria occurs. However, the autoimmune state is short-lived, and disappears in conjunction with the appearance of both Tr and Ts cells. Similar to the experiments in pCONS-induced tolerance the CD8⁺ Ts cells in those experiments suppressed anti-DNA antibody production and nephritis via secretion of TGF- β .

In a second method of inducing CD8⁺ Ts cells in BWF1 mice,⁷ mice were inoculated with DNA encoding each of three MHC class I-binding peptides found in a wild murine antibody to DNA (A6.1 from a nephritic BWF1 mouse). The treatment induced CD8⁺ Ts cells that were cytotoxic for anti-DNA antibody-producing B cells with surface immunoglobulin (sIg) containing the epitopes against which the CD8⁺ Ts were targeted. This strategy was effective in delaying the appearance of anti-DNA antibodies and nephritis, and in prolonging survival.

DISCUSSION

In recent years, the importance of regulatory CD4⁺ Tr cells and suppressive CD8⁺ Ts cells in preventing autoimmunity in normal individuals has been recognized. In our hands, two methods have been effective in inducing Tr and Ts cells that can suppress anti-DNA antibodies and nephritis in BWF1 mice. The methods include a peptide-based tolerizing regimen and DNA vaccination. Both methods use the principle that autoantibodies to DNA, the hallmark of SLE, contain amino acid sequences that are T cell epitopes. We and others have identified several regions in the V_H region of both murine and human autoantibodies to DNA that induce T cells to proliferate or secrete cytokines.^{24,25,28} These sequences are similar in location and in amino acid content in different anti-DNA antibodies from patients of different ethnic backgrounds and from mice of different strains. Individuals predisposed to SLE may have biased selection of B cells bearing surface immunoglobulin containing these amino acid sequences.

To induce effector Ts cells, we induced immune tolerance in young BWF1 mice by intravenous administration of high doses of an artificial peptide, pCONS, that contains amino acid sequences that bind the MHC class II molecule I-Ed and induce proliferation in BWF1 T cells;^{25,28} pCONS contains at least one epitope that binds the MHC class I molecule Kd.⁷ Thus, administration of pCONS as tolerogen can induce both CD4⁺ Tr and CD8⁺ Ts cells. One of these sets, the CD8⁺ effectors that help suppress antibody production, becomes defective spontaneously in BWF1 mice near the time of disease onset.⁹ However, when treated with our regimens, the animals are perfectly capable of raising both Tr and at least two types of CD8⁺ Ts cells that downregulate autoantibody production and delay the onset of nephritis. Similar strategies might be used to develop therapeutic interventions in patients with SLE or other autoantibody-mediated diseases.

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FIGURE 1. CD4⁺CD25⁺ regulatory T cells from tolerized NZB/NZW F1 mice suppress the ability of syngeneic B cells to synthesize IgG anti-DNA antibody *in vitro*, and that suppression depends upon membrane-associated TGF and/or GITR. 1 × 10⁵ B cells from spleens of unmanipulated old BWF1 females were cocultured with 1 × 10⁶ CD4⁺ splenic helper T cells from naive young mice and 1 × 10⁶ pCONS-binding CD4⁺CD25⁺ spleen cells from young BWF1 mice tolerized with 1 mg pCONS i.v. one week prior to harvest. Various cell combinations are shown on the x-axis, as well as inhibition with anti-GITR or anti-TGF-β-LAP antibody. Antibody-forming cells (AFC) per 10⁶ B cells are shown on the y-axis. Information below the graphic indicates the contents of cultures labeled **a**, **b**, **c**, **d**, **e**. CD4⁺CD25⁺ T cells were selected by binding with a soluble dimer of I-Ed and pCONS. Note that in **c**, AFC formation is suppressed compared with **b** (*P* < 0.0004), and the suppression is abolished by inhibiting either GITR (**d**) or TGF-lap (**e**). Addition of CTLA4-Ig to the culture to inhibit surface CTLA4 on Tr cells did not inhibit suppression (data not shown). Statistical analysis by ANOVA showed that the following comparisons were significantly different at *P* < 0.0004: **a** vs. **b**, **a** vs. **c**, **b** vs. **c**, **c** vs. **d**, **c** vs. **e**.

FIGURE 2.

CD8⁺ T cells from BWF1 mice tolerized with 1 mg pCONS suppress anti-DNA antibody production by syngeneic B cells. Data shown are representative of four experiments. Cultures contain 1×10^5 B cells from spleens of unmanipulated old BWF1 females, and pCONS. CD4⁺ T cells are from unmanipulated young BWF1 mice and give help for the B cell production of anti-DNA antibody (**b**). To CD4⁺ B are added CD8⁺ cells from unmanipulated young syngeneic mice (**c**), or CD8⁺ cells harvested from spleens one week after administration of pCONS (**d**). CD8 to CD4 ratios are 1:1. Note that the tolerized CD8⁺ T cells suppress anti-DNA antibody production 10-fold (compare **d** vs. **b**).

TABLE 1

Characteristics of T cells that control autoimmunity

Cell Types	Characteristics	Pathways of Effector Functions
NK T Cells	Coexpress NK markers and TCR. Scant in blood, abundant in certain tissues such as liver. Recognize CD1-restricted glycolipid antigens. Usually CD4 ⁺ and CD4 ⁺ CD8 ⁺ . Certain subsets are regulatory, including those with surface Vα24-Jα18 TCR in humans. Participate in tumor rejection, early response to infections and downregulation of autoimmunity. ^{29–31}	1. Secretion of IFN-γ, IL-4, TGF-β, IL-10. 2. May be cytotoxic for immature immune cells. 3. May regulate autoimmunity by supporting immune deviation in CD4 ⁺ T cells from Th1 to Th2
CD4 ⁺ CD25 ⁺ T cells	Suppress self-reactive T or B cells. Educated in the thymus. Prevent "natural" autoimmunity (organ-specific and systemic) induced by other CD4 ⁺ T cells. Other surface molecules include CD45RBlow, CTLA4 and GITR, all of which are induced by activation but may be present constitutively on some Tr cells. Some Tr cells express membrane-bound TGF-β. Some express Toll-like receptors that allow antigen non-specific activation in innate immunity. Tr cells proliferate poorly. When activated they express high levels of the transcription factor Foxp3, which represses transcription factors that regulate T cell activation.	1. Secretion of TGF-β, IL-10, IL-4, IFN-γ. 2. Suppression of CD4 ⁺ CD25 ⁺ proliferation and of B cells may be contact dependent and may be mediated by lap-TGF-β, GITR, and/or CTLA4. 3. Suppressive effects may depend on Foxp3.
CD8 ⁺ cytotoxic/inhibitory T (Ti) cells	Antigen-specific CD8 ⁺ T cells can kill target immune cells. Naive cytotoxic CD8 ⁺ cells are not effectors; they express CD45RA. Memory CD8 ⁺ T cells are effectors and express CD45RO. When antigen-primed, CD8 ⁺ Ti cells regulate CD4 ⁺ T cells activated by the same antigen, this may depend on CD8 TCR recognition of antigen presented by Qa-1 molecules expressed on activated CD4 ⁺ T cells. Superantigen also induces suppressor CD8 ⁺ cells through the Qa-1 restriction MHC element presented by CD4 ⁺ T cells.	1. Direct cytotoxicity toward T and B cells and APCs. This may be mediated by release of Ca ²⁺ , perforin, and granzyme polarized toward the target after CD8 ⁺ TCR recognize antigen, or by activation of apoptosis in the target via Fas/FasL receptors. 2. Cytokine secretion: TGF-β, IL-10, IFN-γ, TNF-α.