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PERSPECTIVES IN BASIC SCIENCE

An appraisal of T cell subsets and the potential for autoimmune injury

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The initiation of T cell dependent immune responses requires the T cell to utilize a heterodimeric cell surface antigen receptor to recognize an antigenic peptide in association with major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells (APCs). This is a necessary but insufficient signal for T cell activation. T cells must additionally receive a costimulatory signal that can be delivered through a variety of receptor-ligand pairs. Of these, the best characterized is the CD28-B7 (CD80 and CD86) couple. It has been appreciated for decades that the outcome of the T cell-APC interaction is highly variable and can lead to different forms of immune responses. The past decade has witnessed major advances in the definition of how distinct lymphocyte functions are dictated by expression of distinct cytokines, activation of defined signal transduction pathways, and, most recently, expression of distinct transcription factors. The production of ever increasing numbers of induced mutant mouse strains has created new reagents in which to analyze the role of particular cytokines or other proteins in defined immune responses. While the temptation remains to accomodate emerging information into reductionist models of T cell dependent immunopathology, it is our view that *in vivo* immune responses are highly complex and regulated events that defy simple categorization.

The goal of this review is to provide an overview of current information relevant to how different types of T cell responses develop and how such responses relate to the expression of disease. Our review will focus in depth on the concept of a "Th1-Th2" paradigm and how that relates to harmful versus protective immune responses. We will briefly touch on the outcome of costimulation of T cells via distinct receptor-ligand couples, and the effect of a number of other defined cell surface glycoproteins and cytokines on T cell responses. Relevant work in model systems of inflammatory renal injury will be discussed where appropriate.

Key words: immune response, antigen presenting cells, cytokines, signal transduction, transcription factors, Th1-Th2, receptor ligand, glycoproteins, inflammation.

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POLARIZED T CELL RESPONSES: THE TH1 VS. TH2 PARADIGM

Over a decade has passed since initial studies by Mosmann et al defined two distinct subsets of murine $CD4^+$ T cell clones based on their ability to synthesize nonoverlapping profiles of cytokines [1]. Since that time, there has been an enormous amount of work examining the cellular and molecular basis for this polarization of $CD4^+$ T cell responses as well as investigations into the relationship between polarized $CD4^+$ T cell responses and immune responses to infections and self antigens. In this section, we will summarize some of this information. Figure 1 provides a schematic outline for the information discussed in this section.

Th1 and Th2 $CD4^+$ T cells are defined primarily by cytokine production

$CD4^+$ T cell clones, which produce interleukin (IL)-2, tumor necrosis factor-β (TNF-β), and γ-interferon (γ-IFN) and mediate DTH responses, are termed "Th1" clones, whereas those $CD4^+$ clones which express IL-4 and IL-5 and induce immunoglobulin (Ig)G1 and IgE production by B cells are termed "Th2" cells [2]. Although these initial observations were made with long-term cultured clones, subsequent studies established the existence of similar subsets *in vivo* [3, 4]. Although the statement is frequently made that Th1 and Th2 responses correspond to cell-mediated and humoral immunity, respectively, in fact Th1-like CD4+ T cells can provide help for the production of some Ig isotypes, in particular IgG2a in the mouse. The existence of discrete Th1 and Th2 subsets in humans is more controversial, although evidence exists for the paradigm in this setting as well [5].

Since the original description of Th1 and Th2 clones, additional characterization of these populations has been performed. A third subset, denoted "Th0" T cells, has been defined that produces a mixture of the Th1 and Th2 cytokine patterns [6]. IL-6, IL-10, and IL-13 have been added to the group of Th2 cytokines [7]. Although several cell-surface markers, including CD45 isoforms, have been proposed as candidates to distinguish the Th1 and Th2 subsets, the stable definition continues to be based on cytokine profiles and effector function. Two recent publications, however, have provided evidence that Th1 and Th2 subsets additionally exhibit distinct migratory capacities into tissues. These capacities map with distinct cell surface receptor expression. Murine Th1 cells, but not Th2 cells, can bind to P- and E-selectin [8]. The migration of these Th1 cells into inflamed sites can be blocked by antibodies to E- and P-selectin. Human Th2 cells, on the other

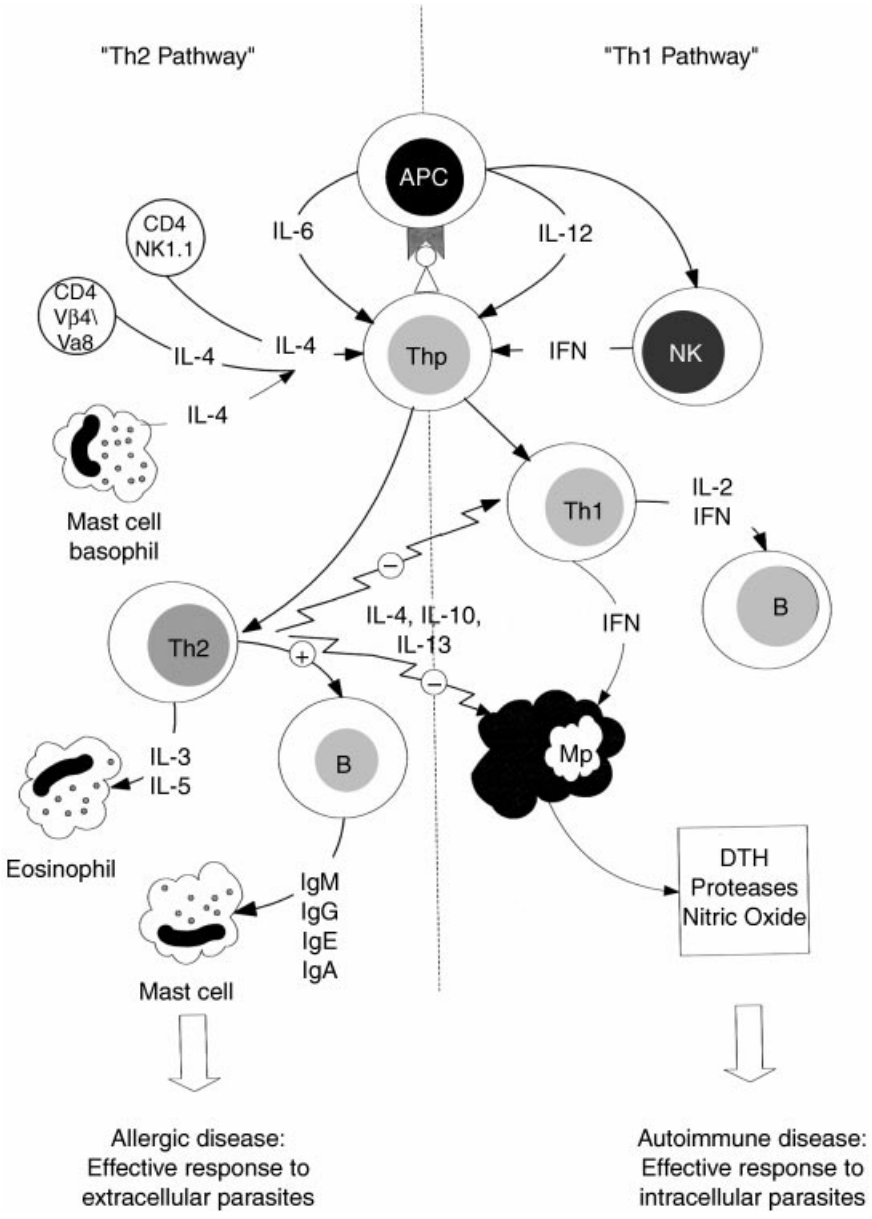

Fig. 1. A diagrammatic representation of T cell differentiation between classical Th1 and Th2 pathways. Details of each step in this diagram are in the text.

hand, express a high affinity receptor for the CC-chemokine eotaxin, called CCR3 [9]. Eotaxin is a potent chemoattractant for eosinophils and basophils. It is produced by phagocytes and epithelial cells. Since Th2-like cytokines like IL-4 and IL-5 are critical growth factors for eosinophils and basophils, the expression of CCR3 on Th2 cells provides a mechanism for the recruitment of this $CD4^+$ subset into sites of allergic inflammation.

Th1 and Th2 cells differentiate from a common precursor under the influence of distinct cytokine influences

How does a T cell *in vivo* become a Th1 or a Th2 cell? Extensive work performed using transgenic mice expressing a single T cell antigen receptor supports the conclusion that there is a single naive precursor population (expressing both γ-IFN and IL-4) that can give rise to cells committed to either the Th1 or Th2 effector lineage [10]. The major *in vivo* factor that has been implicated in directing naive $CD4^+$ cells to the Th1 or Th2 pathway is the cytokine milieu.

IL-12 and γ-IFN promote the expansion of Th1 cells [11, 12], and γ-IFN additionally blocks differentiation along the Th2 pathway. Studies supporting these conclusions have been performed using polyclonal stimuli *in vitro* followed by restimulation to induce cytokine expression and with TCR transgenic T cells stimulated with their relevant antigen. They have additionally been performed by administering IL-12 and γ-IFN to intact animals and studying the resultant populations.

IL-4 is the cytokine that has consistently demonstrated powerful effects in driving differentiation of $\alpha\beta$ $CD4^+$ T cells to the Th2 phenotype [13]. Studies utilizing mice deficient in specific cytokines, be they genetic knockout mice or animals receiving neutralizing antibodies to cytokines, have demonstrated profound influences on Th phenotypes [14–16].

What is the in vivo basis for distinct cytokine milieus? How dominant or selective expression of γ-IFN and IL-12, or IL-4 is achieved *in vivo* is as yet unclear. The cellular sources of these cytokines *in vivo* is focus of much investigation. There has been enthusiasm for the participation of a unique subset of T cells, the $NK1^+$ T cell, in polarizing $CD4^+$ cells to a Th2 response [17]. These T cells can recognize CD1, a family of MHC class I-like glycoproteins, which consist of a 45 kDa heavy chain noncovalently associated with β2-microglobulin. The CD1 molecules only display limited polymorphism, unlike the classical class I and class II MHC molecules. They have been divided into two groups, based on physiologic distribution and sequence [18]. The first group (CD1a, CD1b, and CD1c) are expressed on professional antigen presenting cells, immature thymocytes, and some B cells [19]. These CD1 molecules can present glycolipid and lipid antigens to αβ TCR-expressing T cells [20]. The second group of CD1 molecules (human CD1d and mouse CD1.1 and CD1.2) is expressed on thymocytes, peripheral lymphocytes, intestinal epithelial cells, and hepatocytes [21]. The natural ligands for this group are under intense investigation. However, mouse CD1.1 is recognized by $NK1^+$ T cells. Phenotypically these T cells are unusual, expressing both natural killer receptors (NK1, Ly-49A, Ly-40C) and T cell markers (intermediate levels of αβTCR, and frequently CD4). The TCR usage of these cells is restricted with most using a single TCR alpha chain (Vα14) paired with either Vβ2, Vβ7, or Vβ8.2 [22, 23]. $NK1^+$ T cells are rare among thymocytes (approximately 0.5 to 1.0% of total) but enriched in the liver and bone marrow, compromising 20 to 50% of these T cell populations [23]. These T cells generate large amounts of IL-4 following *in vivo* stimulation with anti-CD3.

This latter observation has led to the hypothesis that one of the *in vivo* functions of $NK1^+$ T cells may be to provide the early IL-4 burst that subsequently biases the immune response to differentiation down the Th2 pathway. This hypothesis is supported indirectly by the fact that some mouse strains (such as, SJL) that express low numbers of $NK1^+$ cells do not demonstrate the early IL-4 burst following anti-CD3 administration *in vivo*, nor do they produce IgE in response to anti-IgD antibodies [24]. SJL and NOD mice (the latter also have low numbers of $NK1^+$ T cells [25]) both display increased susceptibility to a variety of autoimmune diseases. Reports in both experimental and clinical autoimmune diseases have described decreased numbers of $NK1^+$ T cells with disease progression [26, 27]. Finally, *in vivo* treatment with an antibody to the dominant $NK1^+$ T cell TCR alpha chain (Vα14) accelerates murine lupus [26]. However, in mice made genetically deficient in CD1, $NK1^+$ T cells are severely reduced in numbers, and early IL-4 burst is missing, yet the $CD1^{-/-}$ mice generate IgE normally in response to anti-IgD challenge, and they can mount Th2-like recall responses [19, 28]. Since the impact of the mixed genetic background of the knockout mice on these read-out assays is as yet unclear, it is too soon to definitively rule out an important role for $NK1^+$ T cells in driving Th2 responses. It should additionally be noted that $NK1^+$ T cells can also express γ-IFN, but their role in Th1 responses is unexplored [29].

An alternative pathway to early IL-4 expression has been recently described and involves the stimulation of naive T cells by APC-derived IL-6 to make IL-4 [30]. Mast cells and basophils can additionally be induced to make IL-4 following cross-linking of Fc receptors by IgG or IgE [31, 32]. Finally, a recent study investigating the Th2-polarized response of $CD4^+$ T cells from Balb/c mice infected with *Leishmania major*, has strongly implicated a subset of $CD4^+$ T cells in the generation of an early IL-4 "burst" resulting in commitment down the Th2 effector pathway. These cells are of additional interest since they express a highly restricted αβ TCR (Vβ4, Vα8) [33]. Th2 polarization in the *Leishmania major* system additionally appears to be independent of $NK1.1^+$ T cells [34]. In the aggregate, these studies underscore the complexities of polarization of the $CD4^+$ Th response and the potential for distinct mechanisms of regulation in different model systems.

Classic antigen-presenting cells are the likely source of IL-12, which initiates Th1 effector responses. IL-12 is a 70 kDa heterodimer comprised of two covalently linked proteins (p40 and p35). The genes encoding both components must be expressed within the same cell to produce the heterodimer. Although the p35 gene is transcribed in many cell types, p40 gene expression is more highly restricted and tightly regulated. B cells, adherent monocytes, dendritic cells and skin Langerhans cells produce amounts of IL-12, which are likely important in directing the differentiation of $CD4^+$ T cells. A variety of other cell types, including some cells not traditionally regarded as classical APCs or as involved in the innate immune response, likely can also express IL-12 in culture under defined conditions. The *in vivo* significance of these observations has not yet been fully clarified. IL-12 acts directly on undifferentiated T cells and provides an early stimulus for natural killer cells to produce γIFN [35].

The Th1 and Th2 phenotypes involve the activation of distinct signal transduction pathways and transcription factors

How do distinct cytokines generate intracellular signals that result in CD4+ T cell polarization? IL-4 and IL-12 both activate members of a group of latent transcription factors termed signal transducers and activators of transcription (Stat). For example, IL-4 activates Stat-6 in Th2 cells and Stat-6 deficient mice cannot generate Th2 responses [36]. IL-12, on the other hand, induces the phosphorylation of Stat-4 in Th1 cells but not Th2 cells. Stat-4 deficient mice do not generate Th1 responses [37, 38]. The complete connection from activation of different Stat proteins to which genes are expressed in Th1 and Th2 cells is not yet clear. However, within the past year, several transcription factors have been identified to play critical roles in Th1 versus Th2 differentiation. Using cDNA subtraction by representational difference analysis, Zheng and Flavell recently identified the transcription factor GATA-3 as the dominant species present in such an analysis between normal Th1 and Th2 cells induced *in vitro* [39]. GATA-3 is selectively expressed both in differentiating and effector Th2 cells, it is required for the transcription of all Th2 cytokine genes, and the loss of Th2 cytokine gene expression in Th1 cells appears to be related to the down-regulation of GATA-3. Transgenic mice that constitutively express GATA-3 under the control of the CD4 promoter demonstrate Th2 cytokine gene expression in Th1 precursors [39]. This impressive body of work stresses the importance of this transcription factor in committing cells to differentiation along the Th2 pathway.

In complementary studies, recent work employed the combination of the T cell receptor transgenic system with mice lacking the expression of the transcription factor interferon regulatory factor-1 (IRF-1) to determine the importance of IRF-1 in Th1 versus Th2 differentiation [40, 41]. IRF-1 is a transcription factor

induced by interferon (IFN)-$\alpha/\beta/\gamma$. The studies performed in this system suggested that IRF-1 is essential for the development of the Th1 response and that it functions in multiple cell types which participate in the differentiation of Th1 $CD4^+$ T cells. T cells from these mice exclusively undergo Th2 differentiation *in vitro*. The animals demonstrate defects in macrophage function characterized by impaired IL-12 production. Their $CD4^+$ T cells are not responsive to IL-12 and they have abnormal natural killer cell activity [41].

Polarized T cell phenotypes display a complex relationship to the pathogenesis of autoimmune disease

While the molecular mechanisms underlying the polarization of $CD4^+$ Th cells are being meticulously delineated, the larger issue of the relationship between these polarized responses and autoimmunity continues to be more controversial. In this section, we will outline some of the generic issues raised in trying to evaluate polarized Th cell responses in the context of autoimmunity, and give specific examples where appropriate.

A dominant paradigm for infectious disease models, in particular the immune response to *Leishmania major*, has been that cell-mediated immunity critical for the defense against intracellular microorganisms is mediated by Th1-like T cells [42]. Resistance to extracellular pathogens is generated most effectively by activation of Th2-like cells. In some model systems of organ-specific autoimmunity, there is a good correlation between the preferential induction of Th1 T cells and the development of autoimmune pathology with impaired organ function.

Experimental manipulations that examine the relevance of the Th1-Th2 paradigm to organ-specific autoimmunity. How does one experimentally examine the issue of whether the mediation of an autoimmune disease is attributable to a polarized Th response? Supportive evidence can be obtained by examining the cytokine profiles of Ag-reactive T cells obtained from sites of immunization, or, preferably, from the disease site. The need for isolation of cells from disease foci and *ex vivo* culture has been obviated by the use of sensitive *in situ* techniques for detection of cytokine mRNA and intracellular cytokine proteins. Demonstrating organ pathology and functional organ impairment following the adoptive transfer of a T cell clone that displays polarized cytokine expression can provide evidence of relevance for immunopathology. Failure of such a clone to cause disease, however, does not negate the hypothesis, since cultured T cell clones may not traffic normally in the naive host, and they may require other immune cell types or microenvironmental factors only present in the immunized or disease-prone animal.

Some investigators have tested the relevance of polarized $CD4^+$ T cells to autoimmunity by employing maneuvers that lead to a polarized response and examining whether such a maneuver results in directionally consistent changes in disease expression. Examples of this could include the administration of polarizing cytokines, such as IL-12, γ-IFN, or IL-4, or the administration of neutralizing antibodies to such cytokines. Difficulties in the interpretation of such studies include the obvious problem that cytokine administration may trigger effector or regulatory events relevant to disease expression in conjunction with, but unrelated to, the observed polarization of the Th response. The major technical problems with antibody administration center around the duration of therapy required, the timing of the administration, and the development of immune responses targeted to the neutralizing antibody.

Genetically altered mice can be used theoretically to avoid some of the above-described problems with either cytokine or antibody infusion. For example, the incidence and severity of collagen-induced arthritis are significantly reduced in IL-12 knockout mice. These animals also display decreases in IgG2a responses to type II collagen (the immunogen) and depressed collagen-induced secretion of γ-IFN by splenocytes *in vitro* [43]. Yet it is frequently unclear what definitive conclusions can be drawn from studies of autoimmunity in cytokine knockout mice. The majority of cytokine knockouts generated to date are animals in which the cytokine(s) is deleted from birth in all cells of the animal. Given the well recognized redundancy in cytokine function, the failure of cytokine deletion to affect the course of an autoimmune process does not establish that in the intact organism that cytokine has no important role in the disease process. This generic issue has been well summarized recently [44].

The course of experimental allergic encephalitis (EAE) in various cytokine knockout animals provides instructive examples. There is little controversy that Th1-like T cells are important to the pathogenesis of EAE. The $CD4^+$ T cells that differentiate following immunization to produce disease in both mice and rats are Th1-like in cytokine expression. Th1-like T cell clones can adoptively transfer disease into naive hosts. Multiple studies have provided both direct and correlative evidence implicating TNF-α and lymphotoxin (LT)-α in the immunopathogenesis of EAE. Despite this, EAE develops normally in animals in whom both TNF-α and LT-α are inactivated [45]. Gamma-IFN knockout mice display heightened mortality when immunized to produce EAE, rather than protection [46]. Such results may be related to other cytokines substituting for the roles typically played by TNF-α, LT-α, and γ-IFN. Alternatively, cytokines such as γ-IFN may be subserving protective, rather than pathogenic roles that have been underappreciated [47]. In the case of γ-IFN, a strong argument can be made for the ability of this cytokine to induce nitric oxide synthesis through the cytokine inducible isoform and thereby dampen pathogenic T cell responses (see below) [48].

Alternate forms of antigen presentation that protect the host from induction of disease can lead to polarized T cell responses. The phenomenology that alternate, "less immunogenic" means of antigen presentation can elicit "tolerance" when the host is subsequently challenged with antigen in an immunogenic form has been established for years in the field of immunology. Examples of this include preimmunization with the antigen of interest in incomplete Freund's adjuvant (IFA) or administering the antigen orally, intranasally, or via the respiratory tract. When the antigen of interest is the target of an autoimmune disease, preimmunization in IFA or oral feeding can result in protection from disease [49–51]. These alternate forms of antigen presentation appear to preferentially activate T cells expressing Th2-like cytokine expression [50, 52]. Indeed, T cells expressing Th2-like cytokines probably account for some of the phenomenology previously attributed to suppressor T cells. Transforming growth factor-β (TGF-β) appears to play a prominent role in the suppression of disease mediated by these T cells [53]. Likewise, in murine interstitial nephritis, an autoimmune disease dependent on effector T cells, TGF-β plays an important role in the suppression of disease seen following alternate antigen presentation [54–56].

Th2 T cells can cause autoimmune disease in immunocompromised hosts. Although a paradigm based on the ability of Th1-like T cells to cause autoimmune disease and Th2-like cells to be protective is appealing in its simplicity and symmetry, recent studies suggest that the paradigm requires modification. For example, augmented pathology, probably attributable to autoantibodies, is demonstrable following immune deviation to a Th2-like response in a model of encephalomyelitis [57]. In addition, two recent publications have meticulously demonstrated that in immunodeficient hosts, Th2 T cells can cause pathologic lesions that histologically resemble allergic processes [58, 59]. Both studies utilized a similar experimental design of taking T cells bearing single antigen receptors from TCR transgenic mice, culturing them *in vitro* with cytokines designed to polarize the T cells along either the Th1 or Th2 pathway, and then transferring these cells into either immunocompetent or immunodeficient hosts. In one study the T cells were potentially encephalitogenic [58] and in the other study, potentially diabetogenic [59]. Th2 cells could transfer disease to NOD.scid mice, but not neonatal mice. The lesions induced in the NOD.scid mice differed from lesions induced by Th1 cells, in that the leukocytes infiltrated both the endocrine and exocrine tissue, and the lesions were comprised primarily of eosinophils and PMNs [59]. Treatment of NOD.scid recipients of Th2 cells with anti-IL-10, but not anti-IL-4, antibodies resulted in protection from this Th2 mediated lesion. In the encephalomyelitis model system, Th2 cells initiated spinal cord lesions characterized by unusually high numbers of PMNs and mast cells compared to the mononuclear cell infiltrate typically seen following transfer of Th1 cells. These Th2 cells could cause disease following adoptive transfer into RAG-1 or TCR-α knockout mice, but not TCR-δ knockout or immunocompetent mice [58]. These studies heighten concern about the efficacy of therapies based on immune deviation to a Th2 phenotype, particularly in immunodeficient hosts. Since patients with autoimmune diseases are frequently treated with immunosuppressive drugs, these findings are potentially of direct clinical relevance.

Recent information regarding the Th1-Th2 paradigm in models of renal disease

A variety of model systems in inbred rodents have been utilized to examine the immunopathogenesis of various forms of glomerulonephritis and interstitial nephritis. Several recent studies have provided interesting findings with regard to the Th1-Th2 paradigm in glomerulonephritis. A rapid proliferative glomerulonephritis can be produced in rodents if preimmunized (sensitized) with sheep globulin in CFA and then injected intravenously with sheep anti-mouse GBM globulin. The outcome of this protocol in inbred mouse strains which classically display Th1- or Th2-predominant immune responses is quite different. C57BL/6 mice (Th-1 predominant) develop a crescentic glomerulonephritis following this protocol [60]. Involved glomeruli displayed significant accumulations of T cells and macrophages. The severity of this lesion can be diminished through the inhibition of γ-IFN, supporting the notion that Th1-like cells are important in the immunopathogenesis [60]. Further support for the importance of Th1-like cells in glomerular pathology has been provided recently in the Heymann nephritis model of membranous nephropathy [61].

In the murine model of crescentic glomerulonephritis described above, treatment of C57BL/6 mice with IL-4 and/or IL-10, either prior to or following the induction of the immune response resulted in an improved functional outcome, diminished DTH responses to sheep globulin, and diminished glomerular crescent formation [62, 63]. The mechanism underlying this protective effect correlated with selective inhibition of the Th1 response to the antigen, when IL-4/IL-10 treatment was initiated prior to induction of the immune response [62]. With initiation of treatment after the establishment of injury, the mechanism of protection was less clear, as an analysis of the levels of IgG isotypes between experimental groups and splenic production of γ-IFN did not support a deviation of the induced immune response to a Th2-predominant form [63].

Although treatment with IL-4 and IL-10 was protective in a mouse strain selected on the basis of its propensity to display Th1-predominant immune responses, IL-10 alone was ineffective in a related model of accelerated anti-glomerular basement membrane (GBM) disease and crescentic glomerulonephritis in rats [64]. The differences in outcome may relate to differing protocols of administration (including the use of a single cytokine, rather than IL-4 and IL-10), but may also reflect the genetic background.

The outcome of the above described murine model is strikingly different in Th2-biased (Balb/c) mice [65]. When Balb/c mice sensitized to sheep globulin are given a subnephritogenic intravenous dose of anti-mouse GBM globulin, they develop glomerular deposition of Ig and complement, a significant neutrophil influx in the glomeruli, and significant proteinuria. There are no significant crescents or infiltrating T cells and macrophages in contrast to the C57BL/6 mice. The lesion in the Balb/c mice can be attenuated by complement depletion but not $CD4^+$ T cell depletion. The reverse is true for C57BL/6 mice [65]. These studies support the notion that the polarized Th responses may not map phenotypically with injury or protection but rather different forms of injury. Another well studied model system in which autoimmune glomerulonephritis develops in conjunction with a strong induced polyclonal Th2-like response is the $HgCl_2$ induced model of glomerulonephritis in the Brown Norway (BN) rat [66, 67]. Finally, in some transgenic mice that overexpress IL-4, there is demonstrable B cell hyperactivity, elevated IgG1 and IgE levels, and spontaneous glomerulonephritis with complement and antibody deposition within the glomerulus [68]. This is in contrast to the protective effect that constitutive expression of IL-4 has on the lupus-like glomerulonephritis in (NZW x C57BL/6. Yaa)F1 mice [69].

While the argument can be made that many studies in transgenic and knockout mice "muddy the waters" by creating highly artificial and unphysiologic scenarios, the bulk of emerging evidence suggests that the Th1-Th2 (pathogenic-protective) paradigm is too simplistic a model for understanding autoimmunity.

CO-STIMULATION OF T CELLS AND THE INITIATION OR PROPAGATION OF AUTOIMMUNE PATHOLOGY

T cell proliferation is critically dependent on costimulation. This section briefly reviews the process and recent studies on costimulation in the context of autoimmunity are discussed.

The B7:CD28/CTLA-4 pathway

The major ligand receptor pairs functioning as co-stimulatory molecules are the B7:CD28/CTLA-4 proteins. On the B7 side of this interaction, there are two ligands, B7-1 (CD80) and B7-2

(CD86), which are encoded by separate genes and are expressed on different cells with different types of kinetics. Both of these ligands can interact with each of two receptors expressed on T cells, CD28 and CTLA-4. CD28 is expressed on both resting and activated T cells, and following binding B7 molecules results in co-stimulation of T cell activation, augmenting cytokine expression (particularly IL-2) and proliferation [70, 71]. CTLA-4 is only expressed on activated T cells. It has a higher affinity for binding with B7-1 and B7-2 than does CD28. Although initial reports suggested that CTLA-4 was also involved in T cell activation, it is now clear that engagement of CTLA-4 results in the inhibition of IL-2 accumulation and T cell proliferation, especially when co-stimulation by B7-1 or B7-2 is limiting [72, 73]. This inhibitory effect of CTLA-4 engagement probably does not involve cell death, either necrotic or apoptotic, but rather a failure of the T cells to exit G1 [74]. These observations, from studies utilizing naive T cells stimulated *in vitro* with varying combinations of αCD3, αCD28, and αCTLA-4 or CTLA-4Ig, are well corroborated by the observations that blocking CTLA-4 *in vivo* results in augmented anti-tumor immune responses and more severe autoimmune disease [75–77], and by the severe lymphoproliferative disease seen in mice made genetically deficient in CTLA-4 [78, 79]. (CTLA-Ig is a hybrid fusion protein that contains the extracellular domain of CTLA-4 and an immunoglobulin Cγ1 chain (human) or Cγ2a chain (murine CTLA4Ig). It can neutralize both B7-1 and B7-2. Recent studies have further extended the role of the CTLA-4/B7 interaction to include the induction of anergy *in vivo*. In these studies, the induction of functional unresponsiveness in adoptively transferred TCR transgenic T cells was blocked by the administration of CTLA-4Ig, or CTLA-4 mAb, but not antibodies to CD28 [80].

The studies summarized above support the conclusion that the outcome of the T cell-APC interaction will be critically dependent on the nature of the costimulatory interaction, and not simply the absence or presence of costimulation, as was previously hypothesized. The cues that underlie a dominant B7-CD28 versus a B7-CTLA-4 interaction are being intensively investigated. The issues are complex given the potential for interaction between both B7-1 and B7-2 with either CD28 or CTLA-4. In general, the differences in B7-1 and B7-2 expression appear to be largely temporal and in part cell specific. B7-2 is induced earlier than B7-1 in most immune responses. Both molecules have been described to be inducible on dendritic cells, Langerhans cells, B cells, macrophages, and T cells. They are typically up-regulated following macrophage activation and are up-regulated by γ-IFN, LPS, GM-CSF [reviewed in 81].

The relative role of the B7-1 versus B7-2 interaction with CD28 and CTLA-4 has been investigated in a variety of autoimmune model systems. The variety of results obtained underscores the complexities of these interactions. When murine CTLA4Ig is injected into NZB/W F1 lupus prone mice, it blocks the production of anti-DNA antibodies and prolongs life [82]. The role, however, of B7-1 versus B7-2 is harder to generalize between autoimmune models. For example in the lupus prone mice, treatment with anti-B7-2 antibodies blocks production of some anti-DNA antibodies, particularly IgG1 anti-DNA antibodies, but was without effect on nephritis. Injection of anti-B7-1 had no significant effect on anti-DNA antibodies or nephritis. However, the injection of both anti-B7-1 and anti-B7-2 prevented production of anti-DNA antibodies of all subclasses, blocked the development of nephritis and resulted in a longer lifespan [83].

In the spontaneous model of insulin dependent diabetes in non-obese diabetic mice, treatment with CTLA4-Ig or anti-B7-2 blocked the development of diabetes. Antibodies to B7-1 had no protective effect [84]. The timing of this treatment was critical in that only animals treated between 5 to 7 weeks of age were protected. It is most intriguing that using NOD mice bred to the CD28 knockout mouse, or NOD mice bred to a transgenic mouse expressing CTLA-4Ig driven by a skin-specific keratin promoter, the development and progression of spontaneous autoimmune diabetes is promoted, rather than inhibited [85]. The authors propose that this may be attributable to a preferential activation of Th1-like rather than Th2-like autoreactive T cells early during life. This unexpected experimental result raises another caution flag regarding the interruption of this costimulatory pathway as a treatment for autoimmune disease.

In murine experimental EAE, injection with anti-B7-2 antibodies does not affect the severity of disease while injections with anti-B7-1 do [86, 87]. CTLA4-Ig can prevent the onset of collagen induced arthritis in BB rats [88]. These seemingly discrepant results in different model systems would mitigate against being able to broadly generalize the role of these interactive molecules in autoimmune diseases and the types of immune response that are generated. It seems possible that in each particular autoimmune disease the outcome of blocking both B7-1 and B7-2, or either independently, may additionally depend on the types of co-stimulatory molecules expressed on both professional and non-professional antigen presenting cells within the target organ of interest.

Role of the CD40-CD40L pathway

An additional co-stimulatory couple that is important in the expression of autoimmune disease is the interaction between the CD40 ligand on activated T cells and CD40, a molecule expressed on B cells, antigen presenting cells and a whole host of other parenchymal cells. Administration of blocking antibody to the CD40 ligand can block the development of collagen arthritis [89], the anti-DNA autoantibody response and renal disease in NZB/WF1 mice [90], membranous nephropathy [91], autoimmune oophoritis [92], and hapten induced colitis [93]. In genetically manipulated mice, the absence of the CD40 ligand ameliorates the expression of both EAE and the renal disease seen in MRL/*lpr* mice [94, 95]. In many of these model systems, the presumed mechanism of blockade of the CD40/CD40 ligand interaction has been to block autoantibody production. It has also been noted that impaired T cell responses can occur following interruption of this interaction, be that through a neutralizing antibody or through genetic deletion of the CD40 ligand. Blockade of the CD40/CD40L interaction results in an impaired expression of B7-1 and B7-2 on antigen presenting cells [96]. If B7-1 expressing APCs are provided to CD40 ligand knockout mice, which express T cell receptor specific for pathogenic epitope of myelin basic protein, the animals can develop autoimmune disease although they don't in the absence of those antigen presenting cells [95]. Whether blocking of the CD40/CD40 ligand interaction blocks autoimmune pathology through mechanisms other than blocking autoantibody expression and indirectly through blocking T cell activation from impaired B7-1 and B7-2 expression is a continued area of investigation.

Role of other cell surface moieties and mediators in dictating outcomes of immune responses

While the Th2, Th1 paradigm and outcomes of co-stimulation historically have assumed a prominent role in understanding the development of "harmful" versus "harmless" immune responses, there are a number of other cell surface glycoproteins and secreted molecules whose expression may also be important in defining a pathologic T cell response, and these are outlined below.

Role of restricted T cell antigen receptor usage in autoimmune T cell responses. The antigen receptor on the surface of most mature T cells (TCR) is an integral membrane protein comprised of heterodimeric α and β chains. The overall three dimensional structure of the TCR is quite similar to that of immunoglobulin (Ig), namely each α and β chain contains an amino terminal variable region and a carboxy terminal constant region. However, unlike immunoglobulin, which recognizes its ligand in the solution phase either on the surface of B lymphocytes or as a secreted molecule, the TCR only recognizes its ligand as a peptide fragment of the protein antigen in association with major histocompatibility complex (MHC) class I and class II molecules on the cell surface of other cells. In addition, the T cell receptor is not present in the serum as a secreted molecule.

Diversity in antigen recognition by T cells is calculated to be as high as for immunoglobulin due to the inherent diversity accomplished in the process of DNA rearrangements of the TCR variable element genes during T cell development and due to the random sorting of assembled TCR α and β chains [97].

The fact that T cell recognition occurs through clonally distributed TCR chains interacting with MHC-associated peptide fragments of foreign or self proteins, raises the possibility that either expression of unique TCR chains or presentation of unique peptide fragments of self antigens could be factors in determining susceptibility or resistance to autoimmune disease. Support for the idea that unique TCR expression was associated with autoimmunity initially came from observations in both the mouse and rat models of experimental allergic encephalomyelitis (EAE). In both rodent models, it was observed that the majority of T cells responsive to the major encephalitogenic epitopes of myelin basic protein (MBP) used a very limited repertoire of Vα and Vβ genes [98–102]. Furthermore, strategies aimed at deleting or inactivating cells utilizing these families of TCR chains resulted in protection from disease [98, 102–104].

Prompted by these studies, several groups have analyzed T cell responses and TCR usage in a number of clinical autoimmune disease settings. As an example, when studies were performed to assess human T cell responses to MBP, no measurable differences were observed in the frequency of MBP reactive T cells among peripheral blood lymphocytes isolated from normal individuals and patients with multiple sclerosis. Furthermore, there does not appear to be any convincing differences in TCR usage among normal individuals and patients with multiple sclerosis. There are no convincing similarities in TCR usage among MBP reactive T cell clones, isolated from multiple sclerosis (MS) patients, responding to the same epitope of MBP presented by the same HLA-DR or -DQ molecule. One exception of note, however, is the finding of Oksenberg and colleagues who described the presence of a common TCR rearrangement in demyelinating plaques isolated from CNS tissue of deceased MS patients [105]. These unique rearrangements were previously observed in human and rat T cell clones recognizing similar regions of MBP. These results suggest to the authors that certain epitopes of MBP are being recognized in the CNS of patients with MS and perhaps by T cells expressing a limited repertoire of TCR chains.

Overall, the conclusion from these studies and those in other autoimmune settings is that the TCR *per se* is quite likely not going to be a major susceptibility factor with respect to T cell recognition of autoantigens [106]. However, it is equally likely that the diversity of pathologic T cell responses in any individual or in different individuals of similar HLA type and responding to similar epitopes of an autoantigen, may in fact be limited.

If the TCR itself is not a determining factor in the selection of a limited autoaggressive repertoire, then perhaps the repertoire of HLA chains expressed by the individual predisposes towards autoreative T cell development. Inheritance of particular alleles of HLA genes has been associated with several autoimmune diseases over the years. In addition, more recently it has become appreciated that certain alleles are in fact protective towards the development of certain autoimmune diseases. The best example of this phenomenon is seen in the case of juvenile onset diabetes [107]. Because HLA-associated peptide presentation to T cells is critical for both T cell development in the thymus and T cell activation in the periphery, several models have been proposed to account for the apparent HLA linkage to autoimmune disease. It is possible that certain peptide/MHC combinations can cause the deletion of potentially autoreactive T cells maturing in the thymus to a lesser or greater extent depending on amino acid differences in the peptide binding domains of the HLA proteins. Since autoreactivity to many self antigens can be demonstrated in healthy individuals, it is unlikely that thymic deletion *per se* can explain HLA linkage and autoimmune reactivity. Certainly, the overall affinities of allowable self reactive antigen receptors could differ depending on the nature of the peptide/MHC combinations encountered during thymic T cell development and this difference in affinity could be a determining factor in whether a potential autoreactive T cell clone will be triggered or not. An alternative hypothesis has suggested that certain alleles of HLA may have higher affinities for certain self antigens and act as a "peptide sink," thereby removing or competing for particular self antigen epitopes that if presented on a permissive HLA proteins would evoke autoaggressive T cell responses [108]. This model could help account for the observation of protective HLA alleles. A slightly different version of this model would suggest that certain HLA alleles could bind particular regions of an autoantigen with high affinity, thereby altering the proteolytic processing of other epitopes for which autoreactive T cells may exist [109]. Undoubtedly, all of these possibilities as well as others not mentioned will prove to be true, in some form contributing to the overall complexity of autoantigen TCR repertoire development and T cell autoreactive responsiveness.

Role of the L-arginine:nitric oxide system in modulating T cell responses. The quantities of nitric oxide (NO) generated through the cytokine inducible isoform (iNOS, that is, inducible nitric oxide synthase) are an important cytotoxic effector mechanism for macrophages in the context of anti-tumor responses and for eradicating intracellular parasites [110]. Since NO also has profound antiproliferative effects on T cells [111], this system has the potential to be one that alters the expression of autoimmune

diseases dependent on activated T cells. We have recently investigated this hypothesis in two distinct models of organ-specific autoimmune disease, anti-tubular basement membrane disease with interstitial nephritis in the Brown Norway rat [112], and experimental allergic encephalomyelitis in the Lewis rat [48]. In each case the administration of a highly selective iNOS inhibitor, L-N-(1-iminoethyl)lysine (L-NIL), led to a more severe immune injury in animals immunized to produce disease. Interestingly, in adoptive EAE, treatment of the recipients of myelin basic protein reactive T cells with L-NIL led to less severe disease, suggesting that the immunosuppressive effect of endogenously produced NO may be predominantly on the induced T cell response, rather than T cell-target cell interactions. L-NIL augments mitogen-induced T cell proliferation and expression of γ-IFN protein by mitogen activated splenocytes [48]. L-NIL treatment of animals immunized to produce interstitial nephritis leads to augmented IgG responses to the immunogen and augmented expression of IL-2 and γ-IFN (but not IL-4) within the diseased kidney [112]. In the EAE model, treatment with L-NIL was sufficient to convert a typically nonsusceptible strain (F344) to a susceptible strain. These studies suggest that the intensity of cell-mediated immune responses can be potently regulated by endogenously produced nitric oxide. Since Th1-like cytokines such as γ-IFN are important for the up-regulation of iNOS during immune responses, the antiproliferative and other immunosuppressive effects of NO may form one limb of a critical feedback loop.

Lymphocyte migration and susceptibility to autoimmune disease. The importance of lymphocyte migration out of the vasculature and into the target organ as a susceptibility factor for the expression of autoimmune disease has been relatively underexplored. Host-dependent expression of integrins, adhesion molecules, chemoattractants, or their receptors may play critical roles in determining whether potentially injurious effector cells are capable of leaving the vasculature. Two recent studies provide support for an important role of alpha-4 integrins expressed on T cells in this process of T cell entry into target tissues [113, 114].

There is a growing interest in the role of chemokines in the phenotypic expression of autoimmune kidney disease [115–119]. With the recent discovery of genetic polymorphisms in chemokine receptors and the biologic importance of this trait in susceptibility to HIV infection, it is possible that further work will identify these chemokine receptor polymorphisms as additional susceptibility factors for autoimmunity.

CONCLUSIONS

The immune system has evolved to deal with infectious organisms. Major studies over the past ten years have provided an enormous amount of information regarding the various phenotypes of activated T cells, the cytokines they produce, the functions they mediate, their requirements for activation, and how they are differentially regulated. The characteristics which result in a particular T cell response being "host beneficial" in the context of infectious disease can be similar to those resulting in autoimmune pathology. Autoimmunity is a complex process, however, and it is clear that a simple Th1/Th2 paradigm does not fully explain all forms of autoimmune tissue damage. Moreover, although some rodent models of autoimmunity are more widely studied than others, each model system has unique characteristics that preclude generalizing conclusions from investigations into disease pathogenesis or treatment in one or two model systems. Given the available model systems, reagents, and major advances in understanding T cell biology, the next decade will undoubtedly witness innovative approaches to the therapy of autoimmune disease.

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APPENDIX

Abbreviations used in this article are: APCs, antigen presenting cells; CCR3, CC-chemokine eotaxin; EAE, experimental autoimmune encephalomyelitis; γ-INF, γ-interferon; IFA, incomplete Freund's adjuvant; Ig, immunoglobulin; IL, interleukin; iNOS, inducible nitric oxide synthase; IRF-1, interferon regulatory factor-1; L-NIL, L-N-(1-iminoethyl)lysine; MBP, myelin basic protein; MHC, major histocompatibility complex; MS, multiple sclerosis; NK, natural killer; NO, nitric oxide; STATs, signal transducers and activators of transcription; TCR, T cell antigen receptor; TNF-α, tumor necrosis factor-α; TNF-B, tumor necrosis factor-B.

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