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Cytokines Induce the Development of Functionally Heterogeneous T Helper Cell Subsets

Review

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The discovery that subsets of $CD4^+$ T cells could be distinguished according to their ability to produce discrete patterns of cytokines offered a logical explanation for early observations that differential immune responses can be elicited in response to antigen. Early events in an immune response stimulate the production of cytokines that direct the subsequent development of these T helper (Th) subsets with these discrete patterns of cytokine production (Mosmann et al., 1986). The development of these distinct Th subsets is strongly dictated by the type of antigen/microorganism invading a host, as well as the genetic background of the host, and it is likely that the dose and route of immunization of antigen may also be determining factors (Seder and Paul, 1994; Abbas et al., 1996; Constant and Bottomly, 1997).

Heterogeneity of T Cell Populations

Th1 cells, by their production of interferon-γ (IFNγ) and lymphotoxin, are responsible for directing cell-mediated immune responses leading to the eradication of intracellular pathogens (Mosmann et al., 1986; Sher and Coffman, 1992), but they may also cause immunopathology and organ-specific autoimmune disease if dysregulated (O'Garra and Murphy, 1993; Powrie and Coffman, 1993; Scott et al., 1994; Liblau et al., 1995). Because cytokines produced by Th2 cells, such as interleukin (IL)-4 and IL-5, can activate mast cells and eosinophils and in addition can result in elevated levels of IgE, they have been strongly implicated in atopy and allergic inflammation (Romagnani, 1994). The production of B cell growth and differentiation factors by Th2 cells may in part explain why certain immune responses are predominantly humoral, whereas delayed-type hypersensitivity responses, seen in other circumstances, are readily attributable to Th1 cells (Mosmann and Coffman, 1989). The ability of cytokines to stimulate different effector mechanisms and thus differential immune responses is also reinforced by the production of cytokines by each subset, which cross-regulate each other's function as well as development.

For example, IFNγ produced by Th1 cells inhibits the development of Th2 cells (Fitch et al., 1993) as well as humoral responses, whereas the production of IL-4 and IL-10 by Th2 cells inhibits Th1 development and activation as well as macrophage activation and bactericidal activity (Sher and Coffman, 1992; Moore et al., 1993).

One feature, which should be considered with respect to polarized Th1 and Th2 responses, is that they represent endpoints of chronic immunization or chronic disease. Th1 and Th2 clones were generally isolated from hyperimmunized mice (Mosmann and Coffman, 1989; Sher and Coffman, 1992) or during chronic diseases in humans (Romagnani, 1994). This explains why such populations were originally difficult to obtain from human systems, since such attempts involved stimulation of peripheral blood T cells from healthy individuals with mitogens (Romagnani, 1994). Indeed, human Th1- and Th2-type populations have now been isolated from peripheral blood, draining lymph nodes and affected tissues during chronic infectious diseases and allergy (reviewed in Romagnani, 1994).

Although IL-2 production has often been associated with a Th1 phenotype, the production of this cytokine cannot be classified as a hallmark of Th1 cells, since naive $CD4^+$ T cells as well as Th0 cells (described below) also produce IL-2 in response to antigenic stimulation (Sher and Coffman, 1992; Romagnani, 1994; Abbas et al., 1996). Helper T cells and clones that produce both Th1- and Th2-type cytokines (termed Th0) have been described in human and mouse systems (Sher and Coffman, 1992; Romagnani, 1994; Kelso, 1995; Abbas et al., 1996). Whether Th0 cells are precursors for Th1 and Th2 cells or represent a separate, stably differentiated population remains unclear (Kamogawa et al., 1993). It is possible that Th0 cells are involved in eliminating many pathogens, where a balance of both regulated cell-mediated immunity and an appropriate humoral response will eradicate an invading pathogen with minimum immunopathology. However, chronic conditions may result in polarized Th1- and Th2-type responses, which, by the counter-regulatory effects of the cytokines made by the reciprocal subsets, may not only be mutually exclusive but in addition pathogenic (Alwan et al., 1994). To what numerical extent Th1 and Th2 cells dominate such in vivo responses is as yet not clear, but their ability to influence chronic disease or pathology by their production of high levels of regulatory cytokines is not in doubt.

Th1 cells, important for cell-mediated immunity by their production of IFNγ and lymphotoxin, have been implicated in the immunopathology of certain organ-specific autoimmune diseases (Powrie and Coffman, 1993; Scott et al., 1994; Liblau et al., 1995). The hypothesis that protection from autoimmune diseases, such as experimental allergic encephalomyelitis and insulin-dependent diabetes mellitus, could be achieved by a "Th1 to Th2 switch" (Fowell and Mason, 1993; O'Garra and Murphy, 1993; Powrie and Coffman, 1993; Scott et al., 1994; Liblau et al., 1995) and that administration of soluble antigen without adjuvant (De Wit et al., 1992; Burstein and Abbas, 1993) or oral adminstration of soluble antigen (Weiner, 1997) led to unresponsiveness has triggered not only many studies for treatment of autoimmune diseases in mouse models but in addition has influenced the design of therapeutic strategies (Weiner, 1997). Many studies now suggest that alternative regulatory populations exist, which are somehow associated with, but distinct from, Th2 cells (Bridoux et al., 1997; Lafaille et al., 1997; Pakala et al., 1997). Regulatory $CD4^+$ T cell subsets have been described that can inhibit cell-mediated immune responses and/or inflammatory pathologies (Powrie and Mason, 1990; Fowell and Mason,

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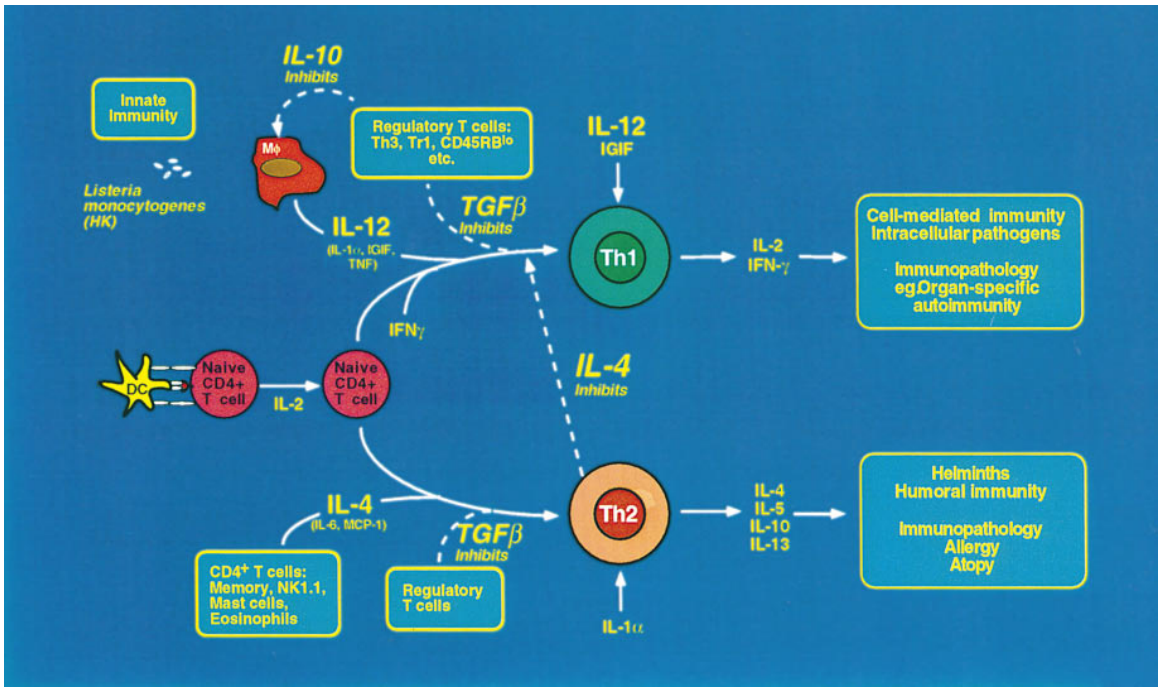

Figure 1. Regulation of Th Responses

Naive CD4$^+$ T cells can develop into Th1 cells responsible for cell-mediated immunity in response to IL-12 with the participation in BALB/c mice of other cofactors, such as IL-1α and IGIF. Th1 development is dependent on IFNγ, and maintenance of phenotype depends on stimulation in the presence of IL-12 and IGIF. Development of Th1 responses can be antagonized directly by IL-4 and indirectly by IL-10, which inhibits the production of inflammatory mediators, such as IL-12 and IGIF, from macrophages stimulated by the innate immune response. Th2 cells dependent on IL-4 to differentiate have been implicated in allergic and atopic manifestations, and in addition through their production of IL-4 and IL-10 they have been suggested to play a role in tolerance; specifically, it has been suggested that a Th1 to Th2 switch may prevent the development of organ-specific autoimmune pathologies. In the last year it has become clear that distinct subsets of regulatory T cells are responsible for regulating both Th1 and Th2 responses and preventing the development of immune pathologies. A common feature of many of these regulatory T cells is that their function is at least in part due to the action of TGFβ; this would be in keeping with the ability of TGFβ to inhibit both Th1 and Th2 development.

1993; Chen et al., 1994; Powrie et al., 1996; Groux et al., 1997; Bridoux et al., 1997; Weiner, 1997). The maintenance of self tolerance, at least in part, appears to depend on active T cell regulation. Although Th2-derived cytokines can sometimes play a role in this process, it is now clear that distinct subsets of regulatory cells exist, which not only inhibit inflammatory responses that lead to autoimmunity (Powrie and Mason, 1990; Powrie et al., 1996; Bridoux et al., 1997; Groux et al., 1997; Weiner, 1997) but also inhibit inflammatory pathologies mediated by Th2-type cells (Bridoux et al., 1997; Lafaille et al., 1997; Pakala et al., 1997). Much of this suppression has been shown to be at least in part attributable to transforming growth factor β (TGFβ) (Powrie et al., 1996; Bridoux et al., 1997; Weiner, 1997), and this is not surprising in view of the ability of TGFβ to inhibit both Th1 and Th2 development (Figure 1) (Swain et al., 1991; Schmitt et al., 1994).

Development of T Helper Cell Subsets

Two main types of systems have helped to determine the factors involved in directing Th1 and Th2 phenotype development. Using T cells stimulated with polyclonal activators or T cells from mice expressing transgenic antigen receptors (Tg-TCR) of known specificities, it has become clear that Th1 and Th2 subsets develop from the same T cell precursor (Hsieh et al., 1992; Rocken et al., 1992; Kamogawa et al., 1993; Seder and Paul, 1994), which is a mature, naive CD4$^+$ T lymphocyte producing mainly IL-2 upon antigen-specific stimulation. The most clearly defined factors determining Th1 and Th2 differentiation from this precursor are cytokines present at the initiation of the immune response at the stage of ligation of the TCR (Seder and Paul, 1994; Abbas et al., 1996). For example, bacterial stimuli activate macrophages and subsequently natural killer (NK) cells of the innate immune response to produce IL-12 and IFNγ, respectively (Hsieh et al., 1993; Trinchieri, 1995), which then drive the development of Th1 cells from naive antigen-specific T cells. This type of innate immune response and its accompanying antigen-specific T cell response are appropriate for the eradication of microbial pathogens (Sher and Coffman, 1992; Trinchieri, 1995). Conversely, production of IL-4 early in an immune response directs the development of a Th2 or allergic/humoral immune response from naive precursors; however, the cell sources producing this early burst of IL-4, and the mechanism for its induction are still not clearly

understood (Seder and Paul, 1994). This area of immune regulation has been the subject of intense research for the last few years.

Cytokine-Induced Th1 Development and Maintenance of Th1 Phenotype

IL-12 is a dominant factor in directing the development of Th1 cells producing high levels of IFNγ (Hsieh et al., 1993; Manetti et al., 1993; Trinchieri, 1995). IL-12 is a 75 kDa heterodimer (Trinchieri, 1995) shown to be produced by macrophages upon their encounter with many microbial products, including lipopolysaccharide and components of viruses, intracellular bacteria such as *Listeria monocytogenes* and mycobacteria, and protozoa such as Toxoplasma via as yet undefined mechanisms (Gazzinelli et al., 1993; Hsieh et al., 1993; Trinchieri, 1995). Interestingly, some organisms, such as measles, have the ability to down-regulate IL-12 production by monocytes and thus evade destruction by the cell-mediated limb of the immune response (Karp et al., 1996). Dendritic cells are professional antigen-presenting cells (APC) specialized in antigen capture, migration to secondary lymphoid organs, and T cell priming (Steinman, 1991), which can under certain conditions also produce IL-12 (Macatonia et al., 1995; Scheicher et al., 1995). Recent studies have demonstrated that ligation of CD40 by the CD40 ligand (Cella et al., 1996; Koch et al., 1996) and/or class II (Koch et al., 1996) on dendritic cells can induce the production of high levels of IL-12. Both IL-4 and IL-10 have the ability to inhibit both dendritic cell (Macatonia et al., 1995; Koch et al., 1996) and macrophage-derived (D'Andrea et al., 1993; Hsieh et al., 1993; Murphy et al., 1994) IL-12 production and thus inhibit the development of Th1 cells.

IL-12 directs Th1 development from antigen-stimulated naive $CD4^+$ T cells (Hsieh et al., 1993; Manetti et al., 1993; Trinchieri, 1995) and activates Stat3 and Stat4 in Th1 cells (Jacobson et al., 1995; Szabo et al., 1995). Gene deletion of IL-12 (Magram et al., 1996) or Stat4 (Kaplan et al., 1996a; Thierfelder et al., 1996) shows that IL-12 signaling through this pathway is required in vivo, since both result in markedly reduced Th1 responses. Responsiveness to IL-12 is impaired in BALB/c mice (Guler et al., 1996), which results in the nonhealing Th2 response to *Leishmania major* infection (Locksley and Scott, 1991). Among $CD4^+$ T cells functional receptors for IL-12 appear to be restricted to recently activated, uncommitted cells and to Th1 cells, and they are lost during differentiation of Th2 cells (Szabo et al., 1995). Th1 development is also dependent on IFNγ (Belosevic et al., 1989; Hsieh et al., 1993), although the requirement for this factor was confusing and controversial for a number of years. The effects of IFNγ on Th1 development may be mediated via action on the macrophage to up-regulate IL-12 production (Trinchieri, 1995) or by direct effects on the T cell. The molecular basis of IL-12 unresponsiveness of Th2 cells has recently been delineated in both mouse and human systems (Rogge et al., 1997; Szabo et al., 1997). This is at least in part due to down-regulation of the IL-12Rβ2 by IL-4 and furthermore the up-regulation of the IL-12Rβ2 by IFNγ, which counteracts the inhibitory effects of IL-4 (Rogge et al., 1997; Szabo et al., 1997). Thus, BALB/c mice, which produce a substantial amount of IL-4 (Guler et al., 1996), may inhibit the IL-12Rβ2 expression, imposing the reported requirement for IFNγ in Th1 development (Szabo et al., 1997).

Recent data have suggested a role for cofactors in induction of both development of Th1 cells and IFNγ production from committed Th1 cells. IL-1-α acts as a cofactor in IL-12–induced Th1 development in BALB/c but not C57BL/6 mice; however, IL-1-α responsiveness is lost by committed Th1 cells and clones (Robinson et al., 1997), in agreement with previous reports of loss of IL-1-α binding by Th1 clones (Lichtman et al., 1988). Interferon-γ–inducing factor (IGIF) was discovered in studies of IFNγ production in a *Proprionobacterium acnes*–induced model of toxic shock (Okamura et al., 1995). This cytokine was subsequently characterized as active in promoting proliferation and IFNγ production by Th1 clones and lines, and NK cells in both mouse and human (Okamura et al., 1995; Ushio et al., 1996). Structural analysis and fold recognition (Bazan et al., 1996) suggest that IGIF belongs to the IL-1 family. IGIF, unlike IL-12, does not drive Th1 development, but like IL-1-α it potentiates IL-12–induced Th1 development in the BALB/c but not C57BL/6 mouse strain (Robinson et al., 1997). IGIF synergizes with IL-12 in inducing IFNγ production from differentiating and committed Th1 cells from both BALB/c and C57BL/6 mice (Robinson et al., 1997), as well as inducing IFNγ production by NK cells in both mouse and human as well as T cell clones (Okamura et al., 1995; Ushio et al., 1996), suggesting that both IL-12 and IGIF are required for significant expression of the Th1 phenotype.

Unlike IL-12, IGIF does not activate Stat4 in Th1 cells (Robinson et al., 1997). IL-1-α signaling is via a receptor-associated kinase termed IRAK, which activates a cascade of kinases leading to activation of NF-κB (Cao et al., 1996). IGIF also signals through the IRAK pathway to induce nuclear translocation of the p65/p50 NF-κB complex in Th1 cells (Robinson et al., 1997). In contrast IL-1-α, which showed no effect on Th1 cells, activated NF-κB and induced proliferation of Th2 cells, which did not respond to IGIF (Robinson et al., 1997). Th1 and Th2 cells thus differ in responsiveness and receptor expression for IL-1 family molecules, and thus IGIF and IL-1-α, by signaling through NF-κB, may differentially amplify Th1 and Th2 effector responses, respectively.

Cytokine-Induced Th2 Development

The development of Th2 cells has been attributed to the exposure of naive $CD4^+$ T cells to IL-4 at the initiation of an immune response (Le Gros et al., 1990; Swain et al., 1990; Seder and Paul, 1994). The effects of IL-4 in inducing Th2 development are dominant over Th1 polarizing cytokines (Hsieh et al., 1993; Seder and Paul, 1994), so that if IL-4 levels reach a certain threshold at the beginning of an immune response, Th2 cells will differentiate, leading to increasing levels of IL-4 progressively. This may explain why chronic stimulation, particularly in the absence of inflammatory signals delivered

during the innate immune response, as well as the magnitude of an immune response, may drive Th2 responses (Abbas et al., 1996). It was originally thought that IL-4 was only produced by differentiated T helper cells, and yet this factor is required early in an immune response for the development of Th2 cells (Abbas et al., 1996). There are now several candidates for IL-4 production early in an immune response, which may be responsible for Th2 differentiation; these include major histocompatibility complex (MHC) class II–restricted $CD4^+$ T cells (memory and possibly naive) (Bradley et al, 1991; Constant et al., 1995; Hosken et al., 1995), the $NK1^+$ subset of $CD4^+$ and double negative (DN) T cells (reviewed in Bendelac et al., 1997), LACK-specific $CD4^+$ T cells expressing Vβ4, Vβ8 TCR (Julia et al., 1996), and non–T cell sources, such as mast cells, basophils, and eosinophils (reviewed in Paul et al., 1993).

Many researchers have suggested a critical role for $NK1^+$ T cells in Th2 differentiation as a result of their ability to produce large amounts of IL-4 rapidly upon activation both in vitro and in vivo with anti-CD3 (reviewed in Bendelac et al., 1997) and of their role in the development of IgE production in response to polyclonal activation with anti-IgD in vivo (Yoshimoto et al., 1995). These T cells ($CD4^+$; and $CD4^-$, $CD8^-$ [DN]) are rather unusual in that they express many NK cell markers, as well as markers typical of activated/memory cells, have a restricted TCR Vα and Vβ usage, and are restricted by nonclassical MHC class I molecules (reviewed in Bendelac et al., 1997). In the mouse, there are two molecules whose expression is dependent on β2M-microglobulin but not the TAP peptide transporter that are candidate ligands for $NK1^+$ T cells: CD1 and the thymus leukemia (TL) antigen, which belong to a large group of nonclassical class Ib molecules and are proposed to function as ligands for $NK1^+$ T cells (reviewed in Bendelac et al., 1997). CD1 expression and assembly are β2M-dependent and, although not dependent on invariant chain and DM, CD1 traffics to the MHC class II compartment and does not require functional TAP-1 and TAP-2 peptide transporters for surface expression (reviewed in Bendelac et al., 1997). A number of recent studies using β2M-deficient mice have failed to support a role for these $NK1^+$ cells in the development of Th2 responses to a variety of parasitic microbes and protein antigens (reviewed in Bendelac et al., 1997). However, β2M-deficient mice not only lack CD1 expression and $NK1^+$ T cells but are also deficient in the surface expression of most classical and nonclassical MHC class I molecules, have a substantial reduction in their numbers of $CD8^+$ T lymphocytes, and have other additional defects, which may influence immune responses (reviewed in Bendelac et al., 1997).

The precise role of $NK1^+$ T cells in the differentiation of Th2 responses has recently been addressed by infection and immunization studies in CD1-deficient mice. Mice deficient in CD1 were found to lack the $NK1^+$ subset but could nevertheless mount a prototypical Th2 response (Smiley et al., 1997). After immunization with antibody to immunoglobulin D (IgD), CD1-deficient mice produced IgE. Thus, although dependent on CD1 for their development, IL-4–secreting NK-like T cells are not required for Th2 responses (Smiley et al., 1997). Further studies ruled out the contributions of TL to the $NK1^+$ T cell repertoire by performing an analysis of CD1-deficient mice that additionally do not express TL in the thymus. In accordance with the earlier study (Smiley et al., 1997), CD1 deficiency led to a marked reduction of $NK1^+$ T cell numbers in the thymus, spleen, and liver, which was accompanied by a dramatic decrease in early IL-4 production induced by anti-CD3 both in vitro and in vivo (Chen et al., 1997; Mendiratta et al., 1997). In addition, comparable levels of IgE were also induced after injection of goat anti-mouse IgD serum in both these CD1-deficient mice and control littermates (Chen et al., 1997, Mendiratta et al., 1997). These studies were all in contrast to studies in the β2M-deficient mice (Yoshimoto et al., 1995). Such differences in findings between the β2M-deficient versus CD1-deficient mice could result from different genetic backgrounds, a compensation for IL-4-dependent IgE switching by an alternative stimulus (Morawetz et al., 1996), or because β2M-deficient mice not only lack CD1 expression and $NK1^+$ T cells but are also deficient in other immunological compartments (reviewed in Bendelac et al., 1997). However, in line with the findings previously reported in the β2M-deficient mice, it was also demonstrated that normal levels of IL-4 were generated in a recall response to the particulate antigen keyhole limpet hemocyanin in adjuvant (Chen et al., 1997). In total, these studies confirm that CD1 is the selecting antigen for $NK1^+$ T cells and that these cells account for the early burst of IL-4 obtained after anti-CD3 activation. However, IL-4 production by NK1.1 cells is not needed for the development of Th2 responses to specific antigens. These data are in agreement with recent studies with mice rendered deficient of $NK1^+$ T cells by depletion with anti-NK1.1 antibodies (von der Weid et al., 1996). Thus, the precise role of $NK1^+$ T cells in the development of Th2 responses remains unclear.

The requirement for IL-4 in mediating both Th2 differentiation and susceptibility to the parasite *L. major* has directed much interest toward the early IL-4 produced in vivo during infection with this organism (Sadick et al., 1990; Locksley and Scott, 1991). It has been recently demonstrated that the early burst of IL-4 peaking in lymph nodes of susceptible BALB/c mice after *L. major* infection occurs within $CD4^+$ T cells that express Vβ4 Vα8 T cell receptors (Julia et al., 1996; Launois et al., 1997). A previously identified antigen, *Leishmania* homolog of receptors for activated C kinase (LACK) was found to be the focus of this initial response (Julia et al., 1996). A role for LACK-specific T cells in the development of a dominant Th2 response to *L. major* infection in BALB/c mice was demonstrated when mice were rendered unresponsive to LACK (Julia et al., 1996). Due to expression of LACK as a transgene, BALB/c mice were made tolerant to this antigen and developed a reduced Th2 response and a protective Th1 response when infected with *L. major*. The mechanism whereby LACK induces a Th2 response in BALB/c mice is still unclear. That early IL-4 induced by LACK is so rapid is reminiscent of a "memory" response (Bradley et al., 1991) and may result from previous activation of T cells by cross-reactive antigens. BALB/c mice depleted of Vβ4 T cells by neonatal infection with a specific mouse mammary tumor virus, MMTV(SIM), were shown to be resistant to

L. major infection and did not produce this early burst of IL-4. The experiments were controlled for the possible adjuvant effect of the virus, since mice deleted for Vβ6 with MMTV(SW) remained susceptible to the parasite. Recombinant LACK antigen from *L. major*, shown previously to activate Vβ4 Vα8 $CD4^+$ T cells (Julia et al., 1996), was demonstrated to cause IL-4 mRNA production with similar kinetics and at comparable levels, as did the intact parasites. IL-4 production from memory T cells could affect the subsequent development of Th2 cells from naive precursors, as previously shown (Bradley et al., 1991; Macatonia et al., 1995). However, it is also possible that this antigen induces a rapid IL-4 response from naive T cells, as has been suggested in other systems (Rocken et al., 1992; Kamogawa et al., 1993; Constant et al., 1995; Hosken et al., 1995; Rincon et al., 1997). Interestingly, injection of recombinant LACK into resistant C57Bl/6 mice results in small, but detectable amounts of IFNγ mRNA at 16 hr, indicating that recognition of this antigen is not invariably associated with IL-4 production.

It was suggested that the genetic susceptibility to *L. major* requires not only a polymorphism in Th subset development (Guler et al., 1996) but also the proper thymic environment for the selection of sufficient numbers of Vβ4 Vα8, $CD4^+$ LACK-specific T cells to direct Th2 development of subsequently activated $CD4^+$ T cells (Launois et al., 1997). The complexity of the genotypes required to mediate susceptibility or resistance to this organism appears to be greater than originally anticipated. Using a novel serial backcross approach to map loci linked (and/or associated) with *Leishmania* resistance, it has been elegantly shown that loci on six distinct chromosomal regions can influence the immune response to this pathogen (Beebe et al., 1997), reinforcing the multigenic nature of resistance to this disease. That non–T cells may also play a role in the initiation of Th2 responses against some pathogens in specific tissues is clear, since mast cells, basophils, and eosinophils can produce cytokines such as IL-4 (reviewed in Paul et al., 1993).

IL-4 ligand binding to its receptor ultimately results in signaling through activation of Stat-6 and in addition through phosphorylation of the insulin receptor substrate 2 (Hou et al., 1994; Ryan et al., 1996). Gene deletion of the IL-4 gene significantly reduces Th2-type responses (Kuhn et al., 1991; Kopf, et al., 1993). Although targeted disruption of the Stat-6 gene (Kaplan et al., 1996b; Shimoda et al., 1996; Takeda et al., 1996) results in deficient Th2 responses, the mechanism by which Stat-6 induces IL-4 production and Th2 development remains unclear. The requirement for Stat-6 to maintain IL-4 production is also supported by a recent study in which it was shown that IL-4R–mediated tyrosine phosphorylation is impaired in Th1 cells (Kubo et al., 1997). A consensus Stat-6–binding site is present in the IL-4 promoter, and a multimer of this site is capable of stimulating transcription of a reporter gene in response to IL-4 (Lederer et al., 1996). This suggests that Stat-6 may activate IL-4 transcription in response to IL-4 itself and in this way play a role in IL-4–driven Th2 development. However, whereas activation of Stat proteins is a transient event that occurs rapidly upon stimulation, the development of effector T helper cells takes several days (Lederer et al., 1996). Therefore, while it is clear that Stats are essential early signal transducers in Th subset differentiation, they are unlikely to determine Th phenotype development directly. Alternatively, it is possible that Stat activation together with TCR signaling results in a cascade of changes of gene expression that ultimately leads to the development of effector phenotypes of Th1 and Th2 cells. Two transcription factors, c-Maf (Ho et al., 1996) and NF-IL-6 (Davydov et al., 1995), have recently been shown to be expressed in Th2 cells and to activate the IL-4 gene promoter. However, the selective expression of these two transcription factors was observed in fully differentiated Th2 cells, and their role in differentiation of Th2 cells from naive precursors is less clear. More recently, a transcription factor GATA-3 was found to be selectively expressed in differentiating and effector Th2 but not Th1 cells (Zheng and Flavell, 1997). Interestingly, GATA-3 was shown to be required for the transcription of all Th2 genes, and the loss of Th2 cytokine gene expression in Th1 cells appeared to be at least in part a consequence of the downregulation of GATA-3. To date, six transcription factors (Stat-6, c-Maf, NF-IL-6, NF-AT, AP-1, and GATA-3) have been implicated in Th2 cell differentiation (Davydov et al., 1995; Rooney et al., 1995; Ho et al., 1996; Kaplan et al., 1996; Rincon and Flavell, 1997; Zheng and Flavell, 1997). However, the precise mechanisms of how these factors interact and lead to Th2 differentiation remain to be clarifed.

Other Factors Affecting Th Subset Development

Although key cytokines, including IL-12 and IFNγ, or IL-4, undoubtedly play a central role in governing the differentiation of Th1 and Th2 effector cells from naive precursors (Abbas et al., 1996), models have been proposed whereby the strength of interaction mediated through the TCR and MHC/peptide complex or the dose of antigen might directly affect lineage commitment of $CD4^+$ T cells (Constant et al., 1995; Hosken et al., 1995; Constant and Bottomly, 1997). The mechanisms underlying the effects of antigen dose on Th subset development are as yet unclear. However, it is likely that some of the effects are mediated by altering the balance of endogenous levels of IL-4 versus IL-12 or IFNγ production by T cells or APC, thus tilting the development toward Th2 or Th1 responses, respectively. This is supported by in vitro studies in which low-dose or very high-dose antigen-driven Th2 development is abrogated by addition of neutralizing antibodies directed against IL-4 (Hosken et al., 1995). In vivo, the nature of the antigen as well as the dose may determine whether macrophages or dendritic cells are triggered to produce IL-12 and thus drive a Th1-type response (Hsieh et al., 1993; Macatonia et al., 1995; Trinchieri, 1995). Other factors suggested to influence T cell subset development are membrane-bound costimulators, such as B7-1 and B7-2, which are signals provided by APC that together with antigen have been shown to enhance specific T cell responses via their interaction with CD28 (Kuchroo et al., 1995). It has been shown in some systems that such costimulators may differentially regulate

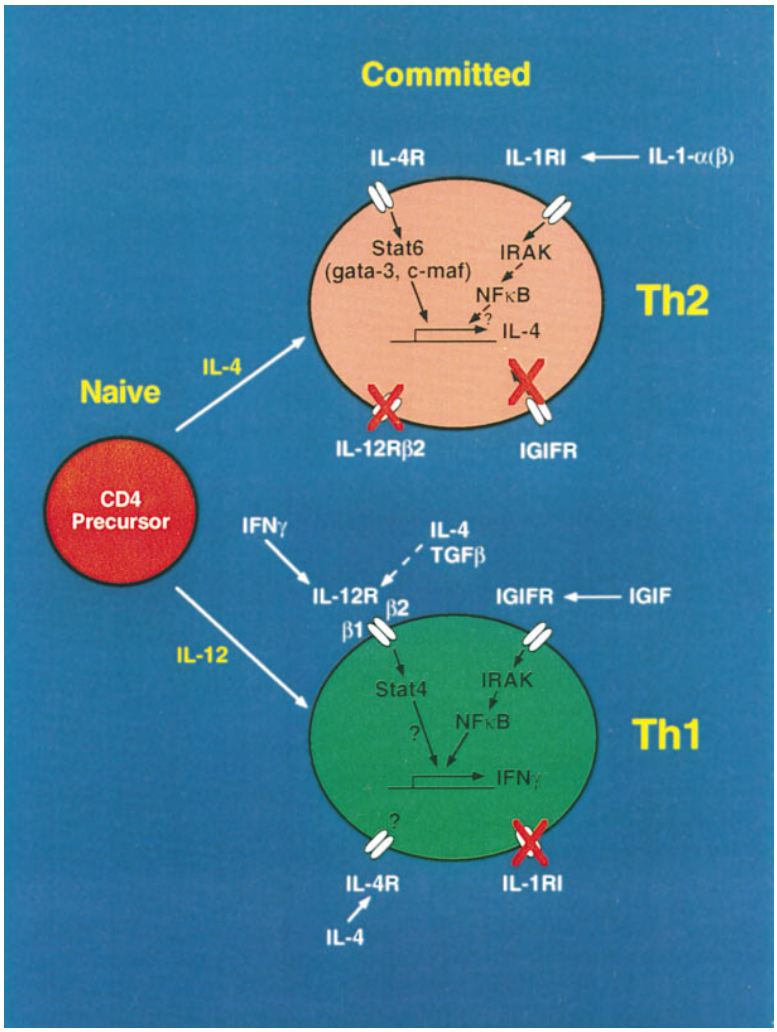

Figure 2. Th1 but Not Th2 Cells Remain Responsive to Both IL-12 and IGIF

Committed Th2 cells lose their ability to respond biologically both to IL-12 and IGIF, perhaps in part explaining why they cannot make Th1-type cytokines such as IFNγ upon stimulation. Loss of IL-12 responsiveness and Stat-4 activation results from preferential loss of the IL-12R-β2. IL-4 can down-regulate IL-12R-β expression, whereas IFNγ up-regulates expression. The inability of IGIF to signal through IRAK to induce nuclear translocation of p65/p50 NF-κB in Th2 cells, in contrast to Th1 cells, suggests a possible loss of the IGIF receptor by Th2 cells, which in contrast maintain expression of the type 1 IL-1R.

Th1 and Th2 development, although the mechanism for these phenomena is unclear (Kuchroo et al., 1995). Furthermore, Th cells that do not express the CD4 coreceptor are deficient in their capacity to differentiate into Th2 cells in response to antigen in vivo or in vitro (Brown et al., 1997; Fowell et al., 1997). This deficiency was evident using both CD4-negative cells from CD4-gene disrupted mice and the DN T cells that developed in MHC class II–restricted TCR transgenic mice. The defect in the ability to produce IL-4 was observed in response to disparate challenges, including intestinal helminths and intracellular parasites. Furthermore, the lack of a Th2 response was also evident when $CD4^+$ T cells were primed by APCs that expressed mutated MHC class II molecules that were unable to bind CD4. It would appear that the coreceptor, perhaps by modulating the strength of the signal delivered to the T cell, may influence Th subset development. Other factors that have been shown to initiate or enhance Th2 development are IL-6 (Rincon et al., 1997) and monocyte chemoattractant protein 1 (Karpus et al., 1997). However, since development of these Th2 responses is neutralized by addition of anti-IL-4 antibodies, it is likely that they may drive Th2 development by augmenting endogenous levels of IL-4 (Figure 2).

It is very likely that the development of T helper subsets may depend on the nature of an invading pathogen or the type of antigen, the route and dose of entry of the antigen/microorganism, as well as the genetic background of the host. However, the most clearly defined factors determining Th subset differentiation from naive $CD4^+$ T cell precursors are cytokines present at the initiation of the immune response at the stage of ligation of the TCR. IL-12 and IFNγ are important factors inducing the development of Th1 cells, and high levels of IFNγ production from committed Th1 cells result from activation in the presence of IL-12 and IGIF. Th1 cells are important effectors involved in the eradication of infectious pathogens, but if inappropriately activated they can cause immunopathology. In contrast, Th2 cells, whose development is induced by IL-4, have been implicated in humoral immune responses and the eradication of helminths, but they may also result in inflammatory damage during allergic manifestations and atopy. Th2 cells have been suggested to play a role in the protection of tissues and organs from autoimmune attack as a result of their production of antiinflammatory cytokines, such as IL-4 and IL-10. Current thinking is that such regulation is achieved by an alternative subset(s) of regulatory T cells by the production of TGFβ, which is able

to inhibit both the development of Th1 and Th2 responses.

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