Abstracts
CD4+ T cells can be subdivided from a functional point of view into two main subsets: effector cells, which provide protection against exogenous offending agents, and regulatory T (Treg) cells whose function is to avoid autoimmune reactions and to stop the effector response against exogenous antigens, when the response itself becomes dangerous for the host. Human effector CD4+ T lymphocytes can be additionally classified into lineages based mainly on their immunological functions that are supported by distinct profile of cytokine, transcription factor, and homing receptors expression. In the last years, beyond the well known populations of human T helper (Th) lymphocytes, Th1 and Th2 cells, other populations have been discovered and phenotypically characterized. These include the Th17 subset, which is certainly the most intensively studied, but also Th22, Th9, and T follicular helper (Tfh) lymphocytes. In addition to their protective functions, these T helper populations are also involved in the pathogenesis of several inflammatory immune-mediated disorders. Th1 and Th17 cells are involved in the pathogenesis of organ-specific autoimmune diseases and other chronic inflammatory disorders, whereas allergen-specific Th2 lymphocytes play a crucial role in allergy. Although classically viewed as distinct lineages, recent evidence indicate that CD4+ T cells, particularly the Th17 subset, are more plastic than previously thought. It is not fully understood how often such plasticity occurs in the course of physiologic responses to pathogens and what its importance is in protective immunity, but in inflammatory conditions Th17 lymphocytes that have shifted towards a Th1 or Th2 phenotype, acquiring the ability to produce IFN-γ or IL-4, and seem to be particularly aggressive and more pathogenic than the unshifted cells. In this context, the possibility to interfere with this modulation of phenotype can be considered a possible target for developing novel therapeutic strategies in the above mentioned diseases.

Key terms
T helper cells; chemokine receptors; cytokine receptors; cytokines

T Helper Subsets

CD4 T cells play a central role in the function of the immune system, firstly by helping B cells to produce antibodies, but also by orchestrating CD8 T cells and macrophage functions against a wide variety of pathogenic microorganisms (1). Human CD4+ Th cells can be subdivided into lineages on the basis of their immunological functions, which are supported by the expression of well defined profiles of specific transcription factors, cytokines, and homing receptors. These lineages include effector cells, which protect from pathogens, and regulatory T cells (Treg), which protect from effector responses when they become dangerous for the host, as it happens for autoimmune responses and, in some circumstances, also for response to exogenous antigens (2).

In this review, we will focus our attention on T helper effector subsets, on their plasticity, and on the opportunity, for clinical immunologists, to interfere with the natural course of several immune-mediated disease by blocking environmental signals that drive the transition of T helper cells towards more aggressive phenotypes, or by promoting the differentiation towards less pathogenic phenotypes. Over 20 years ago, two main subsets of CD4 T helper cells with different functions and patterns of cytokine secretion were identified in both mice and humans, which were
named as type 1 Th (Th1) and type 2 Th (Th2) lymphocytes. (2,3). Recently, additional Th subsets have been identified, the most intensively studied being the Th17 one, which is crucial for host defense against extracellular pathogens (4). Additional subsets are represented by Th22 lymphocytes that play a crucial role in host defense against Gram-negative bacterial organisms (5), Th9 lymphocytes, involved in immune-mediated diseases ranging from autoimmunity to asthma (6), and T follicular helper (Tfh) cells that enter the germinal center to mediate their helper function (7).

In addition to their protective functions, these T helper subsets are also involved in the pathogenesis of several immune-mediated disorders, and therefore represent a potential target for the treatment of such diseases. In particular, both Th1 and Th17 cells have been shown to be involved in the pathogenesis of organ-specific and systemic autoimmune diseases and other chronic inflammatory disorders, whereas allergen-specific Th2 lymphocytes certainly play a crucial role in the pathogenesis of allergy (8).

**Th1 Lymphocytes**

Th1 cells produce high levels of IFN-γ and are responsible for both phagocyte activation and production of opsonizing and complement-fixing antibodies, thus playing an important role in the protection against intracellular pathogens (3).

The key factor needed to obtain Th1 differentiation from naive CD4 T cells is IL-12, but also the presence of IFN-γ has been reported to play an important role, as shown by the observation that its neutralization during IL-12-driven Th1 differentiation partially diminishes this polarization (9). A complex process underlying Th1 differentiation exists, in which both IL-12, through STAT-4, and IFN-γ, through STAT-1, promote activation of the "master regulator transcription factor" of Th1 cells, T-bet, whose activity feeds back to cause additional IFN-γ production and higher T-bet expression, thus amplifying Th1 differentiation (10). At later stages of Th1 differentiation, IL-18Rz is also upregulated. IL-18Rz upregulation requires IL-12/STAT-4 signaling and is further increased by IFN-γ. IL-12 and IL-18 jointly induce IFN-γ production by Th1 cells in the absence of T-cell receptor stimulation. Such antigen-independent cytokine production is probably important for amplifying Th1 responses (11).

In addition to their protective functions from invading pathogens, Th1 lymphocytes also contribute to the development of organ-specific autoimmune diseases, as well as chronic inflammatory disorders (12). Experimental autoimmune encephalomyelitis (EAE), collagen-induce arthritis, and inflammatory bowel disorders (IBD) have been considered for a long time to be the consequence of unchecked Th1 responses on the basis of studies in which disease development was ablated by neutralizing the IL-12p40 chain or targeting the p40 or the IL-12Rβ1 genes. Moreover, it was reported that limiting the differentiation of autoreactive Th1 lymphocytes, through the therapeutic administration of a small interfering RNA specific for T-bet, significantly improved the clinical course of established EAE (13). In addition, in Helicobacter hepaticus-induced colitis, IFN-γ, was the crucial T-cell effector cytokine when T-regulatory cells were absent (14). Finally, in proteoglycan-induced arthritis, Th1 cells are pathogenic (15).

Some years ago, the finding that IL-23, but not IL-12, is critically linked to autoimmunity in mouse models (16,17), generated some doubts on the pathogenic role of Th1 cells in such diseases. However, a series of subsequent studies confirmed the role Th1 cells, as important characters in these disorders.

**Th2 Lymphocytes**

Type 2 immune responses are induced by and confer protection against helminths, but can also promote acute and chronic inflammatory responses against a myriad of allergens (18). Th2 polarization from naive CD4 T cell is achieved thanks to the early production of IL-4 during the primary response (19). However, the cell and the mechanisms responsible for this early IL-4 production have been only recently elucidated, with the observation that IL-4 could be produced by the naive Th cell itself, upon Notch triggering, as a consequence of the expression by dendritic cells (DC) of its ligand Jagged-1 in both mice and humans (20,21). Moreover, it has been reported that a recently discovered cytokine, named as IL-25, produced by mast cells and macrophages in the gut of worm-infested animals or lung epithelial cells, can induce the early production of IL-4 by a non-T, non-B, c-kit+, FcεR1-cell, or by the Th naive cell itself, thus allowing its Th2 polarization (22). In addition, recent studies indicate that IL-33 induces murine and human naive CD4+ T cells to produce IL-5 and IL-13 in absence of IL-4, and that the adoptive transfer of IL-33-differentiated IL-5+IL-4− T cells promotes eosinophilic airway inflammation in naive IL-4−/− mice (23). Finally, the thymic stromal lymphopoietin (TSLP) is an epithelial-derived cytokine that directly triggers DC-mediated allergic inflammation (24,25).

Many experimental works support the critical role of Th2 cells in the initiation, maintenance, and amplification of human allergic inflammation (8). In particular, IL-4 and IL-13 regulate the allergen-specific synthesis of immunoglobulin E, IL-5 the recruitment of eosinophils, IL-9 the growth of mast cells (25). Moreover, IL-4, IL-9, and IL-13 can induce mucosal hypersecretion and contribute, together with IL-5, to the increase in airway hyperreactivity in allergic asthma (8). In addition, other mediators, such as prostaglandin and chemokines largely produced in the context of allergic inflammation, can promote the selective recruitment of Th2 cells through some chemoattractant receptors predominantly or selectively expressed by human Th2 lymphocytes (26). As a consequence, allergen-specific Th2 lymphocytes are present in the lungs of patients with allergic asthma, whereas in chronic obstructive pulmonary disease Th1 lymphocytes have been predominantly found (27,28). In addition, mouse models of allergic asthma gave an important contribution to the understanding of the role of Th2 cells in allergic inflammation. Adoptive transfer of allergen-specific Th2 cells results in the development of airway hyperresponsiveness (AHR) and airway inflammation (29), whereas the transfer of allergen-specific Th1 cells results in a reduction of airway eosinophilia and mucus production (30).
Several recent experimental and clinical observations suggested that different phenotypes of asthma exist, in which Th17, more than Th2 lymphocytes, play a pathogenic role (8). In this context, we and others recently reported the existence in humans of Th17/Th2 cells that secrete both IL-4 and IL-17, coexpress the transcription factors GATA3 and RAR-related orphan receptor C (RORC), and are expanded in asthmatic patients (31,32).

**Th17 lymphocytes**

The main role of Th17 cells is the clearance of extracellular bacteria and fungi, thanks to their capacity to recruit and activate neutrophil granulocytes, either directly through IL-8 production (33) or indirectly by inducing the production of colony stimulatory factors and CXCL8 (34) by tissue resident cells. Th17 lymphocytes also stimulate the production of mucus MUC5AC and MUC5, in primary human bronchial epithelial cells in vitro (35), and the expression of human beta defensin-2 (36) and CCL20 in lung epithelial cells (37).

Human Th17 express the transcription factor RORC (38), the IL-23 receptor (IL-23R), the chemokine receptor CCR6 (39,40), and the lectin receptor CD161 (41). The simultaneous presence of IL-1β and IL-23 is needed to induce Th17 lymphocytes, from CD161+ CD4+ T cell precursors, which are detectable in both human umbilical cord blood and thymus (41). The combination of these two cytokines also induces T-bet and IL-12Rβ2 expression, and the differentiation of Th1 cells, suggesting a possible developmental relationship between human Th17 and Th1 cells (41). Recently it has been suggested that IL-1β was essential for inducing IL-17/IFN-γ double producing cells, the so-called Th17/Th1 subset, a phenotype that is frequently observed in pathological conditions (42). The dispensability of TGF-β signaling for the development of human Th17 lymphocytes (43), has been recently reported also for mouse (44). TGF-β does not have a direct effect on the genesis of human Th17 cells, but it can indirectly favor their development by suppressing both T-bet expression and Th1 differentiation (43,44).

Th17 lymphocytes, beyond their protective role in the clearance of extracellular pathogens, also play a role in the pathogenesis of several autoimmune and inflammatory diseases (45). In particular, a determinant role for Th17 cells has been proposed in multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease (IBD), but also in psoriasis and contact dermatitis (46,47), underevaluating the contribution of Th1 cells (48), previously shown to be crucial. However, more recent articles have reported that both Th1 and Th17 cells can be involved in the pathogenesis of human autoimmune and inflammatory disorders and that these two cell subsets can develop from the same precursors and coexist in the same microenvironment (49,50).

**Other T cell subsets: Th22, Th9, and T follicular helper (Tfh) lymphocytes**

Interleukin-22 (IL-22) belongs to the IL-10 cytokine family and is expressed by innate and adaptive lymphocytes (51). IL-22 binds to a heterodimeric receptor expressed by nonhematopoietic cells such as the epithelial cells of the gastrointestinal tract and skin. IL-22 effects on epithelial cells consist of the induction of the expression of genes involved in antimicrobial host defense such as S100 proteins, defensins, Lipocalin 2, RegIII-family proteins, and of inflammatory molecules such as chemokines and cytokines including IL-6 (5). In addition, IL-22 has an important function in tissue repair via induction of epithelial cell proliferation and improving cell survival. Therefore, IL-22 plays an important role in promoting resistance to extracellular pathogens, particularly to Gram-negative pathogens, such as Klebsiella pneumoniae and Citrobacter rodentium (52–54). Beyond Th17 cells, that are an important source of IL-22 (55), a subset referred to as Th22 cells has been described in humans (56). Human Th22 cells are characterized by production of IL-22 with little or no IL-17 and have been described to be enriched in the healthy human cecum where they would play an important role in maintaining mucosal barrier function (57). An important role for IL-22 has been also reported in a murine model of psoriasis, where neutralization of IL-22 prevented the development of the disease, reducing acanthosis (thickening of the skin), and inflammatory infiltrates. Accordingly, T cells isolated from psoriatic skin of patients produced higher levels of IL-22, and supernatants of lesional psoriatic skin–infiltrating T cells induced an inflammatory response by normal human epidermal keratinocytes (58).

Mouse T helper cells secreting IL-9 are primed in response to TGF-β and IL-4 and are termed Th9. Th9 development is clearly dependent upon the IL-4-activated transcription factor STAT6 (59). Naive human CD4+ T cells also acquire a Th9 phenotype when differentiated in presence of TGF-β and IL-4 (60). TGF-β has also been shown to induce IL-9 production in human Th17 cells, and repeated stimulation under Th17 conditions resulted in the co-expression of IL-17A and IL-9 (6).

Th9 lymphocytes are pro-inflammatory cells that work in a broad spectrum of autoimmune diseases and in allergic inflammation. In mouse, Th9 cells induce inflammation in a T cell transfer colitis and in EAE model (61,62), and also contribute to allergic diseases (6). IL-9 is highly expressed in the lungs of asthmatic patients (63) and was significantly higher in T cells from atopic infants in comparison with nonatopic group (64).

Nevertheless, whether Th9 cells are required as a source of IL-9 for autoimmune inflammation is still not clearly established; also in allergic diseases, how Th9 contribute and how they cooperate with Th2 cells in promoting inflammation is not yet fully established (6).

In recent years, T follicular helper (Tfh) cells have emerged as the key cell type required for the formation of germinal centers in secondary lymphoid tissues (65). Tfh cells in human lymphoid tissues express the chemokine receptor CXCR5 and function primarily to provide help to B cells. Tfh cells can be distinguished from other CD4+ T cell lineages by their low expression levels of cytokines (IFN-γ, IL-4, and IL-17) and transcription factors (T-bet, GATA3, and RORC)
characteristic of Th1, Th2, and Th17 cells, respectively. Furthermore, Thf cells express a unique combination of effector molecules that are critical for their development and function, including high levels of the surface receptors ICOS, CD40 ligand (CD40L), OX40, PD-1, BTLA, and CD84, the cytokine IL-21, the cytoplasmic adaptor protein SLAM-associated protein, and the transcription factors Bcl-6 and c-Maf (65).

Since Thf cells can arise from Th1 and Th2 precursors in response to infection with LCMV (66), or Nippostrongylus brasiliensis (67), respectively, the debate on the possibility that Thf cells are an independent lineage or a functional state of each of the previously described lineages, remains open.

**Th Plasticity**

T cell differentiation was once considered linear and irreversible, but recent findings indicate that this process is flexible, as so-called committed cells can acquire features of different effector fates upon adequate stimuli. There is increasing in vitro evidence that differentiated CD4+ T cell populations can alter the range of their cytokine production, although whether this occurs as readily in vivo remains not fully elucidated (68). In absence of external signals, phenotype stability is ensured by expression of genes turned on by transcription factors but also by the repression of genes conferring alternative fates. **Also, epigenetic modifications and micro-RNA (miRNA) expression contribute to this stability. Both the Il4 and Ifng genes show striking CpG demethylation, an epigenetic modification that is associated with gene expression, in their promoters and in enhancer regions during the process of differentiation to Th2 and Th1 cells, respectively (68). Although all the described mechanisms ensure the stability of different T cell subsets, irreversible commitment might be dangerous considering that T cells migrate in different tissues and are exposed to variable microenvironment. Indeed, there are now many examples of flexible cytokine production by established T helper cells (Fig. 1; 68).

More than 10 years ago, we showed that both IL-12 and the PS-DSP30 oligodeoxynucleotide enabled CRTH2+ allergen-stimulated Th2 cells to produce IFN-γ, thus providing evidence that the cytokine production pattern of fully differentiated Th2 effectors can be changed to a less polarized profile (Th0 cells); (69). In addition, we found that such a modulation of allergen-specific Th2 lymphocytes could also occur in vivo during allergen specific immunotherapy in allergic patients (70). Moreover we reported the presence of T cells producing both IFN-γ and IL-10 in patients treated with specific immunotherapy, allowing us to hypothesize that both Th1-skewing and immunoregulatory mechanism can contemporaneously occur and synergize in these patients (70). The ability of Th2 cells to acquire IL-9–producing capacity in the presence of TGF-β has been also described (60).
Acquisition of IFN-γ-producing potential by human Th17 cells is a common occurrence, particularly in inflammatory conditions (11), as well as the finding of simultaneous production of IL-17 and IFN-γ (39). It has been found that Th17 cells can induce type 1 insulin-dependent diabetes mellitus (IDDM) efficiently in lymphopenic recipient mice only after their shift into Th1 cells (71). Moreover, in a model of IDDM induced by the transfer of highly purified Th17 cells from BDC2.5NOD mice in NOD/SCID recipient mice, the onset of the disease was prevented by treatment with an anti-IFN-γ-neutralizing antibody but not an anti-IL17A-neutralizing antibody (72). Similar conclusions have been more recently drawn also in human subjects affected by autoimmune arthritis and Crohn’s disease (50,73–75). Moreover, it has been reported that treatment with ustekinumab, a monoclonal antibody that binds the shared p40 subunit of human IL-12 and IL-23, thus blocking the activity of both cytokines, resulted in a rapid and significant improvement of symptoms in moderate-to-severe psoriasis and psoriatic arthritis (76,77). In a recent study, we found that Th17 cells are rare in the SF of patients with JIA whereas Th1 cells were highly predominant, which was at least partially due to the property of Th17 cells to shift into Th1 cells in presence of IL-12. The Th17-derived Th1 cells expressed CD161, while the other Th1 cells present in the SF did not, and we named the former cells as nonclassic, as compared with classic CD161−Th1 cells. Moreover, we found an accumulation of Th17 and nonclassic CD161+ Th1 lymphocytes in fistula curettage of patients suffering of fistulising Crohn’s disease (75). Th17 cells can shift to Th1-like cells at inflamed sites, thank to their expression of IL-12Rβ2 and their consequent ability to respond to IL-12 and to coexpress T-bet. Nonclassic Th1 cells can be identified based on CD161 expression, as well as the consistent expression of RORC, IL-17 receptor E, CCR6, and IL-4-induced gene 1, which are all virtually absent in classic Th1 cells. The possibility to distinguish these two-cell subsets by using such a panel of markers may allow the opportunity to better establish the respective pathogenic roles of classic and non-classic (Th17 derived) Th1 cells in different chronic inflammatory disorders (78,79). Since it has been clearly shown that Th17 cells in humans express the chemokine receptor CCR6, whereas Th1 cells poorly express CCR6 and highly express CXCR3 (80), the simultaneous evaluation of these two receptors could help in understanding the different origin of “classic” and “nonclassic” Th1 cells (Fig. 2).

Another evidence of the high plasticity of human Th17 cells also emerges by the recent finding of the existence of a subset of human circulating memory CD4 T cells that produce both IL-17A and IL-4 (31; Fig. 1). This previously unknown population of Th17/Th2 lymphocytes is more represented in the circulation of patients with allergic asthma than in healthy donors, and is enriched in cells specific for the sensitizing allergen, suggesting a possible role in the pathogenesis of the disease. Th17/Th2 cells can derive from Th17 lymphocytes, when they are exposed to IL4-rich microenvironment, thanks to their low, but detectable, expression of IL-4Rα (31). Notably, proinflammatory cytokine IL-1β, IL-6, and IL-21 could induce the up-regulation of IRF4 and RORγt genes expression and the production of IL-17 in classical Th2 memory/effector cells in mice (32). In particular, in a mouse model of induced asthma, transfer of allergen-specific IL-17-producing Th2 cells resulted in profound goblet hyperplasia as...
well as elevated mucin production after antigen sensitization with heterogeneous leukocytes infiltrating the airways, including neutrophils, eosinophils, macrophage, and lymphocytes. In contrast, mice transferred with conventional Th2 or Th17 cells exhibited fewer airway infiltrations of eosinophils or neutrophils, respectively, and limited pathophysiological features (32).

In conclusion, as just reported for Th17/Th1 cells (4), also Th17/Th2 lymphocytes (37) seem to have a more aggressive and pathogenic phenotype than conventional Th17, Th2, or Th1 phenotypes. Even if the real frequency with which Th helper lymphocytes alter their cytokine production in vivo is still not fully known, these findings argue for a high plasticity of Th1 helper cells in both cytokines and transcription factors expression. The first and probably most important consequence of this flexibility concerns the possibility to intervene therapeutically in immune mediated diseases. For example, the allergen-specific Th2 cells of asthmatics might be altered in vivo and thus interrupt the disorder. Similarly, in JIA and Crohn's disease, the transition of Th17 cells toward Th1 phenotype could be blocked by using drugs targeting cytokines involved in this shift. Moreover, the issue is important for a notype could be blocked by using drugs targeting cytokines involved in this shift. Moreover, the issue is important for a Notype could be blocked by using drugs targeting cytokines involved in this shift. Moreover, the issue is important for a notype could be blocked by using drugs targeting cytokines involved in this shift. Moreover, the issue is important for a Notype could be blocked by using drugs targeting cytokines involved in this shift. Moreover, the issue is important for a...

**Literature Cited**


