Type 1/Type 2 Immunity in Infectious Diseases

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T helper type 1 (Th1) lymphocytes secrete interleukin (IL)-2, interferon-γ, and lymphotoxin-α and stimulate type 1 immunity, which is characterized by intense phagocytic activity. Conversely, Th2 cells secrete IL-4, IL-5, IL-9, IL-10, and IL-13 and stimulate type 2 immunity, which is characterized by high antibody titers. Type 1 and type 2 immunity are not strictly synonymous with cell-mediated and humoral immunity, because Th1 cells also stimulate moderate levels of antibody production, whereas Th2 cells actively suppress phagocytosis. For most infections, save those caused by large eukaryotic pathogens, type 1 immunity is protective, whereas type 2 responses assist with the resolution of cell-mediated inflammation. Severe systemic stress, immunosuppression, or overwhelming microbial inoculation causes the immune system to mount a type 2 response to an infection normally controlled by type 1 immunity. In such cases, administration of antimicrobial chemotherapy and exogenous cytokines restores systemic balance, which allows successful immune responses to clear the infection.

HISTORICAL BACKGROUND

At the turn of the 20th century, the new science of immunology was in the throes of a fundamental debate: were phagocytic cells the keys to protection against infection, as proposed by Metchnikoff [1], or were humoral antibodies the true protective factors, as forwarded by Koch’s students, von Behring and Ehrlich [2]? Although the humoralists at first carried the day, the joint awarding of the 1908 Nobel Prize in Medicine to the opponents, Metchnikoff and Ehrlich, was prophetic. By the end of the century, the opposing theories created by these two fathers of immunology would be unified in one paradigm of host defense against infection: type 1/type 2 immunity.

The terms type 1 and type 2 immunity bear no relation to types 1–4 of hypersensitivity reactions described by Gell and Coombs [2a]. Rather, the birth of the type 1/type 2 immunity paradigm can be traced to landmark articles by Parish in the early 1970s [3–5]. Building on studies of immune regulation by Sercarz and Coonz [6], Dresser [7, 8], and Mitchison [9], Parish described a striking inverse correlation between the degree to which a given dose of antigen elicited antibody production or delayed type hypersensitivity (DTH; figure 1). His results, confirmed by other investigators utilizing different experimental models [10–12], were the bedrock of what would become the type 1/type 2 hypothesis: the level of antibody elicited in an immune response is inversely proportional to the level of cell-mediated immunity.

Given the link between humoral and cell-mediated immunity, there was much interest in characterizing the regulation of these alternate forms of host defense. By the early 1980s the 2 major subsets of T lymphocytes had been described: T helper cells, which express the surface protein CD4, and T-cytotoxic cells, which express the CD8 marker (figure 2). Whereas CD8+ T-cytotoxic lymphocytes were known to mediate lysis of autologous cells infected by intracellular pathogens, CD4+ T helper cells were known to induce B cell production of antibodies. Their stimulation of antibody responses made T helper cells the natural subjects of
lymphocytes. By assaying cytokine production of CD4 cells known as T helper type 1 (Th1) and T helper type 2 (Th2) [13] first described the existence of subpopulations of CD4 cells in induction of the DTH reaction that Mosmann et al. investigated into the common regulation of humoral and cell-mediated immunity.

It was in an effort to elucidate the role of T helper lymphocytes in induction of the DTH reaction that Mosmann et al. [13] first described the existence of subpopulations of CD4 cells known as T helper type 1 (Th1) and T helper type 2 (Th2) lymphocytes. By assaying cytokine production of CD4+ T helper cells from inbred mice, the investigators were able to divide the T cell clones into 2 phenotypes. Th1 cells produced IL-2, IFN-γ, and lymphotoxin (LT)-α. Conversely, Th2 cells produced IL-4 as well as other cytokines that had yet to be characterized. Both Th1 and Th2 cells provided help to B cells in antibody assays, but, as reported by Cher and Mosmann a year later, only Th1 cells, and not Th2 cells, could elicit DTH in mice [14].

**TH1/TH2 CELL BIOLOGY**

**Definitions.** Th1 and Th2 cells are now known to exist in human beings as well as mice [15, 16]. All T helper lymphocytes start out as naïve Th0 cells, which, after being activated, are capable of “polarizing,” or differentiating, into either Th1 or Th2 effector cells [17, 18]. Mature Th1 cells secrete IL-2, IFN-γ, and LT-α, and Th2 cells secrete IL-4, IL-5, IL-9, IL-10, and IL-13 [19, 20] (some investigators include IL-6, although this is controversial [21, 22]). In humans this division is not as stringent as in inbred mice. For example, some human Th1 cells secrete IL-10 [23] and IL-13 [24]. In part because of such an overlap of cytokine secretion, the conventional definition of a Th1 or Th2 cell depends strictly on the secretion of IFN-γ or IL-4. Th1 cells secrete IFN-γ but do not secrete IL-4, whereas Th2 cells secrete IL-4 but not IFN-γ. T cells secreting neither IFN-γ nor IL-4 are neither Th1 nor Th2 cells. Some of these cells are naïve Th0 lymphocytes that have never before been activated and thus have not acquired the ability to secrete a mature profile of cytokines.

Aside from Th1 and Th2 cells, several other subtypes of T helper cells have been described (table 1), and indeed the frequency of polarization to the Th1 or Th2 phenotype in vivo is controversial [30, 31]. Like naïve T cells, mature T cells secreting both IFN-γ and IL-4 are also known as Th0 cells, which can lead to some confusion. These latter Th0 cells are lymphocytes that did not polarize during maturation and thus took on attributes of both Th1 and Th2 cells [32, 33]. Additional populations of CD4+ T helper lymphocytes called Th3 cells and T-regulatory 1 (Tr1) cells have been described. Th3 cells secrete transforming growth factor (TGF)-β and are thought to regulate mucosal immunity in mammals [34–37]. Tr1 cells, which appear to be similar to Th3 cells, secrete unusually high levels of IL-10 and lower levels of TGF-β, and they have been implicated in general suppression of immunity [38, 39]. Th3 and Tr1 cells are beyond the scope of this review, which will focus on Th1 and Th2 cells, the crucial cell types in defining the type 1/type 2 paradigm.

**Effects of Th1 and Th2 cells.** Th1 cells are the principal regulators of type 1 immunity. The cytokine chiefly responsible for their proinflammatory effect is IFN-γ. IFN-γ stimulates phagocytosis [40, 41], the oxidative burst [42, 43], and intracellular killing of microbes [44–46]. IFN-γ also upregulates expression of class I [47, 48] and class II major histocompatibility complex (MHC) molecules [49, 50] on a variety of cells, thereby stimulating antigen presentation to T cells (figure 2). Both IFN-γ and LT-α induce other cell types, including non-leukocytes such as endothelial cells [51], keratinocytes [52], and fibroblasts [53, 54], to secrete proinflammatory cytokines, such as TNF and chemotactic cytokines called chemokines [55]. They also stimulate adhesion molecule expression on endothelial cells and induce endothelial cell retraction and vascular smooth-muscle relaxation. The result is accumulation of blood in dilated, leaky vessels, easing diapedesis of leukocytes into areas of danger and allowing recruitment of innate immune cells and opsonins into the interstitium. Thus Th1 cells cause rubor (redness), tumor (swelling), dolor (pain), and calor (warmth), the 4 cardinal signs of inflammation.

Th2 cells, conversely, stimulate high titers of antibody production. In particular, IL-4, IL-10, and IL-13 activate B cell proliferation, antibody production, and class-switching [56–58]. In fact, class-switching from IgG to IgE cannot occur without the presence of IL-4 or IL-13 [59–61], making the production of IgE a perfect bioassay for the presence of Th2 cells in vivo. IL-5 is a potent hematopoietic cytokine that stimulates bone marrow production of eosinophils [62–64], as well as activation and chemotaxis of eosinophils [65, 66] and basophils [67, 68], whereas IL-9 is the equivalent hematopoietic and stimulatory factor for mast cells [69, 70]. It is interesting that IL-
Figure 2. Antigen presentation to T-cytotoxic (CD8+) and T helper (CD4+) cells. In A, a host cell is (1) infected by an intracellular pathogen (a virus, in this example). (2) As progeny virions are produced, viral antigens are pumped into the endoplasmic reticulum, where they bind to class I major histocompatibility complex (MHC I) proteins. (3) Covalent interaction with the chaperone protein, β-2 microglobulin (β-2M), allows surface expression of the MHC I polypeptide, which presents viral antigen bound to a groove at its tip. The CD8 protein on the T-cytotoxic cell binds to the MHC I molecule, stabilizing the interaction between the T-cell receptor (TCR) and the MHC I–antigen complex. (4) The result is activation of the T-cytotoxic cell, which then lyses the host cell to expose the intracellular virus. In B, a phagocyte (1) ingests extracellular microbes and degrades them in the phagolysosome. (2) Degraded microbial fragments are loaded onto class II MHC molecules in the phagolysosome, and the MHC II–antigen complexes are transported to the cell surface. (3) CD4 binds to MHC II, stabilizing the interaction between the TCR and the MHC II–antigen complex. (4) The result is activation of the T helper cell, which then autocrine-stimulates its own proliferation by secreting IL-2.

4, IL-5, IL-9, and IL-13 have been strongly implicated in allergic and atopic reactions, as well as in causing the airway inflammation seen in asthma and reactive airway disease [71–78]. Unlike inflammation stimulated by type 1 cytokines, type 2–mediated inflammation is characterized by eosinophilic and basophilic tissue infiltration, as well as extensive mast cell degranulation, a process dependent on cross-linking of surface-bound IgE.

In addition to their stimulatory effects, Th1 and Th2 cells cross-regulate one another. The IFN-γ secreted by Th1 cells directly suppresses IL-4 secretion and thus inhibits differentiation of naive Th0 cells into Th2 cells [79–81]. Conversely, IL-4 and IL-10 inhibit the secretion of IL-12 and IFN-γ, blocking the ability of Th0 cells to polarize into Th1 cells [82–84]. IL-10 is perhaps the most anti-inflammatory cytokine known [85]. It inhibits the secretion of proinflammatory cytokines [86, 87]; suppresses phagocytosis [88], the oxidative burst [89, 90], and intracellular killing [91, 92]; and inhibits antigen presentation to T cells [93, 94], causing T cell anergy [95]. Like IL-10, IL-4 and IL-13 also inhibit phagocytosis and intracellular killing [91, 92], suppress inflammatory cytokine production [96], and may induce T cell anergy [97].

There are also differences in proliferation requirements between Th1 and Th2 cells. Naive T cells and Th1 cells absolutely require IL-2 for activation and proliferation [98]. However, Th2 cells are perfectly capable of proliferating without IL-2 if IL-4 [98, 99] and/or IL-1 [100, 101] is present, which is convenient for them since they secrete large quantities of IL-4. Therefore, in clinical situations where IL-2 is in limited supply (for example, in patients taking cyclosporine or FK-506 or high-dose glucocorticoids), only Th2 cells will be able to proliferate in response to antigenic exposure.

**TH1 AND TH2 REGULATION**

**Five Factors Inducing Polarization**

Five factors regulate the polarization of newly activated naive T cells into mature Th1 or Th2 cells [19, 102]: the local cytokine milieu; the presence of immunologically active hormones; the dose and route of antigen administration; the type of antigen-
presenting cell stimulating the T cell; and the “strength of signal,” which is an ill-defined summation of the affinity of the T-cell receptor for the MHC-antigen complex, combined with the timing and density of receptor ligation. Of these 5 factors, the most important is the cytokine milieu surrounding the newly activated T cell.

**Cytokines.** The key to polarization into the Th1 phenotype is IL-12 [103–106]. Conversely, IL-4 is the *sine qua non* for Th2 polarization [107–110]. Recent studies have indicated that the phenotype of a newly activated T cell is determined within 48–72 h after activation [110, 111]. This correlates with the in vivo finding that T cells activated in lymph nodes do not polarize until they are exposed to specific cytokine milieus after traveling to peripheral effector sites, which occurs 2–3 days following activation [112]. Thus Th1 or Th2 polarization may not occur until an activated T cell arrives at the site of danger and samples the local cytokine milieu to determine if an inflammatory or antibody response is appropriate. T cells exposed to IL-12 during this time differentiate to become Th1 cells, and T cells exposed to IL-4 differentiate to become Th2 cells. When the immune system is “in doubt” about whether Th1 or Th2 cells should be generated, Th2 outcomes are favored. For instance, if both IL-12 and IL-4 are present at the time of T cell activation, the effect of the IL-4 dominates, and the T lymphocytes polarize to become Th2 effectors [103, 104, 113].

Whether or not established Th1 and Th2 clones can be made to reverse their phenotypes is controversial [113, 114], but it is known that the responses of both Th1 and Th2 cells are reversible at the population level [115, 116], which presents something of a conundrum. If individual T cell clones are nearly impossible to reverse, how can the response of an entire population of T cells be reversed? The answer is that even in highly polarized T cell populations, quiescent clones of the phenotype opposite to the dominant response can be found by fluorescence-activated cell-sorting (FACS) or limiting dilution analysis. That is, quiescent Th1 cells can be found in populations secreting large amounts of IL-4 and IL-10, and quiescent Th2 cells can be found in populations secreting IFN-γ and IL-2 [117–119]. Reversal of a population response involves inhibiting the dominant clones and allowing expansion of the quiescent clones by modulating the cytokine environment.

Therefore, the nature of an immune response cannot be reliably determined from cytokine patterns of individual T cell clones, which may or may not be representative of the dominant population phenotype. Furthermore, B cells, phagocytes, and even nonleukocytes can secrete cytokines typical of a Th1 or Th2 cell (e.g., IFN-γ, IL-4, and IL-10). For these reasons, the terms “Th1 response” and “Th2 response,” which imply only measurement of responses by T helper cell clones, are insufficient. Instead, as originally proposed by Clerici and Shearer in the context of HIV [120], the terms “type 1 response” and “type 2 response” should be used to indicate a summation of the systemic response to a given infection (i.e., a type 1 response is characterized by high in vivo production of the Th1 cytokines IL-2, IFN-γ, and IL-12, without implying that these cytokines are exclusively being produced by T helper cells).

**Hormones.** Glucocorticoids are powerful stimulators of type 2 outcomes and powerful inhibitors of type 1 outcomes, directly inducing IL-4 and IL-10 production from lymphocytes and antigen-presenting cells [121–123] while suppressing the secretion and effects of IFN-γ and IL-12 [124–127]. Furthermore, they suppress secretion of IL-2 [128, 129], which inhibits activated Th1 cells from proliferating while allowing Th2 cells to expand. It is interesting that at high concentrations, glucocorticoids induce lympholysis [130] and inhibit all cytokine secretion, including type 2 cytokines [131, 132]. In order to suppress lymphocyte proliferation and type 2 cytokine production in vitro, glucocorticoids must be present at least 10⁻⁷ M concentrations [132–134]. Although direct comparison with in vivo serum concentrations is problematic, it has been

**Table 1. Subtypes of CD4⁺ T-helper (Th) cells and their characteristic cytokines and effects.**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Defining cytokines</th>
<th>Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive Th0</td>
<td>IL-2</td>
<td>Proliferate and differentiate to effector cells</td>
</tr>
<tr>
<td>Th1</td>
<td>IL-2, IFN-γ, lymphotoxin-α</td>
<td>Cell-mediated immunity, opsonizing antibody</td>
</tr>
<tr>
<td>Mature Th0</td>
<td>IFN-γ, IL-4, others</td>
<td>Unclear</td>
</tr>
<tr>
<td>Th2</td>
<td>IL-4, IL-5, IL-9, IL-10, IL-13</td>
<td>Humoral immunity, inhibits inflammation</td>
</tr>
<tr>
<td>Th3</td>
<td>TGF-β</td>
<td>Regulates mucosal immunity and stimulates B cell secretions of IgA</td>
</tr>
<tr>
<td>T-regulatory 1*</td>
<td>High levels of IL-10, some TGF-β</td>
<td>Suppression of immune response</td>
</tr>
</tbody>
</table>

**NOTE.** TGF, transforming growth factor.

* T-regulatory 1 cells may represent one form of the long-elusive T-suppressor cell [25]. Although the concept of T-suppressor cells has been discredited [26, 27], recent data have implicated both CD4⁺ T-regulatory 1 cells and a separate population of CD4⁺ CD25⁺ T-helper cells in antigen-specific immunosuppression [28]. In the past, CD8⁺ T-cytotoxic cells were widely referred to as T-suppressor cells, especially in the context of AIDS [29]; however, there is no evidence that CD8 cells are inherently suppressive, and use of the term “T-suppressor” to describe CD8 cells should be abandoned.
estimated that serum glucocorticoid levels must reach \(10^{-6} \text{M}\) to be clinically effective in reactive airways disease [135].

A study of patients receiving iv prednisolone indicated that a dose of 1 mg/kg markedly diminished serum T cell concentrations and ex vivo cytokine production, whereas a dose of 0.1 mg/kg mediated minimal changes in serum T cell counts and cytokine production [136]. Thus, at doses used clinically \((40-60 \text{ mg of methylprednisolone per day})\), serum cortisol levels exceed those required to suppress type 2 cytokine secretion, a circumstance that probably explains the efficacy of steroid pulses in the treatment of reactive airways disease.

Like glucocorticoids, estrogens and progestins have been shown to suppress type 1 immunity in favor of type 2 immunity. Both estrogens and progestins inhibit IL-12 and IFN-\(\gamma\) secretion from antigen-presenting cells and T cells, while stimulating IL-4, IL-10, and IL-13 secretion [137–139]. The persistence of the allogeneic fetus in its pregnant mother is perhaps the most compelling example of immunologic tolerance known. Placental secretion of estrogens, progestins, IL-4, and IL-10 [140, 141], which produces type 2 responses to antigenic stimulation during pregnancy [142], is largely responsible for this peripheral tolerance. Aberrant regulation allowing type 1 immunity to occur during pregnancy leads to abnormal labor [143, 144] or maternal rejection of fetal tissues, causing abortion [145–147].

Conversely, the testosterone derivative dehydroepiandrosterone (DHEA) has been shown to potentiate IL-2 secretion as well as the establishment of Th1 clones [148–150]. Therefore, male hormones favor cell-mediated immune responses, whereas female hormones favor humoral immunity. Indeed, in a direct comparison of coxsackievirus infection in male and female mice under identical experimental conditions, male mice mounted type 1 responses to the virus but female mice mounted type 2 responses [151].

Perhaps the most important hormones regulating type 1 and type 2 outcomes are the catecholamines. It has been known for 30 years that lymphocytes express catecholamine receptors [152]. Catecholamines inhibit type 1 cytokine production [153] and stimulate the transcription and secretion of type 2 cytokines from a variety of leukocytes [154–158]. Furthermore, the selective suppression of IL-2 production by catecholamines inhibits Th1 propagation, allowing selective Th2 proliferation [159]. \(\beta-2\) Agonists are extremely potent bronchodilators, a characteristic explaining why they are acutely effective for symptomatic asthma. However, their effects on type 2 cytokines suggest that over the long term they might exacerbate the airway inflammation typical of asthma, which may explain why they must be combined with inhaled steroids to be effective as long-term suppressive therapy.

Finally, other hormones have been shown to modulate type 1 and type 2 outcomes. Chief among these are human chorionic gonadotropin [160], prostaglandins (PGs, especially PGE) [161–164], and \(\alpha\)-melanocyte–stimulating hormone [165, 166], each of which has been shown to suppress type 1 cytokine responses while stimulating type 2 responses.

**Antigen dose.** The effect of antigen dose on the polarization of naive cells to Th1 or Th2 cell types is poorly understood. The use of widely disparate experimental models and an inability to correlate subjective notions of “high” versus “low” dose ranges between these systems have led to confusion in interpreting the literature [102]. As shall be discussed below, however, in vivo animal data and clinical observations consistently indicate that high microbial burdens suppress cell-mediated immunity. Therefore, consistent with Metchnikoff’s findings at the turn of the 20th century [167], it can now be strongly asserted that the highest dose ranges, and therefore the highest microbial burdens in infected patients, suppress cell-mediated immunity.

At first glance the assertion that high infectious inocula suppress immune responses seems counterintuitive. One might predict that higher microbial burdens would stimulate a greater immune response than lower microbial burdens. However, evolution has adapted the mammalian host to be far more concerned about auto-inflammatory destruction than tissue damage wrought by microbes.

Chronic viral hepatitis proves an illuminating model in this regard. It is known that liver necrosis in viral hepatitis is not due to viral cytopathic effect, but rather to CD8\(^+\) T-cytotoxic lymphocytes lysing infected host cells to expose intracellular virus [168]. Acute bursts of type 1 immunity can rapidly interrupt intracellular viral replication and abort infection at the cost of liver necrosis [169, 170]. For example, treatment with IFN-\(\alpha\) can lead to hepatitis virus clearance in some patients by boosting cell-mediated immunity, leading to acute stimulation of CD8\(^+\) T-cytotoxic cell activity [171, 172]. But what if the viral burden in the liver is very high? In such cases, the boosted host response leads to tissue necrosis extensive enough to destroy the liver and cause severe morbidity to the host in the process of clearing the virus, a true Pyrrhic victory [173–176]. In order to prevent this, the mammalian immune system has adapted to suppress cell-mediated immunity when the viral load reaches a certain threshold [177]. Indeed, patients chronically infected with hepatitis B virus are specifically anergic to the virus, and lowering the viral load with lamivudine has been shown to restore T cell–mediated antiviral effects [178].

**Antigen-presenting cell.** The type of antigen-presenting cell stimulating the T cell may also play a role in the polarization of the lymphocyte to a Th1 or Th2 phenotype [179]. Some studies have reported that B cells are particularly prone to inducing type 2 outcomes [180, 181], even in the presence of IL-12 [182]. This is consistent with their need to drive a type 2 response to feed-forward stimulate their own production of antibody. Conversely, macrophages may be more likely to stim-
ulate type 1 outcomes [183]. Dendritic cells, acknowledged to be the most potent antigen-presenting cells in the body [184–186], are capable of driving both type 1 and type 2 outcomes, depending on the local cytokine microenvironment [187].

Prostaglandins and IL-10 suppress IL-12 production by dendritic cells while stimulating secretion of IL-10, which leads to Th2 polarization of responding lymphocytes [188, 189]. Conversely, IFN-γ and microbial fractions such as lipopolysaccharide (LPS) induce dendritic cells to secrete IL-12, stimulating type 1 outcomes [187]. On the basis of these data from dendritic cell studies, it is now believed that all antigen-presenting cells are capable of stimulating either a type 1 or type 2 outcome, depending on the presence of certain “danger signals” (see below) present in the local microenvironment [190]. Such danger signals modulate the profile of cytokines secreted by the antigen-presenting cell, which results in polarization of the responding lymphocyte to the Th1 or Th2 phenotype.

Strength of signal. The affinity of the T-cell receptor for the MHC/stimulating-antigen complex, the presence of certain costimulatory molecules on the antigen-presenting cell, and the timing of T-cell-receptor ligation by the MHC/antigen complex have each been shown to modify type 1 and type 2 outcomes [191–195]. There is much confusion in this area, as evidenced by an abundance of conflicting reports on whether high or low affinity, present or absent costimulation, and short or long ligation mediate Th1 or Th2 polarization. This lack of concordance likely reflects study-to-study variation in the complex interactions between these difficult-to-measure variables.

The Decision to Polarize

Having discussed the mechanisms responsible for polarization to Th1 or Th2 responses, we must now consider how the immune system determines which outcome is superior for a given danger signal. Since the cytokines IL-12 and IL-4 are the key factors responsible for inducing polarization of naïve T cells toward the Th1 or Th2 profile, the question is raised: what factors tell the immune system to preferentially produce IL-12 or IL-4 at the start of a given immune response?

In striking contrast to the prevailing notion of lymphocytes as the “generals” of the immune response and phagocytes as rather obtuse infantrymen, it is now clear that innate immune cells, not T cells, make the “decision” to stimulate a type 1 or type 2 response. Phagocytes instruct T cells to adopt a Th1 or Th2 phenotype by secreting polarizing cytokines, thereby altering the local microenvironment around the newly activated lymphocyte. The information used by innate immune cells to decide which cytokines to secrete include (1) the presence of certain “danger signals” [196] in the local microenvironment and (2) systemic factors affecting the host at the moment the danger signals are revealed.

Microbes contain within them information that directly induces secretion of IL-12 by phagocytic cells. Gram-negative endotoxin, or LPS, and gram-positive cell wall lipoteichoic acid bind to nonpolymorphic receptors on innate immune cells and directly induce secretion of IL-12 and other pro-inflammatory cytokines such as IFN-α and IFN-γ [197, 198]. Furthermore, bacterial DNA is rich in immunostimulatory sequences [199]. Well-described immunostimulatory sequences include poly-G sequences and CpG motifs [200, 201], which are hexamers of purine-purine-CG-pyrimidine-pyrimidine. Eukaryotic DNA contains very few CpG motifs, and even when they occur in eukaryotic genomes they tend to be methylated. Unmethylated prokaryotic CpG DNA is capable of directly inducing IFN-α and IFN-γ and IL-12 secretion from B-cells, natural killer cells, and monocyte/macrophages [202–206].

Other innately stimulatory microbial antigenic fractions have also been described, including antigens from Toxoplasma [207], mycobacteria [208], and fungi [197]. Finally, heat shock proteins, which are synthesized in response to cellular stresses in both prokaryotic and eukaryotic cells, are capable of directly inducing IL-12 secretion from innate immune cells [197]. Even host heat shock proteins induce IL-12, which indicated that the innate immune system is genetically programmed to scan local microenvironments for any sign of danger, even if it is an indirect sign of host tissue destruction rather than direct evidence of a microbial invader [209].

Microbial characteristics are also capable of direct induction of type 2 immunity. When a phagocyte attempts to ingest a particle larger than itself, the leukocyte reorganizes its cytoskeleton in a reaction termed “frustrated phagocytosis” [210, 211]. During this process the leukocyte exocytoses intracellular granules that would normally have fused with the ingested phagosome, spewing these granules toward the uningestible particle [212, 213]. The degradative enzymes and microbial peptides that are released are then able to damage the uningestible particle. Meanwhile, genetic transcriptional changes also occur within the innate immune cell as part of the frustrated phagocytosis complex of activities. There is, for example, a marked upregulation of IL-10 secretion and a decreased secretion of IL-12 in neutrophils undergoing frustrated phagocytosis [214]. Thus innate immune cells exposed to large extracellular pathogens, such as helminths, directly induce a type 2 cytokine environment.

Furthermore, as mentioned above, systemic host states at the time of antigen stimulation also modulate type 1/type 2 outcomes. Initial production of IFN-α and IFN-γ by innate immune cells and early responding γδ T cells and natural killer cells can also upregulate the local production of IL-12 [197, 215–218], thereby inducing a type 1 response. Conversely, pre-existing IL-4, IL-10, and IL-13 inhibit secretion of IL-12, thereby blocking Th1 polarization [197]. Elevated catechola-
mine, glucocorticoid, and estrogen/progesterone levels all directly suppress the development of a local cytokine milieu rich in IL-12, and instead induce IL-4, IL-10, and IL-13. Therefore, hosts who are undergoing physiological stress, are pregnant, or are taking glucocorticoids, cyclosporine, or FK-506 are innately prone to developing type 2 responses, even to antigens that would normally elicit a type 1 response.

The Molecular Genetics of Polarization

The past 5 years have witnessed a revolution in our understanding of the molecular genetics of Th1/Th2 polarization. Specifically, investigators have found that ligation of the IL-12 receptor on Th0 cells activates the transcription factor STAT4 (signal transducer and activator of transcription 4) [219], which triggers regulatory sequences leading to Th1 polarization [220]. Conversely, ligation of the IL-4 receptor on Th0 cells triggers activation of STAT6 [221], which suppresses Th1 polarization and leads to Th2 polarization [222]. Indeed, mice with germline disruptions of the STAT4 or STAT6 genes are unable to mount type 1 [223, 224] or type 2 responses [225, 226], respectively.

Even more recently, downstream targets of STAT4 and STAT6 have been identified. The transcription factor GATA-3 directly induces Th2 polarization and inhibits Th1 polarization, acting in concordance with STAT6 [227–229]. Conversely, activation of the transcription factor ERM by STAT4 induces IFN-γ expression [230]. Most recently, Szabo et al. [231] identified the penultimate effector of the STAT4-induced Th1 polarization, the transcription factor T-bet. T-bet exerts direct control over IFN-γ gene expression, and ectopic expression of T-bet in already polarized Th2 cells redirects the lymphocytes to adopt a Th1 profile. The reversal of phenotype of an already polarized lymphocyte is particularly intriguing, which suggests the possibility that small molecules can be designed to disrupt the genetic pathways controlling Th1/Th2 polarization. The development of such agents would allow clinicians to intervene in diseases resulting from inappropriate type 1 or type 2 immune responses.

Summary

Figure 3 summarizes the processes of Th1 and Th2 induction. Naive Th0 lymphocytes polarize into Th1 cells when they are activated in a microenvironment rich in IL-12. IL-12 is directly induced from innate immune cells by gram-negative LPS, gram-positive lipotechoic acid, prokaryotic CpG DNA motifs, and heat shock proteins, as well as pre-existing DHEA, IFN-α and IFN-γ. Conversely, IL-4, IL-10, corticosteroids and catecholamines directly inhibit newly activated Th0 cells from differentiating into Th1 cells.

Naive Th0 cells exposed to IL-4 differentiate into Th2 cells. Exposure of innate immune cells to large, nonphagocytosable particles induces secretion of IL-4, IL-10, and IL-13. Furthermore, any host state that leads to high levels of circulating catecholamines, glucocorticoids, estrogens, progestins, or prostaglandins will directly induce secretion of IL-4, IL-10, and IL-13 from innate cells and lymphocytes and directly suppress secretion of IFN-γ and IL-12. Exposure of naive Th0 cells to

Figure 3. Summary of Th1/Th2 induction. Cytokines, hormones, and microbial antigens stimulate the innate immune system to produce either IL-12 or IL-4 in the local microenvironment around a newly activated T cell. IL-12 induces Th0 polarization to the Th1 phenotype and inhibits polarization to the Th2 phenotype, whereas IL-4 acts reciprocally. CMI, cell-mediated immunity; CpG, purine-purine-C-G-pyrimidine-pyrimidine DNA hexamer; DHEA, dehydroepiandrosterone; Epi/NE, epinephrine/norepinephrine; HSP, heat shock protein; LTA, lipotechoic acid; LPS, lipopolysaccharide; PG, prostaglandin; est/n/pgstn, estrogen/progesterone; frust. phag., frustrated phagocytosis.
IL-12 inhibits polarization to Th2 cells; however, if both IL-4 and IL-12 are present at the time of naive T cell activation, the effects of the IL-4 will dominate, and a type 2 response will ensue.

RELEVANT DISEASE MODELS: ANIMAL MODELS

Type 1 Protective Models

*Leishmania.* The classic model of type 1/type 2 immunity is *Leishmania major* infection of Balb/C or C57BL mice. Balb/C mice are genetically prone to type 2 immune responses, whereas C57BL mice are prone to type 1 immunity. The genetic mechanisms of these tendencies are not well characterized, but Balb/C mice appear to have a defect in the normal induction of IL-12 [232], possibly due to a tendency for early hypersecretion of IL-4 [233]. The result is that newly activated T cells in Balb/C mice are resistant to Th1 polarization and instead become Th2 cells.

Sadick and Locksley et al. first reported that Balb/C mice are inherently susceptible to *Leishmania* [234, 235], and quickly thereafter they and others showed that C57BL mice are intrinsically resistant to the organism [236, 237]. Subsequent studies confirmed that CD4 cells from susceptible Balb/C mice responded to *Leishmania* infection by differentiating into Th2 effector cells, which could not protect the mice from infection, whereas C57BL CD4 cells polarized into Th1 cells that abrogated infection [238, 239]. Blockade of IL-4 in susceptible Balb/C mice allowed protective type 1 responses to develop, and the mice survived the infection [239–241]. Conversely, administration of IL-4 or abrogation of IFN-γ in normally resistant C57BL mice caused them to mount futile type 2 responses and succumb to disseminated disease [242].

Mosmann’s group (Krishnan et al. [243]) reported that pregnant C57BL mice are unable to mount their normal type 1 immune responses because of placental stimulation of IL-4 and IL-10 and suppression of IFN-γ. Such pregnant mice mounted futile type 2 responses and were unable to control *Leishmania* infections. The evolutionary benefit of suppression of inflammation by the pregnant state was made obvious in a second study by the same group; induction of a type 1 response to *Leishmania* in infected pregnant mice caused fetal resorption and uterine scarring [146].

IL-12 must be present during the initial infection in order for protective type 1 responses to develop, but once a population of Th1 cells is established, IL-12 is not required for the cells to mediate protection [244]. The dispensability of IL-12 after establishment of type 1 responses was strongly supported by a study in which mature Th1 cells adoptively transferred into severe combined immunodeficiency (SCID) mice were able to protect the mice from *Leishmania* infection without addition of recombinant IL-12 [245]. As Th1 cells do not actually secrete IL-12 (IL-12 is secreted only by antigen-presenting cells), IL-12 cannot have been necessary for the protective effect of the Th1 cells against infection.

Although addition of cytokines or anti-cytokine antibodies at the time of initial infection is able to reverse the type 1/type 2 polarity at the outset of the immune response, it has proved difficult to reverse type 1 or type 2 responses in vivo once they have already been established. Conversely, it has been possible to reverse the phenotype of populations of ex vivo T cells taken from animals with established infection [115, 246]. These studies raised an interesting question: if populations of ex vivo T cells taken from mice with established infections can be reverted in vitro, why can’t the phenotype of the overall immune response be reversed in vivo? A likely answer was provided by Nabors et al. [247]. These investigators administered IL-12 with or without a chemotherapy agent to mice with *Leishmania* infections. Only the combination of IL-12 and the antimicrobial agent was able to reverse the established nonhealing type 2 response in vivo, allowing a healing type 1 response to develop. The implication is that decreasing the antigenic burden of the infected animals was crucial to disinhibiting the animals’ type 1 immunity.

In concordance with this finding, inoculation of low infectious doses of *Leishmania* in Balb/C mice induced a type 1 response rather than the expected type 2 response [248, 249]. This is consistent with the notion that high infectious inoculations stimulate type 2 immunity, whereas lower inoculations allow protective type 1 inflammation to develop. These results indicate that antimicrobial chemotherapy is a powerful tool for inducing healing type 1 responses in hosts whose normal type 1 immunity is suppressed by overwhelming antigenic burden.

*Bacterial infections.* Pathogenic *Escherichia coli* and *Salmonella typhimurium* directly induced IFN-γ in vivo in infected mice. Strains of mice producing higher levels of IFN-γ cleared the microbes up to 10-fold more effectively, making them resistant to infection [250], whereas IFN-γ gene knockout mice suffered from severe septicemia following oral inoculation [251]. As well, ex vivo spleen cells taken from mice with acute *E. coli* pyelonephritis produced IL-2 and IFN-γ but no IL-4 during the first 7 days after infection [252]. Thereafter, a gradual switch in profiles was noted, such that by several months after infection the level of type 1 cytokines was decreased. This is consistent with the notion that the immune system uses type 1 responses for protection against acute infection and switches to type 2 responses when the danger is passed in order to reestablish homeostasis and protect the host from autoimmune destruction.

Exogenous IL-12 induced long-lasting protective type 1 immunity in mice infected with *Listeria monocytogenes* [253, 254], and IL-10−/− knockout mice were resistant to infection by Lis-
Such knockout mice quickly developed more intensely polarized type 1 responses than their wild-type littermates, so that by 48–72 h after infection, their microbial tissue burden was 50-fold lower than that of the wild-type mice [255]. An elegant study compared early ex vivo γδ T cell cytokines following murine infection by Listeria or the helminth Nippostrongylus. Within several days of infection, Listeria induced γδ T cells to secrete a type 1 profile of cytokines, whereas Nippostrongylus infection induced a rapid secretion of type 2 cytokines [256]. This is consistent with in vitro data suggesting that intracellular pathogens with innately antigenic fractions induce early IL-12 release from leukocytes, whereas large extracellular pathogens induce frustrated phagocytosis, thereby eliciting a type 2 response. Type 1 responses are also protective against other bacteria, such as Pseudomonas, Yersinia, and Klebsiella [257–261].

**Mycobacterial infections.** Type 1 immunity has also been shown to be protective against mycobacteria. Mice inherently resistant to Mycobacterium leprae produced IL-12 early on at the site of infection [262, 263], but mice susceptible to M. leprae infection failed to produce early IL-12 [262]. When IL-12 was administered to mice with established M. leprae infections, bacteria burdens were markedly decreased.

Orme et al. studied the evolution of an immune response to tuberculosis in infected mice by pulsing ex vivo CD4 cells with microbial fractions and measuring the resultant cytokines released [264]. Early during infection, and peaking with the time of maximum protective inflammation, IFN-γ dominated the cytokine response. By 3–5 weeks later, when the infection had been contained and granulomas had begun to organize in vivo, IL-4 and IL-10 dominated the cytokine profile, and IFN-γ was markedly suppressed. This is in concordance with the recurrent theme that type 1 cytokines are expressed during the protective phase of an immune response, and a switch is made to expression of type 2 cytokines during the resolution phase.

**Fungal infections.** Findings in studies of the role of type 1/type 2 immunity in Candida, Coccidioides, Cryptococcus, and Aspergillus infections parallel closely the findings from the Leishmania model: mice susceptible to fungal infections mount type 2 responses to the organisms, but resistant mice mount type 1 responses [265–270]. Blocking IL-4 or IL-10 at the time of infection of Balb/C mice with Candida increased the level of IFN-γ-induced cell-mediated immunity, which effectively protected these normally susceptible mice from the fungus [89, 271]. Indeed, inhibition of IL-4 allowed 81% of mice to survive the infection; none of the untreated control mice survived [271].

Adding to the notion that high microbial burden suppresses type 1 immunity, normally susceptible mice infected with sublethal inocula of Candida developed type 1 immunity instead of their usual type 2 immune response [272]. This reversal of immunologic phenotype was due to diminished induction of IL-4 secretion by the lower inoculum. Furthermore, lowering fungal burden in infected mice by treatment with either amphotericin B or fluconazole induced a healing type 1 immune response, an effect potentiated by blockade of IL-4 [273, 274]. Therefore, it is the balance of IL-12 and IL-4 induced early after infection that determines the eventual phenotype adopted by the adaptive immune response. High microbial burden tips the balance in favor of IL-4, suppressing cell-mediated immunity and polarizing the immune response toward a type 2 phenotype. Antimicrobial chemotherapy is an effective intervention to favor type 1 immunity by lowering microbial burden.

**Type 2 Protective Models**

**Helminths.** The same tendency toward type 2 immune responses that makes Balb/C mice inherently susceptible to bacterial infections makes them inherently resistant to infection by helminths [275]. In addition, strains of mice prone to type 1 responses, although innately resistant to most bacteria, are inherently susceptible to helminthic infections [275]. Therefore, unlike every disease model so far discussed, it is type 2 immunity rather than type 1 immunity that protects mammals from helminths [275–277]. Type 2 responses correlate with diminished worm burden, whereas type 1 responses allow chronic infection and scarring to develop [278]. Mast cell activation has been shown to be a key component of type 2 immunity to various helminths, including Trichinella and Nippostrongylus [279–281]. IL-9 is important in mast cell activation, which increases gastrointestinal peristalsis that successfully expels parasites from the gut [282]. Indeed, transgenic mice overexpressing IL-9 were highly resistant to trichinella infection [283], and blockade of IL-9 worsened infection in mice normally resistant to trichuris infection [284].

Abrogation of IFN-γ or administration of exogenous IL-4 in mice susceptible to helminths reversed their normal type 1 immunity, and the resultant type 2 response mediated expulsion of the parasite from the gut [285]. Conversely, blocking IL-4 or administering IL-12 in normally resistant mice led to the development of futile type 1 responses, which allowed chronic infection to develop [285–287].

Although the above studies provide convincing evidence that type 2 immunity is protective against helminths, recent revisionist thinking has challenged this notion [288]. Although conceding that type 2 immunity is the natural mammalian response to helminths, the revisionist theory states that type 2 immunity occurs because helminths deviate the host immune response to a nonprotective posture, enabling the worms to successfully infect the host. The evidence in support of this notion derives from studies in which serum IgE and IL-4 levels have failed to correlate with host protection against helminths [289–295]. In fact, IL-4 knockout mice are perfectly resistant
Immunity to Nippostrongylus infection, indicating that IL-4 is not required for protection against this helminth in mice [296].

The notion that type 2 immunity is a maladaptive response to helminthic infection was directly challenged by an elegant study by Bancroft et al. (figure 4) [297]. These investigators compared immune responses in wild-type, IL-4−/−, and IL-13−/− knockout mice following infection with Trichuris muris. Wild-type mice mounted strong type 2 responses that successfully cleared the helminth. IL-4 knockout mice failed to mount type 2 responses; they produced no IL-4 and had markedly diminished IL-5 and IL-13 levels, which resulted in a huge increase in worm burden. It is interesting that in contrast to IL-4 knockout mice, production of IL-4 and IL-5 by the IL-13 knockout mice was only mildly diminished in comparison with that in wild-type animals, indicating that they still mounted type 2 responses. Nevertheless, despite their apparent type 2 response, the IL-13 knockout mice suffered from twice the worm burden than that in the IL-4 knockout mice. Thus a type 2 immune response is protective against Trichuris only if IL-13 is present.

IL-4 is a key inducer of type 2 immunity, but IL-13 can mimic some of IL-4’s function by binding to a shared IL-4/IL-13 receptor [298–300]. Furthermore, much like IL-12 induces type 1 immunity but IFN-γ actually mediates type 1 effects, IL-13 appears to be more important than IL-4 for the actual protective effect of type 2 immunity against helminths. Indeed, whereas IL-4 knockout mice expel Nippostrongylus normally from the gut, IL-13 knockout mice and IL-4/IL-13 double-knockout mice are extremely susceptible to Nippostrongylus infection [301, 302].

An overwhelming majority of data indicate that type 2 immunity is the key to mammalian protection against infection by helminths. IL-4 is important for induction of type 2 immunity, but IL-5, IL-9, and IL-13 are the key effector cytokines in type 2-mediated protection. IL-5 and IL-9 act via eosinophil and mast cell stimulation. Although it has been suggested that IL-13 acts via secondary induction of TNF [303], its definitive mechanism remains obscure.

RELEVANT DISEASE MODELS: CLINICAL DISEASES

Leishmania. The findings of studies of immunity in patients infected with Leishmania parallel the data generated in animal models. mRNA analysis of cutaneous lesions caused by Leishmania braziliensis demonstrated 2 profiles in human patients [304]. Biopsy specimens from patients with localized disease displayed prominent mRNA coding for IL-2, IFN-γ, and LT-α consistent with a protective type 1 immune response. Biopsies of lesions from patients suffering from destructive mucocutaneous American leishmaniasis demonstrated a marked increase in the level of IL-4 mRNA, which is consistent with a failed type 2 host immune response.

Analogous to murine data on infectious burden, expression of IL-10 was found to be higher in patients with active Leishmania donovani infections than in patients who had been cured of disease [305]. T cells from patients who had recovered from limited Leishmania infections expressed high levels of IFN-γ and LT-α, but little or no IL-4, when exposed in vitro to Leishmania antigens [306]. Conversely, ex vivo cytokine production by lymphocytes taken from patients with severe visceral leish-

![Figure 4](https://academic.oup.com/cid/article/32/1/76/311106)
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Figure 5. Change in cytokine pattern over time following BCG vaccination in humans. Ex vivo peripheral blood mononuclear cells (PBMCs) were analyzed by fluorescence-activated cell-sorting (FACS) for intracellular cytokine production on sequential days following administration of BCG vaccine to healthy volunteers. The percentage of mononuclear cells expressing type 1 cytokines (IL-2, IFN-γ, lymphotoxin [LT]-α) is shown on the left y axis, and the percentage of cells expressing type 2 cytokines (IL-4, IL-5, IL-10) is shown on the right y axis (figure reproduced with permission from [328]).

Maniasis was dominated by the type 2 cytokines IL-4 and IL-10 [307]. Thus type 1 immunity is the key to protection against Leishmania infections in humans, and a high infectious burden suppresses the human immune system from mounting type 1 responses.

Bacterial infections. Direct examination of cytokine profiles of patients infected with Haemophilus influenzae and Streptococcus pyogenes revealed that IFN-γ and LT-α were the dominant cytokines elicited at the site of infection, and no IL-4 was found [308]. Similarly, Lactobacillus, S. agalactiae, S. pyogenes, and Listeria directly induced IL-12 and IFN-γ but not IL-4 secretion from human leukocytes [309–311]. Case reports concerning patients with identified genetic defects in cytokine or cytokine receptor genes are illustrative of the role of cytokines in host defense against infection. For example, patients with defects in the IFN-γ, IL-12, or IL-12-receptor genes are unable to produce IL-12 and/or IFN-γ and as a result have developed severe infections caused by Salmonella [312, 313], Streptococcus, or Listeria [314, 315].

Mycobacterial infections. The dichotomy between patients with lepromatous leprosy, which is the disseminated, severe form of the disease, and those with tuberculoid leprosy, which is local disease controlled by the immune system, parallels in vivo cytokine production [316]. Lepromatous leprosy develops in patients who mount type 2 immune responses to the organism, whereas tuberculoid leprosy is synonymous with a successful type 1 immune response to M. leprae [164, 317]. Mononuclear cells from lepromatous leprosy patients secreted high levels of prostaglandin E and IL-10, thereby suppressing IL-12 induction of IFN-γ, whereas cells from tuberculoid leprosy patients did not secrete prostaglandin E and IL-10 [164]. Furthermore, lesion-biopsy specimens from patients with tuberculoid leprosy contained Th1 cells expressing 10-fold higher levels of IL-12 mRNA than in lepromatous patients [316]. Conversely, biopsy specimens from lepromatous patients contained high levels of IL-4 and IL-10.

There is also a dichotomy between a high humoral response and a high DTH response among patients infected with Mycobacterium tuberculosis [318, 319]. Patients with active tuberculosis have been shown to have diminished DTH reactions, cell proliferation, and IFN-γ production in response to PPD, as well as higher levels of antitycobacterial antibodies, than do PPD-positive, uninfected case control subjects [320]. In addition, infected patients produced higher levels of IL-4 [321, 322]. These data are consistent with the notion that active M. tuberculosis infection was related to dominant type 2 immunity, whereas protected patients mounted type 1 immune responses to the organism. Multiple published case reports of severe mycobacterial infections in patients with genetic IFN-γ or IL-12 deficiencies confirm the importance of type 1 immunity in protection against these organisms [312, 313, 315, 323–327].

As in mice, immunity to mycobacteria in humans evolves over time (figure 5) [328]. Within 5 days after BCG vaccination, leukocyte production of IL-2, IFN-γ, and LT-α was shown to increase dramatically, whereas production of Th2 cytokines remained minimal. By a week after inoculation, the production of Th1 cytokines reached a plateau and there was a sudden burst in IL-4 production. By days 10–12 after vaccination, there
was a remarkable suppression of type 1 cytokine activity and a rise in IL-5 and IL-10 production. This elegant study provided powerful confirmatory evidence that type 1 immunity is directly induced by mycobacteria and that the immune system naturally switches over time to a type 2 immune response in order to reestablish homeostasis after the battle is won.

Fungal infections. Similar to antigenic fractions from typical bacteria, certain *Candida* antigens directly induced secretion of IL-2 and IFN-γ, but not IL-4 and IL-10, from human leukocytes [329]. It is interesting that patients with chronic mucocutaneous candidiasis seem refractory to the normal induction of IL-2 and IFN-γ. Cells from such patients produced an altered profile of cytokines, more reminiscent of a type 2 profile, when stimulated in vitro with *Candida* antigens [330]. Since IL-4 inhibits human phagocytes from killing *Candida* [331], this switch to a type 2 profile can explain the inherent susceptibility of such patients to candidial infections.

The importance of IFN-γ and type 1 immunity in host defense against fungal infections is made clear by observations of patients with chronic granulomatous disease. Such patients suffer from an inability to generate a respiratory burst in phagocytic cells and therefore commonly develop invasive pyogenic and fungal infections, often caused by *Aspergillus* [332]. Treatment with recombinant IFN-γ stimulates killing of fungi by phagocytes of patients with chronic granulomatous disease [333] and thereby reduces the frequency and severity of clinically apparent fungal infections [334]. Therefore, IFN-γ, and by extension type 1 immunity, is protective against fungal infections.

HIV. Numerous studies have found that during the progression of AIDS, mononuclear cells lose the ability to secrete IL-2, IL-12, and IFN-γ and produce increased levels of IL-4 and IL-10 [335–340]. Because of the loss of IL-2 secretion, T cells from HIV-positive patients are typically unable to proliferate when stimulated by common antigens. However, addition of recombinant IL-12 to in vitro cultures of T cells restored not only the lymphocytes’ ability to proliferate but also their production of IL-2 and IFN-γ [341]. Analyses of the impact of highly active antiretroviral therapy on immune reconstitution in patients with AIDS have been recently published [342, 343]. Investigators reported that potent inhibition of viral replication reversed the suppression of IL-2 and IFN-γ production in the patients’ leukocytes while markedly diminishing their overproduction of IL-4 and IL-10. This paralleled a recovery in T cell counts. Similar results have been found in studies of children treated with highly active antiretroviral therapy [344]. Therefore, clinicians may be able to reverse the immune dysregulation in patients with AIDS by affecting viral suppression.

Th1 cells express the chemokine receptor CCR5, whereas Th2 cells express the CXCR4 receptor [345–347]. M-tropic strains of HIV, which are the infectious particles, use CCR5 as a co-receptor for viral entry into the host cell, whereas T-tropic HIV strains, which emerge as the dominant strains during the progression of AIDS, use CXCR4 [348, 349]. This switch in receptor usage during disease progression parallels the frequency with which Th1 and Th2 cells are found in vivo. Thus part of the pathogenesis of AIDS is a selective loss of Th1 cells, which then forces the virus to adapt to infect Th2 cells in order to persist in the host.

Although suppression of IL-12 production by phagocytes is one mechanism by which HIV acts to suppress type 1 immunity, in vivo studies have demonstrated an additional effect. Hormonal abnormalities, such as a loss of serum testosterone derivatives, are commonly seen in patients with AIDS and result in lean-muscle-mass wasting [350–352]. In fact, decreases in serum DHEA were linearly related to loss of CD4 cells during AIDS progression [353, 354], and patients with serum DHEA levels <180 ng/mL had a 2.3 RR for progression to AIDS within 2 years [355, 356]. Conversely, loss of CD4 cells and AIDS progression were inversely correlated with serum cortisol levels [350, 354, 357]. Therefore, AIDS is associated with hormonal conditions—namely, low DHEA and high glucocorticoid levels—which suppress IL-2, IFN-γ, and IL-12 production and stimulate IL-4 and IL-10 production [358]. This is the perfect recipe for systemic suppression of type 1 responses and stimulation of type 2 responses.

These studies provide the theoretical underpinning for the hypothesis that HIV induces a gradual paralysis of type 1 immunity, allowing expansion of Th2 cells at the expense of naive T cells and Th1 cells. This theory was confirmed by clinical observations in several key studies. As IgE is a highly specific bioassay for type 2 immune responses, Vigano et al. compared serum levels of IgE in 58 vertically infected HIV-positive children and 35 serorevertant control subjects (figure 6) [359, 360]. They found that HIV-positive children had significantly higher levels of IgE antibody than did the serorevertants, and 75% of the children whose IgE levels were abnormally high had a precipitous drop (by ≈30%) in their CD4 counts over the subsequent year. Therefore, HIV-positive children suffer from dysregulated immunity in which type 2 responses overwhelm type 1 responses, and the rate of loss of type 1 immunity parallels the loss of T helper cells.

HIV is unique among the disorders so far discussed in that several interventional cytokine trials involving humans have been performed. Kovacs et al., for example, published an impressive randomized trial of IL-2 plus zidovudine versus zidovudine alone in the treatment of 60 patients with AIDS [361]. After a year of follow-up, the CD4 cell counts of patients in the IL-2 group doubled from a mean of 428 cells/μL to 916 cells/μL, whereas those of the patients treated with zidovudine alone dropped from a mean of 406 cells/μL to 329 cells/μL. There was no difference in the viral load between the 2 groups.
at the end of the year. Thus the difference in T helper cell
counts at the end of the year was unrelated to the degree of
viral suppression. Rather, the exogenous IL-2 restored the im-
mune system’s ability to produce more peripheral T cells, a
finding confirmed by more recent clinical trials [362–364].

Smaller studies of subcutaneous IL-2 in HIV-infected pa-
tients by Davey et al. [365] and De Paoli et al. [366] yielded
similar results. The latter study examined the effect of IL-2 on
naive T cells, identified by staining for the surface marker
CD45RA. Like Kovacs et al. [361], De Paoli et al. [366] reported
a doubling of CD4 cell counts at 1 year in AIDS patients treated
with IL-2 and antiretrovirals and no significant change in the
CD4 counts of patients treated with antiretroviral therapy alone.
Like the total T cell population, the numbers of naive T cells
also doubled in the IL-2-treated patients, whereas there was no
change in the number of naive T helper cells in the group
treated with antiretrovirals alone. Therefore, IL-2 not only en-
ables activation of and expansion of the number of Th1 effector
cells but also promotes survival of naive Th0 cells in patients
with AIDS. In addition, Khatri et al. [367] studied ex vivo
cytokine production by T cells in patients with AIDS who were
treated with IL-2. They reported that the patients’ T cells pro-
duced twice as much IFN-γ and half as much IL-10 during
the IL-2 therapy as they did before or after treatment.

The final proof of a shift from type 1 to type 2 immunity
in patients with AIDS derives from an elegant study by Norbiato
et al. [368]. These investigators studied 10 patients with AIDS
who developed Addsonian symptoms and were found to be
glucocorticoid-resistant, as determined by low-affinity binding
of their glucocorticoid receptors to dexamethasone. The glu-
cocorticoid-resistant AIDS patients were compared to 10 case-
control AIDS patients with normal steroid receptors and 10
HIV-negative control subjects. Glucocorticoid-resistant AIDS
patients had urinary cortisol levels 5 times higher than those
in the HIV-negative patients. However, because their resistance
to cortisol spared them from its immunosuppressive effects
[369], T cells from the glucocorticoid-resistant patients were
able to secrete high levels of IL-2 and maintain a normal ratio
of serum IFN-γ to IL-4 (figure 7).

Conversely, although patients with AIDS who had normal
steroid receptors had urinary cortisol levels somewhat lower
than those of the steroid-resistant patients with AIDS, the cor-
tisol completely suppressed endogenous IL-2 production in
these patients (figure 7). This loss of IL-2 secretion almost
totally abrogated IFN-γ production, whereas IL-4 and IL-10
production were strongly upregulated. A comparison of serum
IgE levels in the cortisol-resistant and normal-receptor patients
with AIDS closes the argument. The IgE levels of glucocorti-
coid-resistant patients with AIDS were barely elevated in com-
parison with those of HIV-negative control subjects, which in-
dicates an essentially normal ratio of in vivo type 1/type 2
immunity. Conversely, patients with AIDS with normal steroid
receptors had IgE levels 100-fold higher than those of HIV-
negative control subjects. Clearly, these patients with AIDS were
suffering from an overwhelming excess of type 2 immunity.

Thus in vitro and in vivo data indicate that HIV, beyond
simply killing T cells, disrupts normal homeostasis of type 1
and type 2 immunity. Abnormally high levels of glucocorticoids
and suppressed levels of DHEA, along with direct viral sup-
pression of IL-12 production, create a host environment that
suppresses differentiation of type 1 effector cells and stimulates
development of type 2 immune responses that are ineffective
at controlling a broad range of pathogens.

![Figure 6. Ex vivo cytokine production by peripheral blood mononuclear cells and serum IgE levels in 58 vertically infected children with HIV infection or AIDS, compared to that in 35 serorevertant HIV-negative control subjects (figure reproduced with permission from [359, 360]).](https://academic.oup.com/cid/article/32/1/76/311106)
RELEVANT DISEASE MODELS: TYPE 1/TYPede HOMEOSTASIS

As repeatedly discussed, the immune system restores homeostasis by switching a type 1 response into a type 2 response once an infection has been cleared. Recent data from Gett and Hodgkin shed new light on the mechanism of this switch [370]. These investigators developed a sophisticated experimental system to determine how many cell divisions a given T cell had undergone after activation. They simultaneously measured in vitro cytokine production by the lymphocytes, allowing correlations to be drawn between cell division number and cytokine production (figure 8).

Immediately after activation of naive T cells, only IL-2 was produced. During the subsequent cell divisions, polarization began to occur, and IFN-γ was first secreted at cell division number 4. IL-4 secretion began at division 6, accompanied by the gradual waning of IL-2 secretion. IFN-γ production reached a plateau by cell division 7, at which time IL-4 production began to logarithmically increase. Finally, at cell division 8, there was a sharp burst in production of IL-10. Unfortunately, the limit of the technology is about 8 cell divisions. However, with a sudden upswing in IL-10 production at division 8, it can be hypothesized that cell divisions occurring beyond 8 would be associated with a loss of IFN-γ secretion and the establishment of a polarized Th2 profile of IL-4 and IL-10 secretion.

Thus there is a molecular genetic basis for the clinical phenomenon of switching from a type 1 to a type 2 response over time during infection. T cells appear to gradually lose the ability to secrete pro-inflammatory cytokines as they mature after multiple cell divisions.

VACCINES AND IMMUNOTHERAPY: PARADOX AND PROMISE

Clinicians traditionally measure vaccine-induced protective immunity by following antibody titers. Furthermore, passive immunization, or administration of exogenous antibody, mediates protection against a variety of infections. Thus, although the model outlined above indicates that type 1 responses are the keys to protection against most infections, paradoxical observations suggest that vaccines and passive immunization rely on type 2 immunity to mediate protection.

Three concepts resolve this paradox, fitting the mechanisms of vaccines and passive immunization with the type 1/type 2 model. The first concept is that type 1 immunity does not actively suppress antibody responses. Although titers are lower in dominant type 1 responses than in type 2 responses, Th1 cells are quite capable of inducing antibody production by B cells [13]. Thus antibody production is consistent with either a type 1 or type 2 response, depending on the subtypes of antibody present. The IFN-γ produced by Th1 cells causes antibody class switching from IgM to the IgG1 and IgG3 subtypes, rather than the IgG2, IgG4, and IgE that are induced by IL-4. IFN-γ–induced IgG1 and IgG3 bind avidly via their Fc portions to the Fcγ receptor of phagocytes. Thus, by serving as opsonins, antibody induced during a type 1 immune response synergistically increases the effectiveness of cell-mediated immunity.

The second concept is that type 2 immunity, in addition to inducing antibody, actively suppresses cell-mediated immunity. Thus administration of exogenous antibody is not equivalent to induction of a type 2 immune response. Rather, passive

Figure 7. Twenty-four-hour urine cortisol, serum cytokine, and serum IgE levels in glucocorticoid-resistant patients with AIDS (AIDS-GR) and glucocorticoid-susceptible control patients with AIDS (AIDS-GS) and HIV-negative control subjects (HIV−; figure reproduced with permission from [368]).
immunization with exogenous antibody garners the immunologic benefit of a humoral response without the added deficit of suppressing cell-mediated immunity. Instead, passive immunization synergizes with type 1 immunity by providing extra opsonins to assist activated phagocytes. Passive immunization therefore does not fit into either the type 1 or type 2 description of endogenous immunity. This is logical, since passive immunization is obviously not a normal, physiological component of mammalian immune systems.

Although antibody titers are used clinically to determine the efficacy of vaccines, this correlation of high titers with protective immunity cannot be reliably interpreted as an indication that vaccines work via induction of type 2 immunity. Although there is an inverse relationship between the degree of cell-mediated and humoral immunity elicited by a given antigen, the ratio of type 1/type 2 immunity can range from highly polarized to equivalent (figure 1). Indeed, Parish demonstrated 2 zones of humoral and cell-mediated equivalency in his seminal article [3]. Thus the third concept is that since most experimental studies of vaccines examine only the humoral response, and clinically there are no useful cell-mediated immune assays, the correlation of high antibody titers with protective immunity cannot be interpreted in terms of the type 1/type 2 paradigm. There is no way to know to what degree, on the scale demonstrated by Parish [3], a given vaccine tilts the balance of type 1/type 2 immune response in a patient. High antibody titers might reflect a state of type 1/type 2 equivalence, or they might reflect stimulation of antibody by Th1 cells rather than Th2 cells. Indeed, antibody induced by vaccines may be of the IgG1 or IgG3 isotype, consistent with a type 1 immune profile [371].

Only after directly analyzing cytokine patterns elicited by vaccines or concurrently studying humoral and cell-mediated responses can one comment on the relative effects of a vaccine on inducing type 1/type 2 immunity. When such studies have been undertaken, a dominance of type 1 immunity has been found to be elicited by vaccines [371, 372].

Type 1 outcomes generate both cell-mediated and humoral responses that act synergistically, whereas type 2 outcomes generate humoral responses but actively suppress cell-mediated responses. Thus the type 1/type 2 immunity model indicates that vaccines intended for virtually all infections, save those directed at large, nonphagocytosable eukaryotes, should be designed to skew the induced response toward a type 1 profile. Multiple mechanisms to mediate vaccine-induced type 1 polarization have been described, including the use of IFN-γ and IL-12 as vaccine adjuvants [373–376] and the inclusion of genes coding for IFN-γ, IL-12, or CpG motifs in DNA vaccines [377–381]. In animal models, vaccines inducing type 1 immunity have been proven highly effective at preventing infections, whereas vaccines inducing type 2 immunity increase susceptibility to infection [270, 373, 374, 380, 382–386].

**CONCLUSIONS: A UNIFYING HYPOTHESIS**

Type 1 immunity is the default response to all infections by normal inocula of intracellular or phagocytosable microbes oc-
currying in nonimmunosuppressed hosts. Type 1 immune responses clear such pathogens, thereby diminishing further antigenic stimulation for type 1 immunity. In addition, T lymphocytes naturally switch from production of type 1 cytokines to production of type 2 cytokines as they progress through multiple cell divisions. Therefore, over time a type 1 immune response will tend to convert into a type 2 response, allowing homeostasis to be reestablished. However, with persistent antigenic stimulation—for example, if the type 1 immune response is never able to completely clear the infection—continual stimulation of T cells can induce chronic type 1 responses, leading to host tissue destruction.

Conversely, type 2 immunity is the default response to infections by large, extracellular pathogens that cannot be phagocytosed, such as helminths. Type 2 immunity naturally develops over time from type 1 immune responses. However, in patients who have excess sympathetic stimulation before infection, have excess glucocorticoids or high estrogen or progesterin levels, are IL-2-deficient because of cyclosporine or FK-506, or are inoculated by an overwhelming microbial burden, the usual type 1 response is suppressed and a type 2 response occurs instead.

Clinical factors that induce glucocorticoid or sympathetic responses will tend to make patients susceptible to infections that would normally be dealt with by type 1 immune responses. Malnutrition has been shown to suppress serum DHEA levels and directly induce high systemic levels of glucocorticoids and catecholamines [387–390], as has the presence of malignancy, even independently of malnutrition [391]. Indeed, patients with malignancies are prone to type 2 immunity at the expense of type 1 responses [392, 393]. Furthermore, the clinical conditions of congestive heart failure [394–396], chronic obstructive pulmonary disease [397], and hepatic cirrhosis [398] are all associated with hypersympathetic stimulation and high circulating catecholamine levels, conditions suppressive to type 1 immunity. Finally, severe systemic stresses induced by traumatic injury [399, 400], extensive surgery [401, 402], and the use of total parenteral nutrition [403] have been shown to suppress type 1 immunity and favor type 2 immunity.

The implications for clinicians from these data are three. First, the paradigm of type 1 and type 2 immunity provides a pathophysiological explanation for why patients with the above systemic ailments are prone to severe infections. Second, the model suggests new potential weapons in the clinical battle for host defense. For example, the utilization of β-blockers to reverse catecholamine-induced suppression in patients such as those with AIDS, trauma, congestive heart failure, or cirrhosis or those in intensive care units is intriguing. We are unaware of any published study reports describing the incidence of infections in patients receiving β-2 agonist bronchodilators, catecholamine pressors, or β-blockers for heart failure or hypertension. Such data would be invaluable in the study of host immunity to infection and could easily be obtained by careful reporting of the incidence of infections in future clinical trials of β-2 agonists, pressors, or β-blockers. Third, the model explains an important and previously unknown effect of antimicrobial chemotherapy: lowering antigenic burden by treating with antibiotics disinhibits protective type 1 immunity.

Reports have already been published of phase I/II clinical trials utilizing several different type 1/type 2 cytokines to polarize patients’ immune responses toward appropriate phenotypes. Early studies on the use of IL-2 and IL-12 in humans revealed their potential for severe systemic adverse effects [404, 405]. Despite the initial setbacks in patients with cancer, the use of newer schedules of dosing of IL-2 and IL-12 have demonstrated impressive effects in patients with infectious diseases. The use of low-dose adjuvant IL-2 in patients with multidrug-resistant tuberculosis led to a 60% cure rate in these difficult-to-treat patients [406], and the benefits of IL-2 for patients with AIDS have already been described.

In addition, IL-12 has demonstrated antiviral effects with minimal toxicity in patients with chronic hepatitis [407, 408]. Conversely, the anti-inflammatory effects of IL-10 in humans [409–411] have led to its use in hyperinflammatory states ranging from psoriasis [412] to organ transplantation immunosuppression [413] to Crohn’s disease [414], with promising results. Blockade of cytokine effects has also been attempted. Specifically, inhaled recombinant IL-4 receptor has shown promise as an anti-asthmatic agent, serving to soak up IL-4 in the airways [415]. Finally, as mentioned earlier, the targeted design of small molecules capable of disrupting or inducing transcription factors regulating Th1/Th2 polarization may provide an additional set of clinical weapons to intervene in pathological states resulting from aberrant type 1 or type 2 immunity.

However, exogenous administration of cytokines is a systemic intervention, whereas the immune system normally regulates itself on the basis of the cytokine milieu present at local microenvironments. Clinicians must develop techniques to administer cytokines in a localized fashion rather than flooding the vascular compartment with them. Such techniques might include the inclusion of genes coding for IL-12 or CpG motifs in DNA vaccines against microbes and the use of liposomally coated cytokines targeted toward in vivo–activated leukocytes [416, 417].

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