TH1 and TH2 Lymphocyte Development and Regulation of TH Cell– Mediated Immune Responses of the Skin

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Since the first description of the subpopulations of TH1 and TH2 cells, insights into the development and control of these cells as two polarized and physiologically balanced subsets have been generated. In particular, implications of the TH1-TH2 concept for TH cell–mediated skin disorders have been discovered. This article will review the basic factors that control the development of TH1 and TH2 cells, such as the cytokines IL-12 and IL-4 and transcription factors, the possible role of costimulatory molecules, and specialized dendritic cell populations. These regulatory mechanisms will be discussed in the context of polarized TH1 or TH2 skin disorders such as psoriasis and atopic dermatitis. Also presented are the principles that govern how chemokines and chemokine receptors recruit TH1 and TH2 cells to inflammatory sites and how they amplify these polarized TH cell responses. All of these concepts, including a novel role for IL-4–inducing TH1 responses, can contribute to the design of better therapeutic strategies to modulate TH cell–mediated immune responses.
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The regulation of T helper (TH) cell differentiation and associated effector responses have been important subjects of study initiated by the identification and characterization of cell-mediated versus humoral immunity (Parish, 1972; Cherwinski *et al*, 1987). Following the initial activation by immunogenic peptides presented in the context of major histocompatibility (MHC) molecules by antigen-presenting cells (APC), naïve Th lymphocytes can differentiate into two major phenotypically distinct memory TH cell populations, TH1 and TH2. TH1 cells characteristically produce interleukin-2 (IL-2) and interferon-γ (IFN-γ); induce macrophage activation; and are very effective in controlling infection with intracellular pathogens. In contrast, TH2 cells secrete IL-4, IL-5, and IL-13 as key signature cytokines; are excellent helpers for B cells in producing antibodies; and are required to eradicate helminths and extracellular parasites. In addition, other CD4+ T regulatory cell populations exist that secrete high levels of IL-10 and TGF-β and display the suppressive potential to dampen down both TH1 and TH2 responses. The first half of this review will focus on different factors that control the development of TH1 and TH2 cells; the second half will present some new approaches to redirecting TH1 and TH2 cells responsible for pathological settings, particularly those associated with skin disorders such as atopic dermatitis and psoriasis. Also discussed will be the role of chemokines and chemokine receptors in TH1- or TH2-mediated immune reactions of the skin, indicating new and specific therapeutic strategies.

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Abbreviations: APC, antigen-presenting cells; CCR, CC chemokine receptor; CHSR, contact hypersensitivity reaction; CL, cutaneous lymphocyte antigen; CTLA, cytotoxic T lymphocyte–associated antigen; CXCR, CXC chemokine receptor; DC, dendritic cells; DTHR, delayed-type hypersensitivity reaction; ICOS, inducible costimulator; IFN, interferon; IL, interleukin; MDC, macrophage-derived chemokine; MHC, major histocompatibiliy molecule; SLAM, signaling lymphocyte activation molecule; TCR, T cell receptor; TH, T helper cell.

DOMINANT CYTOKINES IN THE DEVELOPMENT AND CONTROL OF TH1 AND TH2 CELL RESPONSES

Data generated from *in vitro* and *in vivo* experiments using human and murine cells demonstrate that the cytokine composition of the milieu in which TH cells are activated plays a crucial role in determining the outcome of the TH cell response. IL-12, produced by activated monocyte/macrophages and dendritic cells (DC), is the dominant factor promoting TH1 cell polarization in human and mouse systems. Accordingly, gene-modified mice deficient in either IL-12p40 or IL-12 receptor β2 proteins impair TH1 responses. In addition, IFN-α is a strong TH1-polarizing factor in humans (Brinkmann *et al*, 1993; Hsieh *et al*, 1993; Manetti *et al*, 1993). Conversely, IL-4 is essential for the differentiation of IL-4–producing TH2 cells in humans and mice, although the initial source of this cytokine is still a subject of debate (Le Gros *et al*, 1990; Rocken *et al*, 1994; Breit *et al*, 1996; Sornasse *et al*, 1996; Himmelrich *et al*, 1998).

Besides IL-12, IFN-α, and IL-4, other cytokines have been demonstrated to play a role in TH cell differentiation. IFN-γ itself can further enhance IFN-γ production and therefore enlarge the pool of TH1 cells. This effect can be the result of direct IFN-γ signaling and activation of TH1-specific transcription factors, or it can be the indirect consequence of IFN-γ–mediated IL-12 production by macrophages. Ligation of the IL-1 receptor (R) family member IL-18R by IL-18 has been demonstrated to synergize with IL-12 in enhancing IFN-γ production by TH1 cells (Murphy *et al*, 2000; Robinson *et al*, 1997). Other members of the IL-12 cytokine family, such as IL-23 and IL-27, can also play

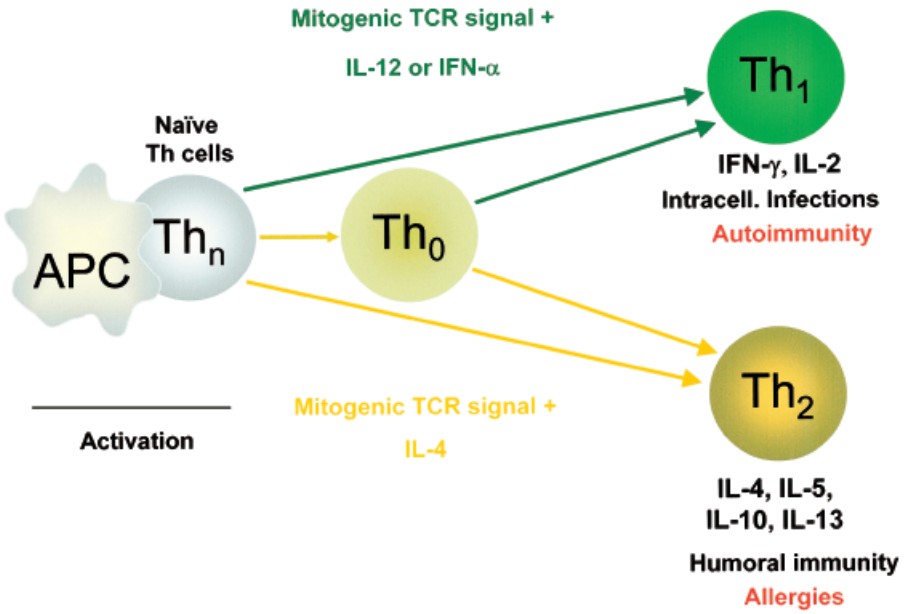

Figure 1. TH1 and TH2 differentiation is dominated by cytokines. Naïve TH cells are activated by antigen-presenting cells (APC), presenting an appropriate peptide via MHC class II molecules. Activation by APC in the presence of costimulatory signals or other mitogenic T cell receptor (TCR) stimuli can lead to the development of TH0 cells that produce an array of cytokines. When IL-12 or IFN-α dominates the microenvironment, TH cells differentiate into TH1 cells, which are defined by the cytokine pattern they produce upon stimulation. TH1 cells produce IL-2 and IFN-γ and are devoid of IL-4, IL-5, and IL-10. Responses dominated by TH1 cells mediate effective immunity against intracellular microbes. If directed against autoantigens, however, TH1 cells can be responsible for autoimmune diseases. When the mitogenic stimulus is given in the presence of IL-4, TH cells become polarized TH2 cells, which produce IL-4, IL-5, IL-10, and IL-13, but no IFN-γ, upon activation. TH2 cells are potent mediators of antibody responses, but are also involved in allergic reactions and atopic diseases.

an instructive role in TH1 development (Oppmann *et al*, 2000; Pflanz *et al*, 2002). In the case of TH2 development, IL-4 is dominant, but APC-derived IL-6 plays an instructive role in TH2 differentiation by inducing early IL-4 production in TH cells (Rincon *et al*, 1997). Furthermore, a member of the IL-1R family, T1/ST2, was recently discovered and demonstrated to be selectively expressed on TH2 cells. T1/ST2 expresses naïve TH cells differentiated into TH2 cells and even naïve TH cells from IL-4–deficient mice. Cross-linking of T1/ST2 was shown to enhance TH2 cytokine production, just as IL-18 enhances TH1 cytokine production (Lohning *et al*, 1998; Meisel *et al*, 2001).

TRANSCRIPTION FACTORS IN TH1 AND TH2 CELL DIFFERENTIATION

In general, cytokine signaling requires heterodimerization of receptor chains. This triggers phosphorylation of Janus kinases and recruitment of signal transducers and activators of transcription (Stat), which translocate to the nucleus and mediate the activation of cytokine-responsive genes.

Some of the Stat molecules have been identified as playing a dominant role in TH1 and TH2 cell polarization. Stat4 is activated in response to IL-12 via IL-12R and mediates the upregulation of IFN-γ transcripts, leading to a TH1 cytokine pattern. Strikingly, deleting IL-12R or Stat4 in mice results in similar phenotypes (Cooper *et al*, 1993; Kaplan *et al*, 1996; Thierfelder *et al*, 1996). Recently it was shown that mice deficient in Stat1 also have impaired IFN-γ functions. Stat1 is activated by IFN-γ signaling and upregulates T-bet, a transcription factor that induces IFN-γ production and TH1 differentiation (Szabo *et al*, 2000; Ihle, 2001; Lighvani *et al*, 2001; Afkarian *et al*, 2002). IFN-γ-mediated induction of T-bet results in upregulation of IL-12R, which in turn increases T-bet levels via Stat4 activation (**Fig 2**; Murphy *et al*, 2000). The cross-talk between these pathways leads to coamplification and further IFN-γ production.

IL-4R ligation activates Stat6, which selectively upregulates GATA-3, especially early in TH2 development, and directly induces gene transcription of TH2 cytokines (**Fig 2**; Zheng and Flavell, 1997; Murphy *et al*, 2000; Farrar *et al*, 2002). The GATA-3–dependent effects are further augmented by GATA-3 autoactivation and signaling through costimulation by CD28 (Farrar *et al,* 2002). GATA-3 is recognized to be the most dominant factor regulating TH2 cytokines, but it mainly controls transcription of IL-5 and IL-13 and, to a lesser extent, IL-4 (Zhang *et al*, 1997; Lee *et al*, 1998; Kishikawa *et al*, 2001). The most important transcription factor for IL-4 is c-Maf, which is primarily induced by signaling through the TCR-CD4 complex (**Fig 2**). Overexpression of c-Maf leads to a spontaneous TH2 phenotype and to increased IgE levels *in vivo* (Ho *et al*, 1998). Interestingly, c-Maf-deficient TH cells can develop into TH2 cells, but always lack IL-4 production (Kishikawa *et al*, 2001; Ho and Glimcher, 2002).

In general, the cytokines and transcription factors augmenting TH1 and TH2 differentiation also have an effect on the reciprocal TH cell subset. Thus, GATA-3 inhibits IL-12Rβ2 expression, resulting in decreased TH1 development even under TH1-inducing conditions. Accordingly, resting TH2 cells fail to express IL-12Rβ2 (Hilkens *et al*, 1996; Szabo *et al*, 1997; Rogge *et al*, 1999), although recent data showed that this state may not be completely irreversible (Hondowicz *et al*, 2000; Smits *et al*, 2001). Furthermore, GATA-3 represses T-bet expression and IFN-γ secretion and therefore TH1 differentiation (**Fig 2**; Ferber *et al*, 1999; Murphy *et al*, 2000; Farrar *et al*, 2002). Stat6 and c-Maf can suppress TH1 cell functions, c-Maf even independently of IL-4 (Ho *et al*, 1998; Kurata *et al*, 1999). On the other hand, TH2 development can be controlled by factors involved in TH1 differentiation, as GATA-3 is inhibited by IFN-γ and IL-12 through Stat1 and Stat4 signaling. T-bet also suppresses GATA-3 and the development of TH2 cells. Even *in vivo*, IL-4 levels increase and mice tend to develop asthma-like airway changes in the absence of T-bet (**Fig 2**; Murphy *et al*, 2000; Finotto *et al*, 2002). This understanding of the reciprocal control mechanisms of TH1 and TH2

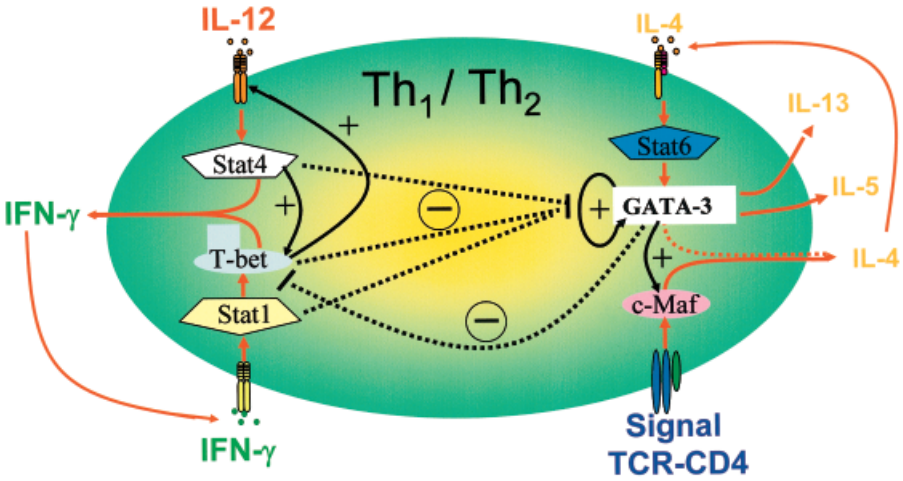

Figure 2. The role of transcription factors in the differentiation of TH1 and TH2 cells. Two major pathways for TH1 cytokine production have been identified. IL-12 signaling via its receptor activates Stat4, which upregulates IFN-γ transcription. IFN-γ, on the other hand, activates Stat1, which upregulates the leading TH1 transcription factor, T-bet, further enhancing IFN-γ production. Both pathways upregulate each other via positive feedback mechanisms (solid black arrows, +). For TH2 cytokine production, two major pathways are identified: IL-4–mediated signaling through the IL-4 receptor activating Stat6 and GATA-3, leading to IL-5 and IL-13 production; and signaling through the TCR-CD4 complex upregulating c-Maf, which in turn initiates and enhances IL-4 transcription. The response controlled by GATA-3 is further enhanced by an autoactivation process and a positive feedback on c-Maf expression. All three factors for TH1 cytokine production (Stat4, Stat1, and T-bet) inhibit GATA-3, which in turn downmodulates T-bet (dotted black line; -).

differentiation, on both the biochemical and cellular levels, can potentially lead to new therapeutic strategies to modulate disorders resulting from aberrant TH1 and TH2 polarization.

COSTIMULATORY MOLECULES AND THE CONTROL OF TH1 AND TH2 DEVELOPMENT

Appropriate Th lymphocyte stimulation requires two signals. The first is delivered by interaction of the T cell receptor (TCR) on the TH cell and the peptide-MHC complex on the APC. This signal is not sufficient to activate T cells because, in the absence of a second signal provided by costimulatory molecules, TCR binding may result in anergy or cell death (Schwartz, 1990). Adhesion and costimulatory molecules, which deliver the second signal to T cells, not only stabilize intercellular binding but also influence TH1 and TH2 differentiation. The most important costimulatory molecule is CD28, which interacts with members of the B7 family.

B7.1 (CD80) is only expressed on activated DC, whereas B7.2 (CD86) shows constitutive expression and is further upregulated upon stimulation. During the interaction of T cells with APC, B7.1 and B7.2 bind to CD28, which results in T cell activation. In contrast, engagement of CTLA-4, the other ligand for B7.1 and B7.2, is a negative regulator of T cell activation. Interestingly, B7 ligands have a higher affinity for CTLA-4 than for CD28, but CTLA-4 needs to be induced on the T cell surface by activation of the T cells via TCR and CD28. Furthermore, B7.1 has a higher affinity for CD28 than does B7.2. These differences in expression and interaction with ligands may explain why these two molecules exert distinct functions *in vivo* (Abbas and Sharpe, 1999).

The differentiation of naïve TH cells is strictly dependent on CD28/B7 costimulation (McAdam *et al*, 1998). In this regard, early *in vivo* data suggested that B7.1 leads to TH1 development and B7.2–mediated signals are involved in the differentiation toward the TH2 phenotype. This effect may be indirect and does not apply to all situations (Kuchroo *et al*, 1995; Lenschow *et al*, 1995; Perrin *et al*, 1995; Schweitzer *et al*, 1997). IL-4 can differentially upregulate B7.2 expression on DC, which was shown to block CD8 T cell–mediated immunity (King *et al*, 2001), but IL-4–mediated upregulation of B7 molecules also activates T cell–mediated immunity (Bagley *et al*, 2000). In contact hypersensitivity reactions (CHSR) of the skin, anti-B7.1 mAb inhibits CHSR and amplifies the regulatory CD4+ TH2 population, which is consistent with the B7.1/TH1 and B7.2/TH2 paradigm. On the other hand, anti-B7.2 mAb also reduces CHSR via downregulation of cytokine production by effector CD8+ and regulatory CD4+ TH cells, thereby challenging this simple analogy (Xu *et al*, 1997).

A series of new members of the B7 family were discovered recently (Abbas and Sharpe, 1999). Among them, the new murine B7. h, also called B7-related protein 1 (B7RP-1) binds a CD28-related protein called inducible costimulator (ICOS), which is induced on T cells following activation. The engagement of ICOS enhances TH cell proliferation, secretion of cytokines, and antibody help, and it particularly favors induction of IL-4 and IL-10 but not IL-2 (Hutloff *et al*, 1999). Accordingly, several studies have reported that ICOS favors TH2 development (Coyle *et al*, 2000; Dong *et al*, 2001; McAdam *et al*, 2001; Tafuri *et al*, 2001), but ICOS-mediated IL-10 production was recently also linked to the induction of T regulatory cells (Akbari *et al*, 2002). In addition, data showed that ICOS can play an important role in TH1-mediated immune responses, such as graft rejection (Sperling and Bluestone, 2001). Other members of the B7 family are B7.H1 and B7.DC, which inhibit activated T cells after ligation of the PD^{-1} receptor, and B7.H3, which can enhance IFN-γ production via a still unknown receptor (Liang and Sha, 2002).

The conflicting results of various experiments analyzing the role of B7 molecules in TH cell differentiation may reflect a dominant interference with alternative binding structures during TH cell–APC interactions. Several other pairs of adhesion or costimulatory molecules, such as CD27/CD70, CD40L/CD40, CD2/CD58, OX/OXL, and LFA-1/ICAM-1, participate in the cross-talk between TH cells and APC and also influence the outcome of the response. These molecules modulate the duration and strength of TH cell activation and, similarly to that recognized for the antigen dose and the affinity of the peptide MHC complex to the TCR, have an effect on TH1 and TH2 cell differentiation (Carballido *et al*, 1992; Nicholson *et al*, 1995; Pfeiffer *et al*, 1995; Carballido *et al*, 1997; Constant and Bottomly, 1997; Gause *et al*, 1997; Ruedl *et al*, 2000).

THE NOVEL ROLE OF ANTIGEN-PRESENTING CELLS IN TH1/TH2 REGULATION

Increasingly the data suggest that the functional phenotype of APC determines the cytokine production profile of activated TH cells. DC generally mediate the priming of naïve TH cells. Following activation, immature DC undergo differentiation and migrate from peripheral tissues to draining lymph nodes. These new immigrants, as well as the resident DC, may activate naïve TH cells. Importantly, only activated DC may lead to TH1 or TH2 cell differentiation whereas immature or quiescent DC may induce tolerance or anergy in TH cells (Banchereau and Steinman, 1998; Shortman and Liu, 2002). Among activated DC, type 1 and type 2 have been described regarding their role in TH cell differentiation toward either TH1 or TH2 cells.

Alternative models of the origin or generation of these functionally distinct DC subtypes have been proposed. One is called the specialized lineage model (Rissoan *et al*, 1999; Shortman and Liu, 2002), and it implies that specialized DC lineages give rise to either DC1 or DC2. Human and murine DC lineages and DC populations are defined according to the markers they express. Accordingly, murine DC are subdivided into two major populations. The lymphoid lineage DC population, which is CD8α+, is almost identical to the CD11c+ and CD11b$^{dull/-}$ DC populations described by others. These DC induce primarily TH1 cell differentiation. The other murine subset is derived from myeloid DC precursors, which are CD8α– but CD11c+ and CD11b+. The CD8α– DC can act as DC2 on TH cell polarization. In humans, there are also two accepted precursor cells for DC: one from the myeloid lineage, giving rise to Langerhans DC and interstitial DC; and the other from the lymphoid lineage, developing into plasmacytoid DC. Monocyte-derived DC are considered the best equivalent of the interstitial DC with myeloid origin and become DC1. Lymph node- or tonsil-derived DC are plasmacytoid and can act as DC2 (Moser and Murphy, 2000; Shortman and Liu, 2002). Despite the existence of DC1 and DC2 subsets, it was recently postulated that the DC subsets are not necessarily predetermined but rather are flexible in regard to their own phenotype differentiation (Biedermann *et al*, 2001; Shortman and Liu, 2002).

It is not yet completely clear how the DC subsets induce either TH1 or TH2 cells, because DC provide several signals to TH cells. These signals are antigen specific and are delivered via the MHC-peptide complex to the TCR, signals induced by the cytokine production of the DC in combination with different costimulatory molecules, which all influence the outcome of TH cell differentiation, along with other factors that are probably still unknown. Nevertheless, human and murine DC1 seem to induce TH1 cells primarily via IL-12 (Moser and Murphy, 2000; Shortman and Liu, 2002). DC2 were initially thought to induce TH2 cells by either B7.2 costimulation or IL-6 production, but DC2 can still induce IL-4 production in TH cells under IL-6 neutralizing conditions (De Becker *et al*, 1998). Thus, for the pathway of TH2 induction by DC2, no major influencing factor has yet been identified.

CONTROLLING TH1 AND TH2 LYMPHOCYTE POPULATIONS IN IMMUNE DISORDERS OF THE SKIN

In humans, strongly polarized TH1 and TH2 cells are not frequent but can be isolated from patients with autoimmune or allergic diseases, respectively (Kapsenberg *et al*, 1991; de Vries *et al*, 1995; O'Garra, 1998). TH1 cells mediate effective delayed-type hypersensitivity reactions (DTHR) such as contact hypersensitivity reactions (CHSR) and psoriasis. Other TH1 effector mechanisms include the induction of complement-binding immunoglobulins in B cells. TH2 cytokines promote IgE and non-complement-binding IgG production by B cells (Biedermann and Rocken, 1999). Allergen-specific IgE and TH2 cells mediate allergic responses such as atopic dermatitis, leading to the increased presence and activation of mast cells and, in some cases, eosinophilic granulocytes. Moreover, TH2 cell–derived cytokines exhibit anti-inflammatory functions and TH2 cells inhibit TH1 responses. Consequently, adoptive transfer of TH1, but not TH2, cells can induce DTHR in animals with a normal immune system (Rocken *et al*, 1996). The strong pathogenic association of polarized TH1 and TH2 subsets with immune disorders has led to the development of strategies for redirecting these polarized TH cell forms. TH cells require activation in order to undergo differentiation toward TH1 or TH2 cells, however, and once TH1 or TH2 polarization has been reached these phenotypes are relatively stable (Rocken *et al*, 1992).

REDIRECTION OF TH2 CELLS IN HUMANS

Most available therapies for atopic diseases suppress symptoms, but do not offer a real cure to the patient. In contrast to immunosuppression, several immunotherapies have been proposed that would deviate TH cell responses with long-lasting effects. In one of these therapies used in allergic diseases, especially in patients with bee venom allergy or allergic rhinitis, desensitization is achieved by systemic application of increasing doses of allergen, which has proved effective in a large number of patients. This therapy is as yet limited to a group of allergic reactions, however, and the immunological mechanisms leading to therapeutic success remain enigmatic (Aebischer and Stadler, 1996; Biedermann and Rocken, 1999; Lewis, 2002).

Epidemiological studies have demonstrated that the prevalence of atopic diseases is increasing in industrialized countries with a "western" life style. Moreover, it was found that large numbers of siblings, especially when sharing bedrooms, or early entry into day care reduces the risk of IgE-mediated allergies (von Mutius *et al*, 1994; von Mutius *et al*, 1994; Strachan, 1997; Kramer *et al*, 1999). The data suggest that infections during early childhood reduce the risk of unwanted TH2 responses. IL-12 production by APC during infections may therefore not only prime immune responses for a TH1 phenotype but also protect against TH2-dominated reactions. The IL-12-mediated control of TH2 responses suggested by the epidemiological data is supported by experimental *in vivo* data showing that IL-12-inducing agents can prevent and even revert TH2-dominated responses and induce TH1 cells (Erb *et al*, 1998; Zimmermann *et al*, 1998). Consequently, redirection of TH2 into TH1 responses provides a new therapeutic approach, which may be beneficial as a therapy for allergic individuals.

Among other atopic diseases, TH2 cells specific for environmental antigens are believed to be involved in the development of atopic dermatitis (Biedermann and Rocken, 1999). To test the flexibility of human TH2 cells from atopic dermatitis lesions, skin-derived TH2 cell lines were stimulated in the presence of IL-12 for proof of concept. This activation restored responsiveness to IL-12 through induction of the IL-12Rβ2 chain. Subsequently, IL-12 initiated IFN-γ production and upregulated the TH1 transcription factor T-bet in these TH2 cells.[1]

TH2 cells are involved in the pathogenesis of atopic dermatitis and allergic asthma. Mice with TH cells carrying a deletion in T-bet develop asthma-like airway changes as well as high IL-4 levels (Finotto *et al*, 2002). Accordingly, not only does transfection of polarized TH2 cells with the T-bet strongly induce IFN-γ in murine (Szabo *et al*, 2000; Afkarian *et al*, 2002) and human TH2 cells,[2] but T-bet also suppresses TH2 cytokines. Thus, direct induction of T-bet in TH2 cells may be a new therapeutic rationale for controlling TH2 pathology. The degree of suppression of the different TH2 cytokines by T-bet varies between experimental studies, however (Afkarian *et al*, 2002; Szabo *et al*, 2000). TH2 counter-regulation can also be achieved by inducing IL-12 *in vivo.* Bacteria and certain CpG-containing DNA sequences counter-regulate TH2 responses by inducing IL-12 production and subse-

[1]Schwarzler *et al:* manuscript in preparation.
[2]Lametschwandtner *et al*: manuscript in preparation.

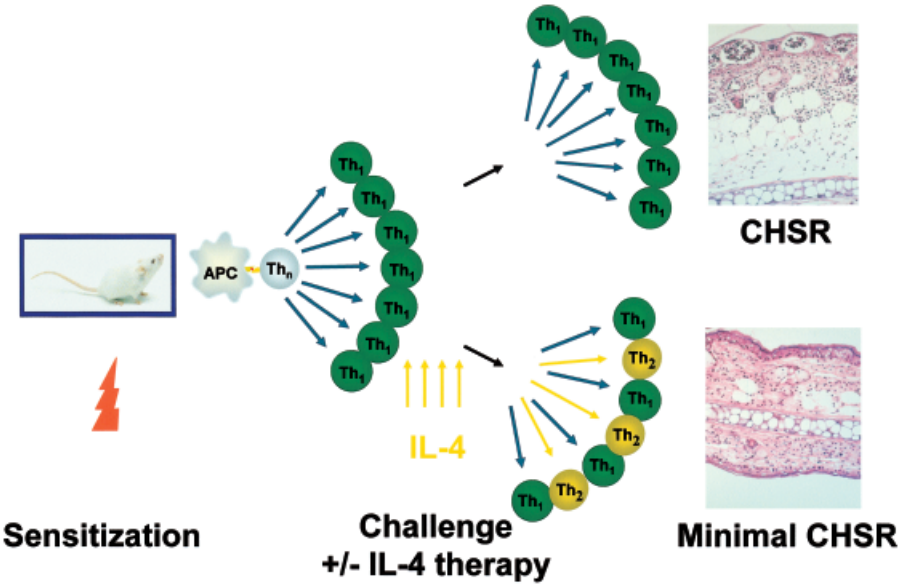

Figure 3. Deviation of TH1 cells into TH2 cells as therapy for TH1-mediated inflammation. Sensitized mice were challenged with the specific hapten, and one group of mice received four therapeutic doses of IL-4. One hapten-specific challenge later, mice that had received hapten plus IL-4 earlier expressed high levels of IL-4 in the skin shortly after challenge, indicating the presence of hapten-specific TH2 cells in the skin. Whereas mice sensitized to a hapten develop strong hapten-specific inflammatory responses after challenge (CHSR), mice treated with IL-4 show only little hapten-specific disease activity (minimal CHSR).

quently TH1 differentiation (Erb *et al*, 1998; Zimmermann *et al*, 1998). Immunotherapy promoting IL-12 or T-bet may thus prove to be beneficial for atopic patients because it targets not only naïve but also memory TH cells (Smits *et al*, 2001).[3] Other factors may be included in new therapeutic strategies for atopic diseases, such as the signaling lymphocyte activation molecule (SLAM, CD150), a member of the CD2 family. SLAM associates intracellularly with the SLAM-associated protein (SAP), which blocks SLAM signaling. SAP-deficient mice show increased TH1 and inhibited TH2 cytokines as well as very low serum IgE levels (Czar *et al*, 2001; Wu *et al*, 2001). Consequently, ligation of SLAM restores TH1 cytokines in polarized TH2 cells. Moreover, SLAM is of special interest because its binding also abrogates the ability of TH2 cells to induce the immunoglobulin switch to IgE in B cells (Carballido *et al*, 1997).

DEVIATION OF TH1 CELLS INTO TH2 CELLS AS THERAPY FOR TH1 CELL–MEDIATED INFLAMMATION

TH1 cells are believed to be involved in the pathogenesis of organ-specific autoimmune diseases, such as psoriasis, rheumatoid arthritis, and multiple sclerosis. In DTHR-like psoriasis or CHSR, TH1 cells mediate harmful inflammation by secreting proinflammatory cytokines and activating downstream effector cells (Biedermann *et al*, 2000). The antagonistic effects of TH1 and TH2 cells on several DTHR have long been established (Paul and Seder, 1994; Abbas *et al*, 1996; Rocken *et al*, 1996). In most approaches, cytokines such as IL-4 or IL-10 suppress the TH1 effector functions of DTHR only transiently without reversing the phenotype (Asadullah *et al*, 1998; Gautam *et al*, 1992; Powrie and Coffman, 1993). One of the major controversies in Th immunology still is whether the TH1/TH2 phenotype of immune responses remains stable (Paul and Seder, 1994; Abbas *et al*, 1996) or whether TH1 cell lines and even clones retain some flexibility and can be deviated into TH2-like cells (Bradley *et al*, 1995; Rocken *et al*, 1992; Rocken *et al*, 1992; Mocci and Coffman, 1995; Breit *et al*, 1996). Redirecting TH cell polarization is a potentially important strategy for treating TH1 cell–mediated autoimmune diseases such as psoriasis, but the feasibility of this concept still needs experimental proof. We investigated the therapeutic potential of IL-4–induced immune deviation in mice with a well-established and advanced CHSR, a prototypic DTHR. Using TNCB-induced CHSR as a model of TH1 cell–mediated skin disease (Biedermann *et al*, 2000), we were able to show that IL-4 therapy during allergen application reverts even established CHSR and dramatically reduces tissue damage during subsequent antigen exposures (**Fig 3**; Biedermann *et al*, 2001). Treatment of established DTHR with IL-4 therapy resulted in responses characterized by increased IL-4 expression during very early phases. This indicated that the presence of TH2 cells at the site of inflammation antagonizes TH1 cell–mediated inflammation, a finding further supported by the fact that adoptive transfer of TNCB-specific TH2 cells also downregulate CHSR (Biedermann *et al*, 2001). Similar results were obtained in the treatment of experimental allergic encephalitis (Racke *et al*, 1994), leading to the conclusion that changing ongoing immune responses in an antigen-specific mode provides a new specific therapeutic strategy to revert and treat inflammatory autoimmune diseases such as lichen planus, psoriasis (including psoriatic arthritis) and perhaps even allergic contact dermatitis (**Fig 3**). A very recent clinical study on IL-4 therapy in psoriasis demonstrated that this therapeutic approach is also very successful in humans (Ghoreschi *et al*, 2003). Thus, exact analyses of underlying mechanisms provide a solid base for the development of this novel approach that not only is more efficient but also seems to be capable of providing long-standing protection (Biedermann *et al*, 2001; Ghoreschi *et al*, 2003).

A CYTOKINE PARADOX: INDUCTION OF DC1 AND TH1 CELLS WITH IL-4

The central role of IL-4 in suppressing TH1 responses in CHRS was first recognized during infection of mice with *Leishmania (L.) major*, where IL-4 inhibited protective immunity against this intracellular pathogen (Reiner and Locksley, 1995). Subsequently, it was shown that inhibition of TH1 development and DTHR by IL-4 and IL-4-producing TH2 cells is one of the most important features of IL-4 physiology (Paul and Seder, 1994; Liblau *et al*, 1995; Reiner and Locksley, 1995; Abbas *et al*, 1996; Rocken *et al*, 1996; King *et al*, 2001). This concept was challenged by results obtained with IL-4 transgenic and IL-4–deficient mice and neutralizing IL-4 antibodies (Bagley *et al*, 2000; Salerno *et al*, 1995; Noben-Trauth *et al*, 1996; Erb *et al*, 1997; Mencacci *et al*, 1998; Schuler *et al*, 1999). Paradoxically, under certain conditions IL-4 may promote TH1 development and the initiation of DTHR. For instance, IL-4-deficient mice are defective in developing

[3]Schwarzler et al: manuscript in preparation; lametschwandtner *et al:* manuscript in preparation.

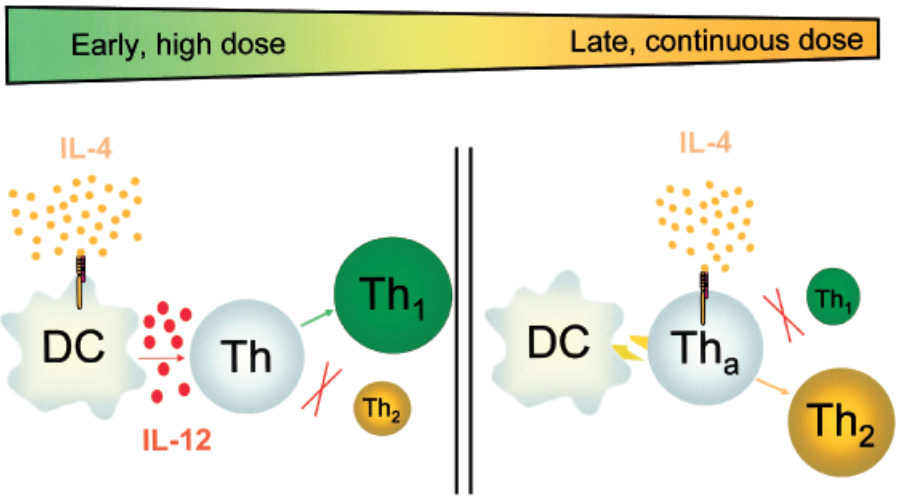

Figure 4. A cytokine paradox: induction of DC1 and TH1 cells as well as differentiation toward TH2 cells with IL-4. When IL-4 is present during dendritic cell (DC) maturation early after DC stimulation, it induces IL-12 production in DC. These IL-12–secreting DC can activate TH cells and instruct a TH1 phenotype (left). When IL-4 is present later during TH cell differentiation, it primarily acts on TH cells by triggering IL-4R and inducing a TH2 phenotype in these activated TH cells.

TH1 responses when infected with certain strains of *L. major* or *Candida albicans* (Mencacci *et al*, 1998; Noben-Trauth *et al*, 1996). Similarly, a critical role for IL-4 and Stat6 was demonstrated in the development of efficient TH1 responses against haptens, autoantigens, alloantigens, and tumor antigens (Golumbek *et al*, 1991; Salerno *et al*, 1995; Schuler *et al*, 1999; Traidl *et al*, 1999; Bagley *et al*, 2000; Yokozeki *et al*, 2000; Biedermann *et al*, 2001). Although the effects of IL-4 on TH2 differentiation are well known, the mechanisms underlying the opposite effects of IL-4, the instruction of TH1 responses, has remained elusive. Modulation of costimulatory molecules on DC has been proposed as one potential mechanism, because IL-4 may affect the expression of B7.1/B7.2 on DC. In view of the questionable role of B7-related molecules in the regulation of TH1 and TH2 cells, these explanations remain uncertain (Bagley *et al*, 2000; King *et al*, 2001). A more solid explanation was provided by the observation that IL-4 can upregulate IL-12 production in DC when given during DC maturation (Hochrein *et al*, 2000; Kalinski *et al*, 2000; Ebner *et al*, 2001). Based on these preliminary data, we could show that DC indeed differentiated into a TH1-inducing DC1 phenotype when primed either *in vitro* or *in vivo* in the presence of IL-4. Blocking experiments confirmed that under these conditions DC–derived IL-12 is in fact the molecule responsible for the instruction of TH1 cells (Biedermann *et al*, 2001).

TH1 cells, not TH2 cells, can orchestrate effective immune responses against intracellular bacteria. Therefore, only TH1 cells mediate immune responses that can control leprosy or leishmaniasis. The antagonistic effects of TH1 and TH2 are in part due to their antagonistic effects on macrophages and monocytes (Racke *et al*, 1994; Rocken *et al*, 1996). TH1-derived IFN-γ stimulates the production of inducible NO synthetase (iNOS), IL-1, IL-12, and TNF, whereas TH2 cytokines suppress these mediators of inflammation and promote the production of IL-10 by macrophages (Moore *et al*, 1993; Bogdan, 1998). Progressive or even fatal courses of infectious diseases caused by intracellular microbes are associated with an immune response dominated by TH2 cells both in humans and in mice (Reiner and Locksley, 1995; Salgame *et al*, 1991; Yamamura *et al*, 1991). The pivotal role of the TH1-inducing cytokine IL-12 for the clearance of infection has long been recognized (Heinzel *et al*, 1993; Guler *et al*, 1996; Zimmermann *et al*, 1998), and severe immunodeficiency syndromes with infections, even after bacillus Calmette-Guerin vaccination of patients with IL-12 receptor deficiencies, have demonstrated the important physiologic role for IL-12 and IL-12-mediated TH1 cell differentiation in humans as well (Altare *et al*, 1998). To address whether IL-4 can induce IL-12-producing DC1 *in vivo* capable of instructing protective TH1 responses, we tested its biological role in infection in Balb/c mice. These mice are genetically susceptible to *L. major* and develop increasing skin lesions and ultimately systemic disease (Reiner and Locksley, 1995) as they mount TH2 responses to these intracellular parasites. IL-4 application to infected mice generally promotes susceptibility; however, when IL-4 treatment is given during DC activation and prior to TH cell activation, it induces resistance to *L. major* in these susceptible mice, leading to parasite clearance and cure (Biedermann *et al*, 2001). As expected, the IL-4 regimen induced IL-12 production by DC from draining lymph nodes within hours and shifted the cytokine balance toward a TH1 response (Biedermann *et al*, 2001). This observation has critical implications for IL-4–mediated immune therapies. Dose and time point of application determine the outcome of Th response. Early application of high doses of IL-4 during the priming of DC and prior to T cell activation leads to the differentiation of TH1 cells, as the DC1-inducing effect dominates (**Fig 4**). In contrast, when IL-4 is present during TH cell activation, it induces a TH2 phenotype in the activated TH cell population (**Fig 4**).

CONTROL OF TH1 AND TH2 EFFECTOR MECHANISMS BY CHEMOKINES

In the skin, TH1 cells elicit immune reactions like CHSR or psoriasis. The skin-infiltrating TH1 cells are recruited into the skin, where they activate downstream effector cells to mount full skin inflammation (Biedermann *et al*, 2000). After IL-4–mediated immune deviation of CHSR, specific TH2 cells obviously precede the TH1 population into the skin and control inflammation (Biedermann *et al*, 2001). TH1 and TH2 cells can be found in the skin under a variety of conditions. In leishmaniasis or leprosy, depending on the course of the infection, both TH1 and TH2 cells migrate to the skin and elicit either a protective immune response or susceptibility (Reiner and Locksley, 1995). In atopic dermatitis, allergen-specific TH2 cells home to the skin and play a crucial role in the initiation of disease. To be recruited into the skin, TH cells follow a multistep process, must adhere to vessel walls, and leave the circulation (Robert and Kupper, 1999). The first step of extravasation is the rolling of TH cells along the endothelial lumen mediated by interactions of E-selectin and its ligands. Skin-homing TH cells express the cutaneous lymphocyte–homing antigen (CLA), which is their ligand for endothelial E-selectin and a skin-homing marker. Initial data indicated that this may be valid only for TH1 cells (Berg *et al*, 1991; Austrup *et al*, 1997), but it was recognized later that CLA also marks TH2 cells in patients with TH2 cell–mediated atopic dermatitis, and now it is proved that TH2 cells definitively use CLA/E-selectin interactions to home to the skin (Biedermann *et al*, 2002; Santamaria

Babi *et al*, 1995). Chemokines can activate rolling TH cells, which results in their firm arrest. This is a prerequisite for Th cell migration. Secretion of chemokines and expression of chemokine receptors are tightly regulated during the multistep migration process (Campbell *et al*, 1998; Sallusto *et al*, 2000). The clinical importance of chemokine receptors in the selective recruitment of either TH1 or TH2 cells was suggested by the observation that TH1 or TH2 cells preferentially express certain receptors. CCR5 and CXCR3 are expressed by TH1 cells; CCR3, CCR4, and CCR8, by TH2 cells (Sallusto *et al*, 1997; Bonecchi *et al*, 1998; D'Ambrosio *et al*, 1998; Loetscher *et al*, 1998; Zingoni *et al*, 1998; Imai *et al*, 1999). Surprisingly, TH2 cells derived from atopic dermatitis express CCR4 but not CCR3 or CCR8 (Biedermann *et al*, 2002). MDC/CCL22 is the dominant CCR4 ligand (Biedermann *et al*, 2002; D'Ambrosio *et al*, 2002) and interactions with CCR4+ TH2 cells therefore play an important role in the induction of atopic dermatitis inflammation. Indeed, CCR4 and MDC/CCL22 have been identified in atopic dermatitis lesions (Campbell *et al*, 1999; Vulcano *et al*, 2001), and TH2 cells produce significantly higher levels of MDC/CCL22 than do TH0 or TH1 cells (Galli *et al*, 2000). In addition, the hallmark cytokine of TH2 cells, IL-4, can markedly upregulate MDC/CCL22 production in macrophages and DC, whereas MDC/CCL22 is downmodulated by IFN-γ (Bonecchi *et al*, 1998; Vulcano *et al*, 2001).

As expected, the TH2 cells that grow out of atopic dermatitis skin explants are CXCR3 whereas TH1 cells tend to express CXCR3+ and may or may not express CCR4+ as well (Kim *et al*, 2001; Biedermann *et al*, 2002). Thus, CLA+ TH cells of both subtypes may utilize CCR4 to migrate to the skin. Using a model in which we transplanted human skin onto severe combined immunodeficient (SCID) mice and adoptively transferred human TH cells to these mice, we confirmed the functional importance of CCR4, because *in vivo* atopic dermatitis–derived TH2 cells only migrate toward CCR4-specific chemokines whereas chemokines binding CCR3, CCR8, or CXCR3 are not capable of attracting these TH2 cells into human skin (Biedermann *et al*, 2002; Carballido *et al*, 2003). In contrast, TH1 cells migrated preferentially toward CXCR3-binding chemokines (Carballido *et al*, 2002). Expression of CXCR3 or its ligands is well described for TH1-mediated skin disorders (Biedermann *et al*, 2000; Flier *et al*, 2001; Rottman *et al*, 2001), and all CXCR3 ligands are inducible by IFN-γ—the main TH1 product (Farber, 1997; Widney *et al*, 2000). This implies that activation of TH1 cells in the skin upregulates the secretion of CXCR3 ligands via IFN-γ and leads to further recruitment of CLA+ CXCR3+ TH1 cells into the skin. In contrast, TH2 cells secrete MDC/CCL22 and IL-4 within the skin and induce further recruitment of CLA+ CCR4+ TH2 cells.

Chemokines control TH1 and TH2 cell–mediated inflammation by activating Th cells rolling along the endothelial wall and by inducing firm Th cell adhesion to endothelial cells. Extravasation and trans-tissue migration are accomplished by concentration gradients of specific chemokines. Chemokines also regulate activation of Th cells by professional APC, as dermal DC not only recruit CLA+ CCR4+ TH2 cells to the skin but also secrete MDC/CCL22 to direct the encounter of TH2 cells and DC (Katou *et al*, 2001). Moreover, constant concentrations of chemokines may keep TH cells within the peripheral tissue because, without a specific attractant, TH cells leave the tissue. The indicated chemokine-mediated amplification loops for TH1 and TH2 cell–mediated skin inflammation therefore control several important steps in TH cell–dependent effector functions and are key mechanisms of disease maintenance. Targeting chemokine–chemokine receptor binding specific for TH1 or TH2 cells with low molecular weight antagonists is thus a very promising approach to treating Th cell–mediated diseases. In addition, specific chemokine receptor antagonists block the vicious circle in which TH1 or TH2 cells further amplify the inflammatory process, and they offer a new therapeutic approach for TH1 and TH2 cell–mediated skin disorders.

CONCLUSION

Multiple control pathways of TH1 and TH2 cell development are now known, and our understanding of the dynamics and complexity of the *in vivo* regulation processes is growing. Among other concepts, the role of cytokines redirecting polarized Th cell responses has been investigated with the finding that IL-12 or IL-12–inducing agents, such as CpG, are very potent in redirecting TH2 cells. Conversely, IL-4 has the potential to reverse ongoing TH1-mediated inflammatory autoimmune diseases, including psoriasis. Also, the immunostimulatory effects of IL-4 have been discovered, leading to its use as a vaccine adjuvant. Inhibiting interactions between CLA and E-selectin have proved to be very promising for interfering with skin homing of TH1 and Th2 cells. On the other hand, harmful effects of defined Th cell subsets at peripheral sites can be treated by blocking chemokine–chemokine receptor ligations with specific antagonists. CCR4 in particular is a promising drugable target for atopic dermatitis and perhaps for other inflammatory skin diseases.

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