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Role of Cytokines in the Pathogenesis and Suppression of Thyroid Autoimmunity

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Autoimmune thyroid diseases (AITD) are one of the most common organ-specific autoimmune disorders, of which Hashimoto's thyroiditis (HT) and Graves' disease (GD) are 2 of the most common clinical expressions. HT is characterized by hypothyroidism that results from the destruction of the thyroid by thyroglobulin-specific T cell-mediated autoimmune response. In contrast, GD is characterized by hyperthyroidism due to excessive production of thyroid hormone induced by thyrotropin receptor-specific stimulatory autoantibodies. Cytokines play a crucial role in modulating immune responses that affect the balance between maintenance of self-tolerance and initiation of autoimmunity. However, the role of cytokines is often confusing and is neither independent nor exclusive of other immune mediators. A regulatory cytokine may either favor induction of tolerance against thyroid autoimmune disease or favor activation and/or exacerbation of autoimmune responses. These apparently contradictory functions of a given cytokine are primarily influenced by the nature of co-signaling delivered by other cytokines. Consequently, a thorough understanding of the role of a particular cytokine in the context of a specific immune response is essential for the development of appropriate strategies to modulate cytokine responses to maintain or restore health. This review provides a summary of recent research pertaining to the role of cytokines in the pathogenesis of AITD with a particular emphasis on the therapeutic applications of cytokine modulation.

Introduction

Autoimmune diseases are a group of heterogeneous disorders characterized by abnormal lymphocytic activation directed against self-tissue (Davidson and Diamond 2001; Marrack and others 2001). These diseases occur essentially due to a breakdown in immunological self-tolerance. According to the clonal selection theory (Burnet 1959), self-reactive lymphocytes are deleted at the early developmental stage by negative selection and constitute what is called "central tolerance." However, it is believed that weakly reactive clones sometimes escape clonal deletion and migrate to the periphery. Physiologically, these potentially self-reactive clones remain either nonresponsive to antigenic stimulation (ignorance) or are rendered anergic (Nossal 1996). In some instances, they undergo activation-induced cell death upon exposure to self-antigen (Green and others 2003). Collectively, these mechanisms of self-tolerance are referred to as cell-intrinsic mechanisms of "peripheral tolerance" (Schwartz 2005). In recent years, another mechanism of peripheral self-tolerance has been described involving forkhead box P3 (Foxp3) expressing regulatory T cells (Tregs) that actively and dominantly suppress self-reactive T-cells (Sakaguchi and others 2007). This constitutes a cell-extrinsic mechanism of self-tolerance (Schwartz 2005).

Autoimmune disease can thus occur when both central and peripheral tolerance mechanisms fail, leading to a pathogenic immune response against a self-antigen. In general, autoimmune diseases can be characterized as T-cell mediated or autoantibody mediated based on the primary effector mechanism and cell type involved in the pathogenesis of the disease. T cell-mediated diseases are characterized by infiltration of T cells into and destruction of the target tissue as seen in Hashimoto's thyroiditis (HT), type 1 diabetes (T1D) and multiple sclerosis (Crane and Forrester 2005). Autoantibody-mediated diseases are characterized by disruption of function as in Graves' disease (GD) or destruction of the target tissue as seen in myasthenia gravis, pemphigus vulgaris, systemic lupus erythematosus, rheumatoid arthritis (RA), etc. (Yanaba and others 2008).

Autoimmune thyroid diseases (AITD) are the most common organ-specific autoimmune disorders affecting approximately 5% (Caturegli and others 2007) of the overall population. HT and GD are 2 of the most common clinical expressions of thyroid dysfunction but differ in their clinical presentations as well as pathophysiology. HT is a T cell-mediated organ-specific

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autoimmune disease that results in clinical hypothyroidism due to thyroid destruction and is mediated by infiltrating and/or locally activated thyroglobulin (Tg)-specific T cells. In contrast, GD is characterized by hyperthyroidism due to excessive production of thyroid hormone induced by specific autoantibodies to thyrotropin receptor (TSHR). There is considerable evidence implicating that the actual destruction of thyroid cells in AITD may be caused by different and multiple mechanisms, including auto reactive T-lymphocytes, natural killer (NK) cells, and cytokines. Several studies in animal models have concluded that organ-specific autoimmune thyroiditis (AIT) should be regarded as a polygenic disease that is strongly influenced by environmental factors (Prabhakar and others 2003; Tomer and others 2003; Klecha and others 2008). Although individuals may be genetically predisposed to AIT, the disruption of immune system homeostasis by environmental factors results in thyroid dysfunction. The most significant factor that is likely to be involved in the induction of autoimmunity is a defect or deficiency in the immune regulation, particularly a perturbation in the balance between the effector T cells (Teff) and Tregs that prevent the development of autoimmunity.

A murine model of HT called experimental autoimmune thyroiditis (EAT) exhibits key features of HT, including mononuclear cell infiltration that destroy thyroid follicles, presence of autoantibodies, and autoreactive T-cells to thyroid autoantigens (Kong and others 2009). Although this disease is induced by immunization of experimental animals with mouse thyroglobulin (mTg) emulsified in complete Freund's adjuvant (Vasu and others 2003), it can also be induced with mTg in conjunction with bacterial lipopolysacchharide (LPS) or interleukin (IL)-1β as adjuvant (Esquivel and others 1977; Nabozny and Kong 1992). Antigen presentation in the thyroid draining lymph nodes lead to the differentiation of Tg-specific T-cell subsets that migrate to the thyroid and cause tissue destruction (Fig. 1). This vigorously explored murine model has given us many insights into the molecular mechanisms underlying HT and also enabled us to identify potential therapeutic treatments. For instance, using this model, it was shown that lymphocytic infiltration of the thyroid depends upon the chemokine CCL21 and its receptor CCR7 (Martin and others 2004; Lira and others 2005). On the other hand, our laboratory has used this murine model to show that the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) can suppress EAT by mobilizing Tregs that secrete IL-10, an immunosuppressive cytokine.

Immunological Events in AITD

In order to understand the role of cytokines in the initiation or suppression of AITD, it is critical to understand the immunological events that trigger the autoimmune response, which eventually results in the associated pathology.

In HT, cell-mediated immunity promotes the induction of auto-antibodies and self-reactive T cells against Tg, and other auto-antigens, including thyroid peroxidase. HT is characterized by infiltration of lymphocytes and other immune cells, thyroid enlargement and fibrosis, and progressive destruction of thyrocytes that eventually results in hypothyroidism (Weetman 2003). Upon initiation of the immune response to Tg, thyroid-specific T lymphocytes migrate to the thyroid and through interferon (IFN)-γ production induce thyrocyte expression of major histocompatibility complex (MHC) class-II molecules. This results in further expansion of autoreactive T cells and the inflammatory response leading to the accumulation of activated CD4+ and CD8+ T cells, B cells, plasma cells, and macrophages in the thyroid.

Induction of autoimmune responses is believed to be initiated by an environmental trigger (Weetman 2003) such as LPS and cholera toxin. These microbial products can induce antigen presenting cells (APCs) such as dendritic cells (DC) to produce inflammatory cytokines and activate various other cells of the innate immune system by increasing the levels of

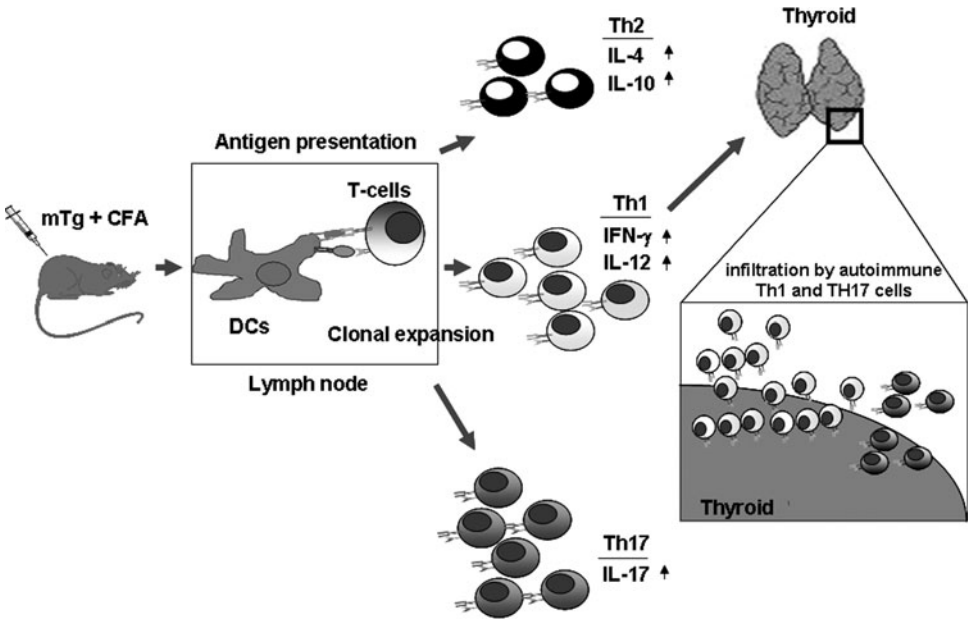

FIG. 1. The role of cytokines in EAT. EAT in mice is induced by immunization with mTg emulsified in the presence of Complete Freund's adjuvant. The antigen is taken up by the antigen presenting cells such as DCs, which undergo maturation to become potent antigen presenting cells. These cells present mTg-derived peptides to T cells in the draining lymph nodes. The activated DCs secrete pro-inflammatory cytokines that initiate a T helper response. Based on the type of cytokines secreted by these DCs, a Th1, Th2, or a Th17 response can be initiated. The Th1 cells predominantly secrete IFN-γ and IL-12, whereas the Th2 cells secrete IL-4, and Th17 cells secrete IL-17. The Th1 and Th17 cells have been shown to infiltrate the thyroid resulting in inflammation and ultimately death of the thyrocytes in EAT. EAT, experimental autoimmune thyroiditis; CFA, complete Freund's adjuvant; DCs, dendritic cells; mTg, mouse thyroglobulin; IFN, interferon; IL, interleukin.

expression of co-stimulatory and/or MHC molecules. These cytokines can also up-regulate expression of MHC molecules on the target cells. Highly activated APCs present self-antigens and activate self-reactive naïve T cells and initiate an autoimmune response that can be sustained by antigen presentation by the target cells.

In GD, loss of tolerance to TSHR results in the production of autoantibodies against TSHR and upon affinity maturation some of these antibodies bind to particular regions of the TSHR with high affinity and act as TSH agonists, and cause hyperthyroidism. Although patients with GD may show lymphocytic infiltration and some damage to the thyroid, the disease is primarily caused by the stimulatory antibodies. Since immunoglobulin class switching and affinity maturation would require TSHR-specific T cell help, modulating TSHR-specific T cell function might also be beneficial in GD.

CD4+ T cells are the major type of infiltrating cells in AITD. However, CD4+ T cells comprise of a functionally heterogeneous population of Teff responsible for both development of thyroiditis and a smaller population ($\sim$10%) of Tregs expressing CD25 (IL-2Rα) that are critical for maintaining peripheral tolerance (Sakaguchi and others 2008). Tregs are identified by their expression of Foxp3, a transcription factor that is necessary and sufficient for Treg development (Zheng and Rudensky 2007). Besides, natural Tregs (nTregs), there is another component of CD4+ Tregs that do not express Foxp3 but secrete the cytokines IL-10 and transforming growth factor-β (TGF-β) to induce tolerance. Cytokines secreted by various components of the immune system, primarily the DCs, Teff, and Tregs have profound effects on the function of each other and play a crucial role in determining the ultimate outcome of an immune response.

Cytokines and T-Cell Subsets

Cytokines are small molecules secreted by cells of the immune system that serve to regulate various other components of the immune system, and they play a crucial role in health and disease (Brown and others 1989; Kim 2004). Each cytokine signals by binding either to a unique or a shared receptor, triggering an intracellular signaling cascade that can cause up-regulation or down-regulation of transcription factors that regulate the expression of various other genes. This can result in the production of other biologically active molecules, including other cytokines, alteration in the number of surface receptors, or a feedback inhibition loop that leads to self-regulation.

A model proposed by Mosmann and others (1986, 1989) suggested that CD4+ T-cells can be phenotypically and functionally characterized into 2 distinct subsets, Th1 and Th2, with distinct cytokine secretion profiles. Th1 cells secrete IL-2, IFN-γ, and tumor necrosis factor-α (TNF-α), and support the activation of APCs, delayed type hypersensitivity response, and immunoglobulin isotype switching to immunoglobulin G (IgG)2a in mice (Kroemer and others 1996). Th1 cells play an important role in mounting host defense against intracellular pathogens as well as in inducing delayed type hypersensitivity responses. Th2 cells, in contrast, are characterized by the secretion of IL-4, IL-5, IL-6, IL-10, and IL-13, and provide efficient help for B cell activation, antibody production, and immunoglobulin class switching to IgG1 and IgE isotypes (Kroemer and others 1996). While Th1 cells are associated with host defense against intracellular pathogens and T cell-mediated autoimmune diseases, Th2 cells are essential for clearing extracellular parasites and helminths. In addition, Th2 cells play a crucial role in bringing about eosinophilic inflammation and IgE production in allergic reactions and asthma (Murphy and Reiner 2002). If a perturbation in the balance between Th1-Th2 immune responses leads to the activation of Th1-cell-mediated autoimmune response against Tg, it can cause thyrocyte destruction and result in hypothyroidism as seen in patients with HT. Conversely, if the perturbation leads to a Th2-mediated stimulatory antibody response against TSHR, it can cause hyperthyroidism associated with GD.

In a physiological setting, professional APCs (eg, DCs, macrophages, and B-cells) recognize microbial components through certain receptors such as Toll-like receptors. Activation of these receptors results in the secretion of a specific set of cytokines that lead to the induction of a particular T-cell subset. For instance, secretion of IL-12 and IL-18 by APCs can lead to the induction of a Th1 response. In contrast, Th2 differentiation is mediated by T-cell-derived cytokines like IL-4 after interaction with APCs (Yoshimoto and others 1998; Chang and others 2000). Thus, inflammatory APCs can contribute to autoimmunity by influencing the activation of a particular subset of T cells. Conversely, suppressor cytokine-mediated modulation of APCs can be potentially used to treat autoimmunity.

More recently, cytokines have been grouped further into Th17 as well as regulatory cytokines released by T cell subsets like type-1 Tregs (Tr1) cells, Th3 cells, and Tregs. The Th17 Teff population produces pro-inflammatory cytokines IL-17A and IL-17F (Langrish and others 2005) and play an important role in mediating host defenses against bacteria like *Citrobacter* and *Klebsiella pneumoniae,* and fungi such as *Candida albicans*, and their inflammatory properties can initiate autoimmunity. Th17 cells share a reciprocal developmental pathway with Foxp3+ Treg cells. The regulatory cytokine TGF-β plays a critical role in the differentiation of both pathogenic Th17 cells and induced Tregs upon T cell receptor (TCR) activation. TGF-β along with the cytokine IL-6 activates Smad3 and STAT3, which induce activation of ROR-γt required for Th17 cell differentiation. In contrast, TGF-β alone induces Smad3, a transcription factor, which induces Foxp3 expression in the presence of retinoic acid (Ziegler and Buckner 2009).

Th17 responses can contribute to immunopathogenesis of AITD, whereas regulatory cytokines such as IL-10 and TGF-β have been shown to prevent AITD. Although several factors, including genetic, hormonal, environmental, and nutritional factors, have been implicated in the initiation and/or development of AIT, the changes associated with the pathophysiology of AITD are brought about by inflammatory cytokines. In contrast, regulatory cytokines can prevent the development of autoimmune diseases. Interestingly, recent studies in humans with AITD, as well as in established murine models of AITD, have shown that the role and function of various cytokines are neither independent nor exclusive. Furthermore, cytokines in many cases are pleiotropic, and can often have both immunostimulatory and immunosuppressive functions depending upon the specific context in which the immune response is taking place. For example, cytokines such as IL-2, IFN-γ, and TNF-α have been shown to both enhance and suppress autoimmune diseases (O'Shea and others 2002).

Th1 Cytokines in AITD

Th1 cells are generated from naive T helper cells by TCR engagement and STAT1 signaling initiated upon IFN-γ binding to its cognate receptor (IFN-γR). Phosphorylated STAT1 induces the expression of the transcription factor T-bet, which drives the differentiation of Th cells into Th1 cells by transactivating IFN-γ and the specific subunit of IL-12Rβ2, the receptor for IL-12. Upon expression of IL-12Rβ2, the cell becomes responsive to IL-12, which may be produced by activated DCs, macrophages, or other immune cells. Subsequent IL-12 signaling through STAT4 further stabilizes the Th1 phenotype (Yang and others 1999).

Analysis of cytokine expression has shown that Th1 cytokines are commonly prevalent in HT as well as in EAT (Drugarin and others 2000) and that the proportion of peripheral Th1 cells was higher in patients with severe HD than in patients with mild HD (Nanba and others 2009). APCs such as macrophages and DCs form the link between innate and acquired immune responses and produce considerable amounts of pro-inflammatory cytokines; TNF-α and IL-1β play important roles in initiating an adaptive immune response. DCs produce large amounts of IL-12, an important cytokine involved in inducing Th1 type of adaptive immune response (Moser and Murphy 2000). Increased expression of IL-12 by APCs, *in vitro* as well as *in vivo*, has been linked to EAT and T1D susceptibility and other autoimmune conditions (Trembleau and others 1995; Zipris and others 1996; Braley-Mullen and others 1998; Mannon and others 2004; Gangi and others 2005; Cheatem and others 2009; Ganesh and others 2009). More recent studies have implicated IL-12 in overcoming immune tolerance by suppressing Foxp3+ Tregs. In a recent study, Brahmachari and Pahan (2009) have shown that the p40 subunit of IL-12 can down-regulate Foxp3 expression via the production of nitric oxide. Our observation that IL-12 abrogates Foxp3 expression in T cells during activation further confirms the pro-inflammatory and the potential pathogenic effects of this cytokine in autoimmunity (Ganesh and others 2009).

IL-1β, on the other hand, has pleiotropic effects and can alter cell signaling, migration, and cytokine production, and influence T cell differentiation differently under different conditions (Johnson and others 2005; Dinarello 2009; Sutton and others 2009). Like IL-12, IL-1β has been shown to break peripheral tolerance by facilitating the expansion of Teff, and is implicated in autoimmune diseases such as RA (O'Sullivan and others 2006). Lately, IL-1β has been shown to be critical for the generation of IL-17-secreting T helper cells in humans. IL-23 when combined with IL-1β can induce and maintain pathogenic Th17 cells (Chung and others 2009; Lee and others 2010).

It is, however, interesting to note that the role of pro-inflammatory cytokines is not limited to the induction of AITD. In our recent studies we have observed that while IL-1β in the presence of IL-12 drives a pro-inflammatory response, IL-1β alone in the absence of IL-12 is capable of inducing Foxp3+ Tregs *in vitro.* These Tregs when adoptively transferred into mice immunized with mTg can prevent the development of EAT with greater efficiency than Tregs that were not treated with IL-1β (in press at PLOS1). These contradictory effects of IL-1β both in the induction of Tregs and in the activation of autoreactive T cells have been observed with other pro-inflammatory cytokines as well. Although pro-inflammatory cytokines like IL-6 and TNF-α have been generally associated with the induction of inflammation and autoimmunity, they may provide positive signals for the induction of Tregs (Verginis and others 2005; Nakagawa and others 2010). IL-2 is required for the development and maintenance of Tregs (Fontenot and others 2005; Burchill and others 2007) and provides long-term protection against autoimmune disease. We have seen that induction of IL-2 in the CD25− population and TGF-β in the Foxp3+ Tregs by IL-1β is required for the expansion/maintenance of Foxp3-expressing Tregs *in vitro*. However, this view is in contrast to an earlier finding which showed that IL-2 is required for the development of autoimmune response, although some aspects of autoimmune response are not regulated by IL-2 (Sharma and others 2009).

Other studies have implicated hepatitis C virus (HCV) infection and IFN-α therapy in the development of AITD. Chronic HCV infection has been shown to be associated with increased incidence of clinical and subclinical AIT (ie, the presence of thyroid antibodies in euthyroid subjects). Moreover, IFN-α therapy of chronic HCV infection is associated with subclinical or clinical thyroiditis in up to 40% of cases—in some cases, necessitating discontinuation of therapy. However, the mechanisms causing these conditions are still poorly understood (Tomer 2010).

IFN-γ is the archetype Th1 cytokine produced by CD4+ Th1 cells, CD8+ T cells, and NK cells. IFN-γ alone or in combination with other inflammatory cytokines induces MHC class I and II on APCs and other cells, and up-regulates the expression of adhesion molecules as well as certain chemokines and chemokine receptors to recruit T cells to the site of inflammation. It also activates macrophages and promotes IgG2A antibody production (Boehm and others 1997). A combination of IFN-α, produced by the thyroid, and increased levels of IFN-γ produced by the thyroid infiltrating lymphocytes have been shown to facilitate apoptosis of thyroid follicular cells through caspase activation (Wang and others 2002). Neutralizing antibodies to IFN-γ could prevent the disease and decrease Tg-specific T cell responses, suggesting that this cytokine plays a significant role (Tang and others 1993). However, the role of IFN-γ is controversial and studies from other groups show that IFN-γ may not be essential for the induction of AITD. Systemic administration of IFN-γ has been shown to suppress EAT (Vladutiu and Sulkowski 1980), and mice deficient in IFN-γ (IFN-$\gamma^{-/-}$) (Tang and others 1998) or the receptor for IFN-γ (IFN$\gamma R^{-/-}$) (Alimi and others 1998) are susceptible to EAT.

Although GD is considered a Th2-type disease, a role for both IFN-γ and IL-4 in some murine models of experimental autoimmune Graves' disease (EAGD) has been proposed. Knockout of IFN-γ generally does not prevent development of EAGD (Nagayama and others 2003, 2004), whereas knockout of IL-4 inhibits disease development (Nagayama and others 2004). In another study, IFN-γ was shown to play an important role in EAGD induced by immunization with TSHR antigen (Pichurin and others 2001). Differences in animal models, modes of immunization, and interaction with other cytokines may thus alter the final outcome of cytokines in both HT and GD.

Th2 Cytokines in AITD

Naive T cells differentiate into Th2 cells by activation of the STAT-6 signaling pathway. Engagement of T cells via

TCR and IL-4 receptor leads to the phosphorylation of STAT6, which is critical for the induction of the Th2 transcription factor GATA3. GATA3 transactivates Th2-specific cytokines such as IL-4, IL-5, and IL-13, and down-regulates STAT4 and IL-12Rb2, which are essential to generate a Th1 response (Zheng and Flavell 1997). Another transcription factor, c-maf, also contributes to Th2 differentiation by transactivating IL-4 transcription (Ho and others 1996).

GD in humans has long been thought to be a Th2-dominant autoimmune disease where TSHR autoantibodies play a crucial role in the pathogenesis of disease. Most of the information available on the effects of cytokines in GD is from animal models of EAGD. Our extensive studies on EAGD revealed that adjuvant composition as well as the glycosylation of the antigen could influence the titer, subclass, and fine specificity of antibodies to TSHR (Patibandla and others 1996, 1999; Seetharamaiah and others 1996). In our studies we determined whether skewing the immune response in favor of a Th1 or a Th2 type of response could influence the pathogenesis of EAGD. This was accomplished by treating mice with either Flt3L or GM-CSF, respectively. Although we were able to skew the response to TSHR toward Th1 or Th2 type, we were unable to alter the disease outcome. This was most likely due to the inability of Flt3L and GM-CSF to sustain Th1 and Th2 responses, respectively, for a longer duration required for the disease induction. Therefore, we tested to see if total absence of either IL-4 or IFN-γ could affect the development of EAGD. We immunized wild-type, IFN-γ(−/−) and IL-4(−/−) BALB/c mice with TSHR. Nearly 100% of the wild-type and IFN-γ(−/−) mice developed EAGD with optimal TSHR-specific immune responses, whereas IL-4(−/−) mice completely resisted disease development and showed delayed and suboptimal pathogenic antibody response. These data demonstrated that skewing immune responses to TSHR, using either Flt3-L or GM-CSF in favor of Th1 or Th2, respectively, may not be sufficient to alter the course of the disease, whereas the total absence of IL-4, but not IFN-γ, can prevent the development of EAGD (Dogan and others 2003). Thus, the induction of hyperthyroidism can be modified by manipulating immune responses toward Th1 or Th2 using different adjuvants, cytokines, or appropriate knockout mice (McLachlan and others 2005).

Th17 Cytokines in AITD

Th17 cells are differentiated by a combination of the cytokines TGF-β and IL-6 (Bettelli and others 2006; Mangan and others 2006; Veldhoen and others 2006). IL-6 signals via the STAT3 pathway (Yang and others 2007), but Th17 cells can also be induced by TGF-β in the presence of IL-21 (Korn and others 2007). TGF-β and IL-6 induce transcription factor RORγt, which may transactivate many components essential for differentiation of Th17 cells, including IL-17A, IL-17F, and IL-23R (Zhou and Littman 2009). Besides RORγt, the transcription factor ROR α is also involved in Th17 differentiation.

Several new studies have implicated Th17 cells both in HT and in GD in humans and in animal models. Iodine-induced AIT in nonobese diabetic-H2(h4) mice, a spontaneous mouse model of HT, revealed increased numbers of Th1 and Th17 cells in the spleens and thyroid glands of iodine-fed wild-type mice. Furthermore, the incidence and severity of intrathyroidal lymphocyte infiltration was markedly reduced in iodine-treated IL-17(−/−) mice, indicating that both Th1 and Th17 cells are critical for the pathogenesis of spontaneous AIT (Horie and others 2009). Enhanced levels of T cells synthesizing IL-17 and IL-22 in the peripheral blood of AITD patients, expression of IL-17 and IL-22, and an enhanced number of IL-23R+ cells were detected in thyroid glands from HT patients compared with GD patients or controls (Figueroa-Vega and others 2010). These studies demonstrated an increased differentiation of Th17 lymphocytes and enhanced synthesis of Th17 cytokines in AITD, mainly in HT. The discovery of Th17 cells and the recent findings have challenged the notion that HT is solely a Th1-mediated disease. In fact, the expression levels of Th1 cell-related T-bet and IFN-γ mRNA in peripheral blood mononuclear cells (PBMC) from HT were significantly decreased, whereas RORγt and IL-17 were increased, in patients with HT. In addition, the expression of transcription factors T-bet and RORγt mRNA correlated negatively, suggesting that Th17 cells rather than Th1 might be involved in the pathogenesis of HT (Shi and others 2010). In a very recent finding, the Th17 cells were also shown to induce GD in NOD-H2(h4), but not in BALB/c mice, thus showing that the effect of IL-17 may also differ with the genetic background (Horie and others 2011).

Regulatory Cytokines in AITD

TGF-β and IL-10 are the most important cytokines that have been implicated in the induction/maintenance of tolerance and prevention of autoimmunity. Tregs play critical roles in the induction of peripheral tolerance to self- and foreign antigens. Naturally occurring CD4+CD25+ Tregs express Foxp3 and generally mediate suppression by contact-dependent mechanisms (Bhattacharya and others 2011). TGF-β facilitates induction of Foxp3-expressing CD4+CD25+ Tregs and can therefore indirectly influence T cell activation. TGF-β is also a potent regulator of Teff differentiation, and it generally inhibits the acquisition of Th cell functions (Gorelik and others 2002). TGF-β blocks Th1 cell differentiation by reducing IL-12 receptor β2 (IL-12Rβ2) and T-bet expression (Gorham and others 1998; Gorelik and others 2002), and Th2 cells by inhibiting the expression of GATA-3 (Gorelik and others 2000; Heath and others 2000). In AITD, reduced TGF-β levels have been associated with HT (Akinci and others 2008; Vural and others 2009), whereas increased levels of TGF-β secretion by Tregs have been found to suppress EAT (Wang and others 2009). In a recent study, Transgenic NOD.H-2h4 mice expressing TGF-β under the control of the Tg promoter were generated and found to have higher frequencies of Foxp3+ Tregs compared with nontransgenic WT mice (Yu and others 2010) and the development of spontaneous AITD was inhibited.

Additional important mechanisms enforce immunological self-tolerance in the periphery. IL-10 is a regulatory cytokine that plays a central role in controlling inflammatory processes, and IL-10-secreting T cells may constitute additional mechanisms that are responsible for peripheral tolerance. Tr1 are induced upon antigen exposure and exhibit significant regulatory activities. Similarly, Th3 cells secrete both IL-10 and TGF-β and play a role in the induction of peripheral tolerance. IL-10 downregulates the expression of MHC class II and co-stimulatory molecules such as CD54, CD80, and CD86 (de Waal Malefyt and others 1991; Ding and others

1993; Moore and others 2001). Reduced expression of these molecules would therefore significantly affect the T cell-stimulating capacity of APCs (de Waal Malefyt and others 1991; Fiorentino and others 1991; Ding and Shevach 1992). Several studies have shown that IL-10 can protect against the development of HT and GD. In preliminary studies, injection of mTg-activated spleen cells cultured in the presence of recombinant IL-10, and not in its absence, into irradiated CBA/J mice induced a significant decrease in lymphocytic infiltrations in the recipient thyroid glands, but failed to reduce anti-mTg autoantibody production *in vivo* (Mignon-Godefroy and others 1995a). However, another study has shown that the effect of IL-10 is more likely on B-cell proliferation and antibody production and not on the pro-inflammatory cytokines (de la Vega and others 1998).

In our studies, treatment of mTg-primed mice with GM-CSF suppressed EAT and resulted in considerable expansion of Tregs and significantly higher levels of IL-10 compared with mTg-primed, untreated control mice. Administration of anti-IL-10R Ab into GM-CSF-treated mice abrogated GM-CSF-induced suppression of EAT without affecting the GM-CSF-induced expansion of CD4+CD25+ T cells. Moreover, our study showed that the IL-10-induced immunosuppression was due to its direct effects on mTg-specific Teff (Gangi and others 2005).

IL-10 is a key regulator of inflammation, and it can inhibit both Th1- and Th2-type immune responses through the suppression of pro-inflammatory cytokines and T cell proliferative responses (Groux and Cottrez 2003). One of the major mechanisms of IL-10-mediated suppression of T cells is through selective inhibition of the CD28 co-stimulatory pathway (Zhang and Kong 1998). However, in thyroiditis, alternative mechanisms of action of IL-10 have been proposed (Mignon-Godefroy and others 1995b; Batteux and others 1999; Tourneur and others 2002; Zhang and others 2003). Injection of cDNA expression vectors encoding IL-10 into the thyroid can significantly inhibit lymphocyte infiltration and development of EAT, and prevent progression of the disease (Batteux and others 1999). This suppressive effect of IL-10 is mediated either through enhancement of FasL expression on thyrocytes and induction of activation-induced cell death of thyroid-infiltrating T lymphocytes (Tourneur and others 2002) or through a potent up-regulation of anti-apoptotic molecules, such as cellular FLIP and Bcl-x_L, which can prevent CD95-induced apoptosis of thyrocytes (Stassi and others 2000; Stassi and De Maria 2002). Conversely, direct injection of IL-1 and TNF-α into the thyroids of mTg-primed mice can induce thyrocyte apoptosis, indicating that pro-inflammatory cytokines may play a critical role in thyroid destruction (Wang and others 2002). These observations suggest that IL-10 could be mediating its effects through suppression of pro-inflammatory cytokine production.

Cytokine Modulation As Potential Therapeutic Approach in AITD

Pathogenesis of autoimmune diseases is frequently characterized by pro-inflammatory cytokine production such as TNF-α and IFNs. Consequently, there are 2 major approaches for treatment of autoimmune diseases. One is to either block the action of the pro-inflammatory cytokine or interfere with its production, whereas the other is to use immunomodulatory cytokines that can restore the Teff/Treg balance. One of the major breakthroughs that contributed to the first approach was the development agents that can inhibit TNF-α function including monoclonal antibodies and soluble receptors. This approach has been successfully used in treating RA and Crohn's disease (O'Shea and others 2002) and 3 licensed agents, adalimumab, etarnecept, and infliximab, are

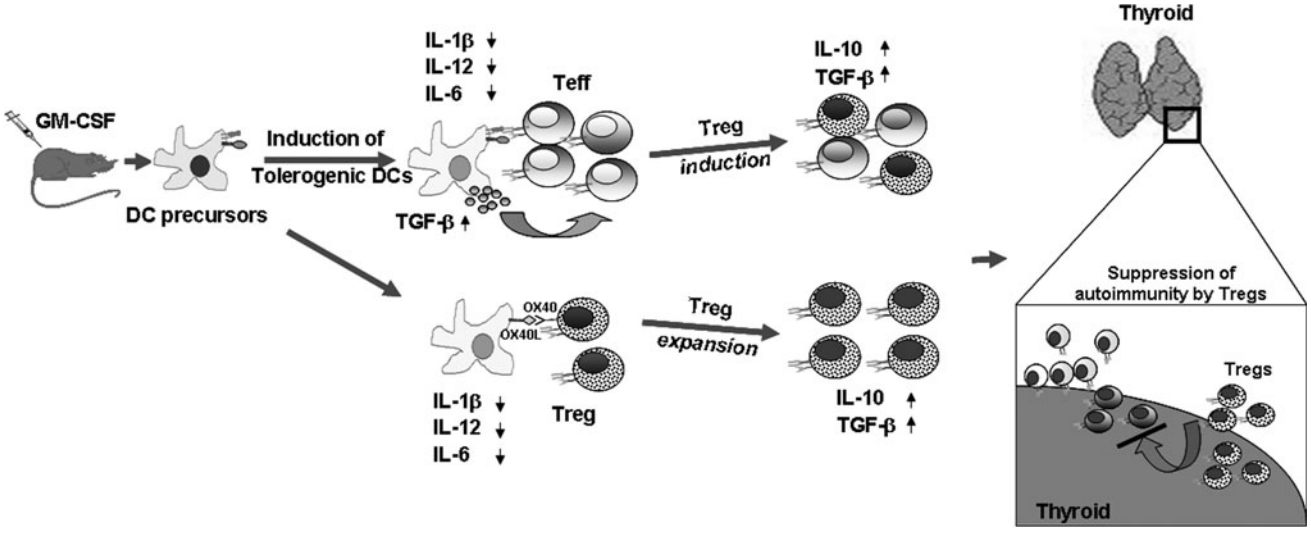

FIG. 2. GM-CSF induces tolerogenic DCs and suppresses EAT. Pro-inflammatory cytokines play a crucial role in the generation of an inflammatory response and the subsequent induction of EAT. Treatment of mice with GM-CSF induces semi-matured tolerogenic DCs that are characterized by the reduced levels of pro-inflammatory cytokines such as IL-1β and IL-12. These tolerogenic DCs, instead of activating pathogenic Teff, induce or expand Tregs that produce IL-10 and TGF-β. These regulatory cytokines counteract the role of the pro-inflammatory cytokines resulting in the suppression/prevention of EAT. GM-CSF, granulocyte-macrophage colony-stimulating factor; Teff, effector T cells; Tregs, regulatory T cells; TGF-β, transforming growth factor-β.

currently in the market for the treatment of immune-mediated inflammatory diseases (Silva and others 2010). In this context, the efficacy of an anti-TNF-α monoclonal antibody has been tested in a mouse model of granulomatous experimental thyroiditis. Although the disease severity in antibody treated mice was comparable to that of untreated control mice, antibody-treated mice showed less fibrosis and were able to clear thyroid lesions earlier (Chen and others 2007).

Our laboratory has been actively investigating the pathogenesis of AITD and exploring means to suppress them by using cytokine modulators of DCs. Studies from our laboratory showed that administration of GM-CSF could prevent, as well as suppress ongoing, EAT (Vasu and others 2003; Gangi and others 2005; Ganesh and others 2009). On the other hand, treatment with Flt3-L, which enhanced Th1 type of response, exacerbated the disease (Vasu and others 2003). GM-CSF-induced suppression of EAT was associated with a selective expansion of CD4+CD25+ Foxp3+ T cells (Tregs) that suppress mTg-specific responses through increased production of IL-10 (Gangi and others 2005; Ganesh and others 2009). These observations have been substantiated by others (Morris and others 2003). Similarly, treatment with GM-CSF reversed experimental autoimmune myasthenia gravis (EAMG) in C57BL/6 mice (Sheng and others 2006, 2008, 2009; Meriggioli and others 2008) and prevented the development of T1D in NOD mice (Gaudreau and others 2007; Cheatem and others 2009). Subsequent studies showed that GM-CSF acted primarily on DC precursors and caused an expansion of CD8a-DCs (Ganesh and others 2009). These DCs expressed very low to negligible levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, but expressed higher levels of TGF-β. Other studies using G-CSF saw a similar induction of Tregs that suppressed the development of T1D through the induction of TGF-β1 (Kared and others 2005). Although the mechanism by which GM-CSF imparts these tolerogenic phenotype to CD8a- DCs is not fully understood, we believe that GM-CSF-treatment of mice maintains DCs in a semi-matured phenotype, as indicated by high levels of expression of MHC class II and B7 molecules, but lower levels of expression of pro-inflammatory cytokines compared with untreated control mice (Lutz and Schuler 2002). Antigen presentation by these semi-mature DCs possibly led to the differentiation/expansion of IL-10 producing Treg cells (Gangi and others 2005) (Fig. 2). In contrast, Flt3L treatment led to an enhanced production of IL-12 and IFN-γ, indicating a predominantly Th1 response against mTg and thus did not suppress EAT (Vasu and others 2003). We speculate that antigen-specific Tregs possibly migrate to the thyroid and actively and dominantly suppress autoimmune Teff function, thus leading to disease amelioration.

In our very recent studies, we have found that bone marrow DCs (BMDCs) derived *ex vivo* through GM-CSF treatment could selectively expand nTregs through a contact-dependent mechanism mediated by OX40L/OX40 interactions (Bhattacharya and others 2011). This mechanism was independent of TCR involvement but required IL-2. Additionally, these BMDCs secreted high levels of TGF-β that were sufficient to convert Foxp3- T cells to Foxp3$^+$ Tregs (i.e. induced Tregs) upon TCR stimulation. In summary, the above information collectively suggests that GM-CSF may be used as a therapeutic agent in AITD. Currently, our laboratory is conducting a clinical trial to test the efficacy of GM-CSF treatment in patients with autoimmune myasthenia gravis.

Summary

Studies from humans and animal models have revealed significant new insights into the complex role of cytokines in the pathogenesis of AITD. Modulating cytokine responses have yielded highly encouraging results and they hold considerable promise in the treatment of autoimmune diseases. Pro-inflammatory cytokines such as GM-CSF and IL1β can contribute to Foxp3+ Treg expansion, whereas a regulatory suppressor cytokine such as TGF-β can initiate a pathogenic Th17 T cell response. These observations highlight the paradoxical effects of cytokines and their critical roles in maintaining a delicate balance between health and disease. Therefore, additional studies to understand the complex interplay between different cytokines and their effects on the different components of the immune system in the context of a particular disease are essential.

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Author Disclosure Statement

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