9-2008

Therapeutic Benefits of Regulating Inflammation in Autoimmunity

Enayat Nikoopour
Jordan Ari Schwartz
Bhagirath Singh
University of Western Ontario, bsingh@uwo.ca

Follow this and additional works at: https://ir.lib.uwo.ca/mnipub

Part of the Immunology and Infectious Disease Commons, and the Microbiology Commons

Citation of this paper:
Nikoopour, Enayat; Schwartz, Jordan Ari; and Singh, Bhagirath, "Therapeutic Benefits ofRegulating Inflammation in Autoimmunity" (2008). Microbiology & Immunology Publications. 1.
https://ir.lib.uwo.ca/mnipub/1
Therapeutic Benefits of Regulating Inflammation in Autoimmunity

Enayat Nikoopour§, Jordan Ari Schwartz§ and Bhagirath Singh*

Department of Microbiology and Immunology and Robarts Research Institute, University of Western Ontario, London, Ontario N6A 5C1 Canada

Abstract: Autoimmunity results from the dysregulation of the immune system leading to tissue damage. Th1 and Th17 cells are known to be cellular mediators of inflammation in autoimmune diseases. The specific cytokine milieu within the site of inflammation or within secondary lymphatic tissues is important during the priming and effector phases of T cell response. In this review, we will address the nature of the inflammatory response in the context of autoimmune disease, specifically we will discuss the role of dendritic cells following stimulation of their innate pathogen recognition receptors in directing the development of T cell responses. We will focus on how dendritic cell subsets change the balance between major players in autoimmunity, namely Th1, Th17 and regulatory T cells. Th17 cells, once thought to only act as pathogenic effectors through production of IL-17, have been shown to have regulatory properties as well with co-production of the anti-inflammatory cytokine IL-10 by a subset now referred to as regulatory Th17 cells. IL-17 is important in the induction of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) and inflammatory bowel disease (IBD). Study of the inflammatory process following encounter with agents that stimulate the innate immune responses such as adjuvants opens a new horizon for the discovery of therapeutic agents including those derived from microorganisms. Microbial products such as adjuvants that function as TLR ligands may stimulate the immune system by interacting with Toll-like receptors (TLR) on antigen-presenting cells. Microbial agents such as Bacille Calmette-Guérin (BCG) or Freund's adjuvant (CFA) that induce a Th17 response are protective in models of autoimmune diseases particularly EAE and type 1 diabetes (T1D). The induction of innate immunity by these microbial products alters the balance in the cytokine microenvironment and may be responsible for modulation of the inflammation and protection from autoimmunity.

Keywords: Th17, inflammation, dendritic cells, autoimmunity, adjuvant, therapeutic use, immunoregulation.

INTRODUCTION

With the discovery of Th17 cells, reinterpretation of the findings linking infection, inflammation and autoimmunity previously interpreted under the Th1-Th2 paradigm is required. The paradigm suggests that Th1 and Th2 cells counter-regulate each other. Microbial agents capable of inducing T helper cell differentiating cytokines from dendritic cells (DCs) have a major impact on immune regulation, specifically in controlling inflammatory and autoimmune processes. In light of the recent findings that expanded the Th paradigm to include regulatory T cells (Treg) and Th17 cells, a new look at how the innate immune system recognizes microbial antigens and promotes various T cell-mediated adaptive responses is required. Microbial infections are known to influence autoimmune diseases in a variety of ways [1]. In this article, we will specifically focus on how microbial agents can influence inflammation that may regulate autoimmune diseases.

THE T-HELPER CELL PARADIGM AND AUTOIMMUNITY

One of the fundamental concepts of the adaptive immune system is the T-helper cell paradigm. The paradigm was first described in 1986, and dichotomized CD4+ T-lymphocytes into two distinct classes referred to as Th1 and Th2, based on their cytokine profiles [2]. Th1 cells are thought to be more of an effector subset, which drives cell-mediated immune responses, and Th2 cells are for class switching and antibody mediated clearance of pathogens. Since then, Th1 cells have been thought to be the main pathogenic T cell subset in most autoimmune diseases. However, gaps in the Th1/Th2 paradigm began to form when some findings were unexplainable by this model. IFN-γ, the prototypic Th1 cytokine is not essential in the development of experimental autoimmune encephalomyelitis (EAE), and IFN-γ receptor knockout mice experience exacerbated disease [3,4]. In fact, IFN-γ plays an important role in down-regulation of EAE at both the effector and induction phase of disease [4]. These issues have generated much speculation for the potential of other subsets that could be more relevant in autoimmunity. These include regulatory T cells that have a significant role in controlling autoimmunity [5]. In addition, a pro-inflammatory T cell subset has emerged in recent years, referred to as Th17 cells for their production of the cytokine IL-17 [6]. Table I summarizes major characteristic of various T cell subsets.

The role of Th1 cells in the development of certain autoimmune diseases was further brought into question when it was discovered that the Th1 inducing cytokine IL-12, contained a common p40 subunit with the cytokine IL-23 [7]. In the EAE model of autoimmune disease, it was assumed that Th1 cells were driving disease because p40 knockout mice were resistant to disease induction. However, knocking out the p19 subunit unique to IL-23 resulted in alleviation of disease in the EAE mouse model, whereas knockouts of the
p35 subunit that is specific for IL-12, had augmented disease [8]. IL-23 was linked to expanding CD4+ cells capable of producing the pro-inflammatory cytokine, IL-17 [9]. These new IL-17 producing cells were shown to be of a separate and distinct lineage from Th1 and Th2, and were named Th17 cells [6]. As Tbet and GATA3 are specific transcription factors for Th1 and Th2 lineage commitment respectively [6], the orphan nuclear receptor RORγt is a transcription factor for commitment to Th17 cells [10]. While TGF-β and IL-6 are cytokines contributing to differentiation of naive CD4+ T cells into Th17 cells [11,12], IL-23 produced by DCs works as a cytokine for maintenance and persistence of the already established Th17 memory population [9]. This is because naïve T cells lack IL-23 receptor expression and therefore cannot respond to IL-23, but IL-6 is able to induce IL-23 receptor expression on the developing Th17 cells [13]. In addition to IL-23, DCs secrete IL-27 [14], which counter-regulates Th17 development [15,16]. The effector functions of IL-23 cytokine have been shown in numerous disease models including EAE [17] and inflammatory bowel disease (IBD) [18].

Despite the central role of Th1 cells in autoimmune diseases, EAE can develop in the absence of the Th1-inducer cytokine, IL-12. More precisely, mice in the absence of Th1 related molecules such as IFN-γ and IL-12p35, and their respective receptors, develop EAE [19-22]. In a new perspective, autoimmune diseases such as EAE are now thought to be due to the presence of Th17 cells since neutralization of IL-17 cytokine can block disease development [23], and IL-23 deficient mice are protected from disease [17,23]. Nonetheless, there are findings showing that Th1 and Th17 cells are able to cross regulate each other and disturbance in this Th1/Th17 cross-regulation could lead to autoimmunity. IL-12 administration during initiation of EAE can have a regulatory role through IFN-γ production and inhibition of IL-17 production [24,25]. However, Th17 cells in the absence of regulation from Th1 cells in IL-12 or IFN-γ deficient mice are pathogenic [17].

**T HELPER CELL SUBSETS AND INFLAMMATION**

IFN-γ and IL-17 are signature cytokines of Th1 and Th17 cells, respectively. There are conflicting findings on cross-talk between Th1 and Th17 cells. *In vitro*, IFN-γ exerts an inhibitory action on IL-17 [6]. However, there may not be a clear dichotomy between these two cytokines as there are reports of *in vivo* co-production of IL-17 and IFN-γ in CD4+ T cells [6, 26, 27]. To reconcile these discrepant results, it should be considered that the development of Th17 cells from naïve precursor cells is potently inhibited by IFN-γ and IL-4, whereas committed Th17 cells are resistant to suppression by these Th1 and Th2 cytokines respectively [6]. We are not sure that co-production of IFN-γ and IL-17 cytokines can exacerbate or reduce disease severity. Stockinger et al. reported an increase in the number of IFN-γ and IL-17 double producer cells in the acute stage of EAE while these cells are decreased in chronic disease [27], suggesting a more pathogenic role for these cells. Contrarily, in our model of adjuvant protection from diabetes protected mice showed a higher frequency of IFN-γ and IL-17 double producer cells, negating their role as more pathogenic (JAS, EN, BS unpublished observations). In confirmation of this, neonatal induction of a vigorous IFN-γ and IL-17 immune response does not result in experimental autoimmune encephalomyelitis and can protect against the disease in adulthood [26]. Furthermore, it has been shown that DCs infected with *Mycobacterium tuberculosis* stimulate production of both IFN-γ and IL-17 from CD4+ cells [28]. Also, an increase in production of both cytokines in antigen specific CD4+ T cells can be seen in mice infected with Mycobacterium [28]. Bringing another finding to attention, that a regulatory subset of Th17 cells co-express IL-10 and IL-17 [29] further complicates interpretation of the immunoregulatory cross-talks between T cell subpopulations.

Our data on cross-talk between T cell subsets are limited to the action of Th1 or Th2 cytokines on Th17 development. Despite an inhibitory effect of IFN-γ and IL-4 on Th17 cells, IL-23 as a Th17 cell expanding factor has limited effects on Th1 or Th2 polarized effector T cells [6]. Th17 cells have a reciprocal relationship with regulatory T cells (Tregs) due to the Th17 cells requirement for TGF-β [11]. At low concentrations, TGF-β synergizes with IL-6 and IL-21 to promote IL-23 receptor expression, favoring Th17 cell differentiation. High concentrations of TGF-β repress the IL-23 receptor expression and favor Foxp3+ Treg cells [30]. As well, the Treg master regulator Foxp3 can bind to the Th17 transcription factor RORγt to prevent the expression of IL-17 [30]. Further evidence for a dichotomy between Tregs and Th17 cells is that Treg-promoting factors such as IL-2 and retinoic acid strongly inhibit Th17 cells [31,32]. The immune response in the intestine is a good model to show cross-talk between Treg and Th17 cells. IL-23 plays a key role in the balance between these two populations by restraining the activity of the Treg population [33]. Mice deficient in IL-23 are resistant to colitis development [33]. This was initially

---

**Table 1.** Major Subsets of CD4+ T Cells

<table>
<thead>
<tr>
<th>T Cell Subset</th>
<th>Differentiating Factor</th>
<th>Survival Factor</th>
<th>Cytokine Antagonists</th>
<th>Transcription Factors</th>
<th>Disease Involvement</th>
<th>Pathogen Association</th>
<th>Ref.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1</td>
<td>IL-12</td>
<td>IL-2</td>
<td>IL-10</td>
<td>Tbet</td>
<td>T1D, IBD, EAE</td>
<td><em>S. aureus, E. coli, T. gondii, K. pneumoniae</em></td>
<td></td>
</tr>
<tr>
<td>Th2</td>
<td>IL-4</td>
<td>IL-2</td>
<td>IFN-γ</td>
<td>Gata3</td>
<td>Allergy</td>
<td>Helminth</td>
<td></td>
</tr>
<tr>
<td>Tregs</td>
<td>TGF-β</td>
<td>IL-2</td>
<td>IL-6</td>
<td>Foxp3</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Th17</td>
<td>TGF-β, IL-6</td>
<td>IL-23</td>
<td>IFN-γ, IL-4, IL-2, IL-27</td>
<td>RORγt</td>
<td>EAE, IBD, Allergic Asthma</td>
<td><em>M. tuberculosis, K. pneumonia, M. pneumoniae, B. burgdorferi, C. albicans, Schistosoma sp</em></td>
<td>[38-44]</td>
</tr>
</tbody>
</table>

*References are only shown for microbial agents that stimulate Th17 response.*
presumed to be as a result of defective development of Th17 response in the gut but there is no significant reduction in IL-17 [33]. Indeed, the role of IL-23 in intestinal inflammation is independent of its role in promoting Th17 response and inversely related to the activity of regulatory T cells. In line with this, adoptive transfer of naïve T cells from IL-23 knockout mice fails to induce colitis and is associated with an increased frequency of Treg population [33]. This indicates an important role for IL-23 in restraining Treg cell responses in the gut to permit development of colitis by Th1 cells. There is still uncertainty in the data about the main pathogenic effector cell in autoimmunity being Th17 or Th1. In the autoimmune disease experimental autoimmune uveitis (EAU), the role of the Th17 effector is redundant with Th1, and each effector phenotype is sufficient to induce pathology [34]. Based on this, it has been suggested that autoimmunity can develop in the context of either Th1 or Th17 effector cells depending on priming conditions present during initial exposure to antigen [34].

**IL-17 PRODUCING CELL SUBTYPES**

Two subsets of Th17 have been identified and these are induced under specific conditions and have particular effector or regulatory properties. The inflammatory/effector T cells include the IL-17 and/or IFN-γ producing T cells and the regulatory Th17 cells include IL-10 and IL-17 double producing T cells [29].

Initial identification of IL-17-producing CD8+ T cells suggested that these cells were non-cytotoxic and had no function in any autoimmune disease models [35]. However, in human studies of multiple sclerosis, IL-17-producing CD8+ T cells have been identified at the site of MS lesions [36]. Further evidence has since been provided suggesting that IL-6-dependant CD8+ T cells are essential in an adoptive transfer model of colitis [37]. The role of IL-17 producing CD8+ T cells in disease or the delineation of their different subsets is still to be explored. In autoimmune diabetes, CD8+ T cells have a pivotal role in destruction of the islet cells, and efficient progression to diabetes requires CD8+ T cells as well as CD4+ T cells [38]. In NOD mice, the frequency of IL-17-producing CD8+ T cells is increased within the spleen during the progression toward diabetes (JAS, EN, and BS unpublished observations).

**DC MICROENVIRONMENT SHAPES THE INDUCTION OF TH17**

The role of priming of T cells for the induction of an adaptive immune response is undertaken by DCs. Toll-like pattern-recognition receptors (TLR), intracellular Nod-like receptors and C-type lectins are innate immune receptors of DCs for detection of microbial patterns. TLR agonists drive DCs to begin maturation and antigen processing essential to activation of CD4+ T cells. Microbial agents such as adjuvants, which function as TLR ligands, may stimulate the immune system by interacting with TLRs on DCs to influence differentiation of naïve cells toward one of the predominant Th1, Th2, Treg or Th17 cell pathways.

Th17 cells are important in the control or immunopathological consequences of microbial infections such as *Mycoplasma tuberculosis* [39], *Klebsiella pneumonia* [40, 41], *Mycoplasma pneumoniae* [42], *Borrelia Burgdorferi* [43] and *Candida albicans* [44] and *Shistosoma* [45]. IL-12 and IL-23, cytokines known to drive differentiation of naïve cells toward Th1 and Th17 cells respectively, are produced by DCs that have been activated by binding of microbial ligands to their pattern-recognition receptors (Table 2). For instance, recognition of a fungal cell wall component called β-glucan curdlan through dectin-1 signaling, induces the secretion of the proinflammatory cytokine IL-23 with little IL-12 in DCs. This preferential production of IL-23 in DCs activated by dectin-1 engagement strongly favors the differentiation of Th17 cells [46]. On the other hand, preferential production of IL-12 by DCs stimulated with LPS and CpG, favors a Th1 response [46]. While TLR4 agonist specifically promotes the production of the Th1-inducing cytokine IL-12, TLR2 stimulation results in preferential induction of IL-23 [47].

Infection with the spirochete *Borrelia burgdorferi*, can lead to development of Lyme arthritis. *B. burgdorferi* induces skewed IL-23/IL-12 cytokine production in DCs in favor of differentiation of Th17 cells [43]. Also, priming of T cells with APCs in the presence of *B. burgdorferi* lysates causes preferential expression of IL-17 mRNA [48]. The Th17 immune response plays a role in the development of the severe destructive arthritis by Lyme spirochete, as IL-17 neutralizing antibody inhibits the development of arthritis [49]. Spirochete-induced Lyme arthritis is a good model connecting inflammatory processes during the course of infection with autoimmune processes. Delineating the role of microbial pathogens in induction of Th17 or Th1 pathways

**Table 2. Differential Induction of Cytokines in DCs by Different Microbial Agents**

<table>
<thead>
<tr>
<th>Microbial Agent</th>
<th>Cytokines Profile of DC</th>
<th>CD4+ Th Phenotype</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curdlan</td>
<td>IL-23 ↑, IL-12 ↓</td>
<td>Th17</td>
<td>[45]</td>
</tr>
<tr>
<td>LPS</td>
<td>IL-12 ↑</td>
<td>Th1</td>
<td>[45]</td>
</tr>
<tr>
<td>CpG</td>
<td>IL-23 ↑</td>
<td>Th1</td>
<td>[45]</td>
</tr>
<tr>
<td>Borrelia burgdorferi</td>
<td>IL-23 ↑, IL-12 ↓</td>
<td>Th17</td>
<td>[42, 47, 48]</td>
</tr>
<tr>
<td>CFA (M. tuberculosis)</td>
<td>TGF-β ↑, IL-6 ↑, IL-23 ↑, IL-12 ↑</td>
<td>Th17, Th1</td>
<td>[27, 47, 49]</td>
</tr>
<tr>
<td>BCG (M. Bovis)</td>
<td>IL-23 ↑, IL-12 ↑</td>
<td>Th17, Th1</td>
<td>[50]</td>
</tr>
<tr>
<td>Zymosan</td>
<td>IL-23 ↓, IL-12 ↓, IL-6 ↓, TGF-β ↑, IL-10 ↑</td>
<td>Th17, Th1</td>
<td>[49, 58, 59]</td>
</tr>
</tbody>
</table>
and cross-talk between these T cell subsets can shed light on the role of infections in pathogenesis of autoimmune diseases.

**MYCOBACTERIAL REGULATION OF TH17 CELL INDUCTION**

Microbial pathogens such as *Mycobacterium tuberculosis* can stimulate IL-17 production in CD4⁺ T cells [28, 48]. Mycobacteria-induced Th17 cell differentiation is dependent on TGF-β and IL-6, because neutralization of these cytokines abrogates Th17 cell generation [50]. Microbial agents have long been used to modulate immune responses and provide protection against pathogens in the form of a vaccine or an adjuvant. Bacille Calmette-Guérin (BCG) has long been used for inducing protective immunity against *Mycobacterium tuberculosis* infections. Low-virulence self-limiting mycobacterial infection by BCG vaccination induces both IFN-γ and IL-17 producing cells initially, but later on induces a negative feedback loop whereby IFN-γ-producing cells limit Th17 cells [51]. Infection of APCs by BCG in an *in vitro* setting results in an increased polarization of T cells to an IL-17 producing phenotype [51]. IFN-γ alters the cytokine profile of BCG-infected DCs to the one that favors IFN-γ producing cells over IL-17 producing cells, by increasing the ratio of IL-12:IL-23 production. In contrast, IL-17 reverses the IL-12:IL-23 ratio [51]. This shows a cross-talk between Th1 and Th17 cells, the balance between which could have pathological consequences in inflammation and autoimmune. There might be qualitatively different T cell responses to different forms of heat killed, attenuated and live mycobacterium as exposure to heat killed mycobacteria can lead to regulatory epitope spreading for mycobacterial heat shock protein (hsp65), and protection in LEW rats rechallenged with self-hsp65 autoantigen [52]. This might explain why killed (CFA) or live attenuated (BCG) forms of mycobacterium have a therapeutic role in autoimmune diabetes disease [53-58]. Dated back to 1990, in our adjuvant therapy model, a single injection of complete Freund’s adjuvant (CFA) given at an early age (5 wk) prevented the appearance of diabetes and greatly increased the life span of NOD mice without additional therapy [57]. Since CFA contains a mycobacterial cell wall that has adjuvant property, this prompted us to investigate the protective role of mycobacterium in young NOD mice. Mice injected with *Mycobacterium bovis* (BCG vaccine) at 4 weeks of age were also protected from diabetes [56]. Later on, our findings were reported by other groups [53,55-58]. The fact that CFA injection can modulate Th17 cell response (EN, JAS, and BS unpublished observations) brings adjuvant therapy into the spotlight and further sheds light on the mechanistic role of microbial agents in changing the course of inflammation in an autoimmune disease background.

Microbial adjuvants are different in their potential to raise a Th17 response and its pathological consequences. Whereas zymosan and CFA have a common ability to stimulate TGF-β production from DCs [50], they differ in their ability to induce IL-23, IL-12 and IL-6 from DCs. This difference in the ability to induce cytokines could result in the differences observed between mice immunized with zymosan or mycobacteria in their potential to induce autoimmune diseases. DCs isolated from mice immunized with encephalitogenic peptides such as MOG in CFA for induction of EAE, produce higher levels of IL-23 and IL-6 compared to those from DCs of mice injected with zymosan as an adjuvant [50]. This may be related to the fact that zymosan induces DCs to secrete an abundance of IL-10 and little IL-12 [59]. This differential cytokine profile of DCs explains why there is disease reversal after the acute phase of disease in mice immunized with encephalitogenic peptide accompanied with zymosan but not CFA as an adjuvant. In general, different ratios of IL-23 and IL-12 produced by DCs in response to adjuvants presumably affect susceptibility to autoimmune diseases. Finally, zymosan is able to induce production of IL-10 [60] in addition to IL-17 in CD4⁺ T cells and this regulatory phenotype can contribute to the disease reversal.

It should be kept in mind that CD4⁺ T cells are not the only source of IL-17 production, and CD8⁺ T cells [37] as well as innate immune cells such as γδT [61] and NKT [62,63] cells are also able to produce IL-17. Specifically, in response to mycobacterial infection, γδT cells are a major IL-17 producing cell type [61]. The production of IL-17 by these cells is stimulated by IL-23, which is produced by *M. tuberculosis*-infected dendritic cells [61]. How IL-17 produced from innate immune cells in the inflammatory response following infection can shape adaptive immune response and contribute to the autoimmune disease process remains to be studied.

Mycobacterial products can also change the course of autoimmune disease in another experimental setting. Adjuvant arthritis (AA), an experimental model for human rheumatoid arthritis, is induced in the Lewis rat by immunization with *Mycobacterium tuberculosis* [64]. Arthritic Lewis rats raise T cell responses to the mycobacterial heat-shock protein (Bhsp65). Bhsp65 contains both arthritogenic and regulatory determinants. During the course of AA in the Lewis rat, there is a diversification of the response to the Bhsp65 C-terminal determinants (BCTD), and pretreatment of naïve Lewis rats with peptides comprising BCTD down-modulate the course of subsequent AA following injection of *M. tuberculosis* [64]. This immunosuppressive effect is not restricted to the mycobacterium. It has been shown that gut microflora can also play a role in modulation of the experimental autoimmune diseases. While chronic arthritis induced by administration of a single dose of streptococcal cell wall (SCW) develops easily in susceptible Lewis rats, another closely related strain, the Fischer 344 (F344) rat is resistant [65]. However, F344 rats that are raised under germ-free conditions become susceptible to the disease and re-exposure to the gut flora helps animals to recover their resistance to the induction of arthritis [65]. In fact, exposure of a susceptible rat strain to the conventional environment leads to a significant reduction in the incidence and severity of an autoimmune disease through spontaneous induction of T cells against regulatory determinants of Bhsp65. It has been suggested that molecular mimicry between mycobacterial hsp65 and microbial agents in the gut microbial flora leads to priming and expansion of T cells against regulatory determinants [64]. Another explanation would be that normal gut flora modulates the adaptive immune response by imposing a new balance between the regulatory T cells and pathogenic Th1 and Th17 subsets. It has been shown that LEW rats predominantly secrete the Th1 cytokine IFN-γ at the highest level during the recovery phase of AA, and pre-treatment of LEW rats with a peptide of self (rat) hsp65 (R465), which
induces T cells secreting predominantly IFN-γ, affords protection against AA and decreases IL-17 expression by the arthritogenic epitope-resitamted T cells [66].

Toll like receptors on DCs and differential expression of Th17-promoting cytokines upon binding of microbial pathogens to different TLRs on DCs can modulate the Th17 cell response. The presence of the adaptor molecule, MyD88, is required for Th17 development. MyD88-/- mice are resistant to EAE and splenic myeloid DCs (mDCs) from MyD88-/- mice fail to express IL-6 and IL-23, resulting in the suppression of a Th17 response [67]. Furthermore, different TLRs can have regulatory effects on IL-17 production. EAE disease was exacerbated in TLR4-/- and TLR9-/- mice, demonstrating a regulatory role for these TLRs in the induction of autoimmune neuroinflammation. There is an increase in the expression of both IL-6 and IL-23 by myeloid DCs in TLR4-/- mice resulting in higher Th17 responses. Similarly, IL-6 expression is higher in splenocytes from TLR9-/- mice when compared to WT mice. Thus, MyD88 mediates peripheral mDC IL-6 and IL-23 expression and Th17 responses with the ensuing development of EAE, whereas TLR4 and TLR9 modulate EAE symptoms [67]. It is noteworthy that TLRs could be present on T cells. Although much evidence implicates the TLR adaptor molecule, MyD88 as a key signaling component of innate responses, this molecule is also expressed in T cells [68]. There is a requirement for MyD88 in T cell-mediated resistance to a pathogen even in the setting of an intact innate response [68]. In a murine model of IBD, TLR signaling via MyD88 is important for effector T cell responses in the intestine, and the absence of MyD88 in adoptively transferred CD4+CD45RBhigh cells results in defective T cell function, especially in Th17 differentiation and in their ability to induce colitis [69].

Myeloid and plasmacytoid subsets of dendritic cells are different in their ability to promote or suppress Th17 response. Myeloid DCs prime naive CD4+ T cells in the CNS to induce a Th17 dominant phenotype. In contrast, depletion of plasmacytoid DCs from the CNS causes exacerbation of EAE severity along with enhanced CNS CD4+ T cell activation and IL-17 and IFN-γ production, which shows a regulatory role for this DC subset in dampening inflammatory response [70]. Interestingly, plasmacytoid DCs are incapable of secreting IL-23 and myeloid DCs seem to be the main source of IL-23 upon TLR ligand triggering [71]. In our model of adjuvant therapy, we have found an increase in the frequency of plasmacytoid DCs along with the increased expression of the IL-27 mRNA transcript in mice injected with mycobacterial adjuvant (JAS, EN, and BS unpublished observations).

**NOVEL THERAPEUTIC MODULATION OF AUTOIMMUNITY BY IL-10 PRODUCING REGULATORY TH17 CELLS**

The introduction of the Th17 cells has changed our understanding of inflammatory processes. Therefore, in addition to Th1 cells, IL-17 producing Th17 cells offer new drug targets to block inflammation and ultimately autoimmunity. Modulations of these cells by microbial agents such as adjuvants that influence innate immune responses through TLRs, offer a good alternative to immunotherapy of autoimmunity via regulatory T cells. Regulatory T cell-derived IL-10 can control inflammation induced by the effector T cells, but Treg cells utilize multiple mechanisms to limit immune responses such as we have shown for their ability to produce Granzyme B [72]. These results have important implications for developing novel therapies to treat organ-specific autoimmune diseases given that levels of IL-23p19 and IL-17 are elevated in many autoimmune diseases such as multiple sclerosis, IBD and rheumatoid arthritis.

Previously, IL-10 was thought of as a cytokine secreted mostly by Th2 and Tregs cells, and to have a major role in the regulation of Th1 cell mediated responses. However, IL-10 production is not restricted to these cells and recent data show the presence of IL-10 in Th1 cells as well. Production of IL-10 in Th1 cells acts as an autocrine negative feedback mechanism to regulate over-reactive immune response to infectious microorganisms [73-75]. Recent findings show that Th17 cells generated with TGF-β and IL-6 are co-producers of IL-17 and IL-10 with regulatory properties [29]. These IL-17 and IL-10 co-producer Th17 cells are not pathogenic and adoptive transfer of these cells along with pathogenic cells can suppress induction of EAE. While cells grown with TGF-β and IL-6, acquire a regulatory phenotype, cells grown with IL-23 gain a pathogenic function. In line with these data, we have found IL-17 and IL-10 double producer CD4+ T cells following adjuvant therapy with the mycobacterial products, CFA and BCG. Surprisingly, IL-23 in vitro can expand these IL-17 and IL-10 producing cells (JAS, EN, and BS unpublished observations). This means that IL-23, known as a factor for expansion of already differentiated Th17 cells [9], can support expansion and persistence of IL-10+ or IL-10–Th17 cells equally. Also, immunization with mycobacterial hsp protects against adjuvant arthritis in rats possibly because of the induction of IL-10 producing T cells [76, 77]. This could also be as a result of inducing tolerogenic DCs that produce IL-10 with exposure to mycobacterial hsp [78]. As cytokines produced by DCs can shape Th17 response, differentiation of naive CD4+ T cells into IL-10 producing cells is also mediated by IL-27 [14,79-81] derived from DCs [14]. Awasthi et al. showed that DCs modified by Treg cells acquire a plasmacytoid-like phenotype (CD11c+CD205+) and secrete IL-10, TGF-β and IL-27 [14]. These DCs require the presence of the IL-27 receptor on T cells to induce IL-10 production, which shows a critical role for IL-27 produced by tolerogenic DCs in induction of IL-10. This finding implicates a role for different subsets of dendritic cells, including plasmacytoid and myeloid, in the modulation of IL-10 production from Th1 and Th17 cell responses. Fig. (1) depicts a model for the mechanism of action of CFA adjuvant in protecting mice from autoimmune diseases. Assuming the presence of regulatory IL-10 and IL-10 producing Th17 cells as a result of cross-talk between Tregs and neighbouring Th17 cells in mucosal immune sites, this might explain the protective role of IL-17 in the gut since IL-17 neutralization can worsen colitis in an experimental model of DSS-induced colitis [82].

Experimental induction of EAE requires MOG or PLP antigenic peptides in an adjuvant containing heat-killed mycobacteria. It is known that injection of CFA alone compared to CFA with antigens has a very different clinical outcome. While injection of CFA alone can protect mice from autoimmune diseases such as diabetes in NOD mice [53-58] and EAE [83], CFA injection with encephalitogenic peptides...
induces disease. Induction and modulation of Th17 response following injection of mycobacterial adjuvant can explain this discrepancy. Differential expression of IL-17, IL-10 and IFN-γ in the two protocols of injection might have a role in discriminating between disease outcomes. Whether or not these apparently different outcomes reflect different patterns of secretion of cytokines from DCs, which regulate the balance between Th1 and Th17 cells, is an issue to be dealt with in the future studies.

Despite successful use of mycobacterial adjuvants in prevention and reversal of diabetes in NOD mice, vaccination with BCG in young children with type 1 diabetes was not promising for reducing disease incidence or progression [84-86]. Difference in quality or quantity of immune responses toward live or attenuated forms of mycobacterium in human and mice due to different genetic composition might affect the expected tolerogenic phenomenon. Furthermore, study of Th1 and Th17 responses in individuals vaccinated with BCG in early life might explain why BCG vaccination is not effective in humans so far. In this regard, it is necessary to look at the immune response during the course of mycobacterial infection and BCG vaccination in humans. Incubation of whole blood from mycobacteria-exposed individuals with BCG induces IL-17 production in CD4+ cells with long-lived central memory phenotype along with Th1 effector response [87]. Also, data on the human immune response to Mycobacterium tuberculosis (TB) shows that CD4+CD25+Foxp3 regulatory T cells are expanded in the peripheral blood of patients with TB along with more expression of TGF-β and IL-10 mRNA, and this may contribute to suppression of Th1-type immune responses [88]. BCG vaccination of human newborns induces mainly type 1 cytokines such as IFN-γ, IL-2 and TNF-α in CD4+ T cells with low frequency of type 2 cytokines such as IL-10 and IL-4 expression [89]. It is noteworthy that children vaccinated with certain strains of BCG show higher expression of IL-27 mRNA [90], a cytokine known to induce IL-10 in T cells [14]. Cytokine analysis on the effect of BCG is focused primarily on IFN-γ or other Th1 related cytokines due to the presumed role of these cytokines in protective immunity against mycobacterial infection. In this respect, a new study centered on the evaluation of IL-10 and IL-17 levels in infants vaccinated with BCG is needed to determine the efficacy of BCG treatment for autoimmune diabetes. It should be kept in mind that the BCG strain [90], time of vaccination, dose, and percutaneous or intradermal route of administration [91] are factors that must be considered to optimize adjuvant therapy.

CONCLUDING REMARKS

As the key regulators of inflammation, Th1 and Th17 cells have a critical role in autoimmune diseases. Although Th17 cells were proposed to act only as effectors through production of IL-17, recent data has demonstrated that a Th17 subset has regulatory properties. In addition, dendritic cell subsets play an important role in shifting the balances between Th1, Th17 and regulatory T cells. The microbial agents can influence the induction of these cells. Therefore, the stimulation of innate immune responses and regulation of inflammatory responses by microbial agents could potentially act as new therapeutic agents in prevention and regulation of autoimmunity. The induction of innate immunity by these microbial products could alter the balance in the local cytokine microenvironment and may lead to modulation of inflammation and protection from autoimmunity. Therefore, the study of inflammatory processes following encounter with microbial agents that stimulate innate immune responses such as adjuvants, could be helpful in the design of new therapeutic agents to control autoimmune disease.

ACKNOWLEDGEMENTS

Work in our laboratory was supported by the Canadian Institutes of Health Research. We thank Katrina Huszarik for helpful comments on the manuscript.

REFERENCES


Fig. (1). Role of adjuvants in the activation of T cells through modulation of DC subsets.


