

Review article

Novel CD4 and CD8 T-cell subsets

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T cells are central to the immune responses as both regulators and effectors of immune function. These functions are largely mediated by the cytokines they produce. Since the discovery of the classic type 1 and type 2 T-cell subset network, studies of the function and interrelationships between T-cell subsets have focused on their ability to regulate different immune responses. However, it is becoming increasingly apparent that other CD4 and CD8 T-cell subsets which do not conform to a classical type 1 or type 2 cytokine/marker profile exist and have an integral role in the regulation of many aspects of the T-cell network. Here we review novel CD4 and CD8 T-cell subsets with respect to cytokine secretion, cell-surface ligand expression, and function.

Established T-cell subsets

Mosmann and others have demonstrated that after chronic exposure to antigen, two subpopulations of CD4 T cells may be identified in mice. These were defined as T helper 1 (Th1) and T helper 2 (Th2) cells. Th1 cells produce the cytokines interferon- γ (IFN- γ), interleukin (IL)-2, and lymphotoxin- α . Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (1–4). IL-3, tumour necrosis factor- α (TNF- α), GM-CSF, and various members of the chemokine family are produced by both subsets. Similar subpopulations have also been identified in rats and in man (5, 6). More recently, researchers have also identified CD8 T cells which exhibit patterns of cytokine production which

approximately mirror those seen in CD4 T cells. Tc1 cells, which secrete IL-2 and IFN- γ , and Tc2 cells, which secrete IL-4, IL-5, and little or no IFN- γ (7–9) (Table 1). Similar, if less clear-cut, subpopulations have been observed in rats and in various human disease states (10–12). Th1/Th2 and Tc1/Tc2 cells represent extremes of differentiation and each develop from common precursors which secrete IL-2 only (13–15). T helper 0 (Th0) and T cytotoxic 0 (Tc0) cells have also been described as lymphocytes which exhibit an unrestricted cytokine profile; i.e., they produce both type 1 and type 2 cytokines (16). It has been suggested that this phenotype represents an intermediate stage of differentiation from their respective precursors into either the type 1 or type 2 cell. Nakamura et al. showed that both IL-4 and IFN- γ genes were activated during Th1-cell differentiation (17). However, it is clear from other studies that the Th0 phenotype exists and is stable (18).

A number of factors affect the differentiation of naive CD4 or CD8 precursors into type 1 and type 2 effector cells. These include the affinity of the T-cell receptor (TcR) for a particular immunodominant peptide (19), the type of coreceptor ligated during activation (20), and the presence of type 1 or type 2 cytokines (21). When present early in the immune response, IL-4 drives naive CD4 and CD8 lymphocytes to become Th2 and Tc2 cells, respectively. IL-4, from the differentiated type 2 cells, inhibits the production of type 1 cytokines by other surrounding cells (14, 21), serving to polarize further the response. The presence in the extra-

Table 1. Summary of classical and nonclassical T-cell subsets as defined by surface marker expression, cytokine secretion, and function

Surface markers	Th1	Th2	Tc1	Tc2	Th3		Tr1	$\gamma\delta$		
	CD4 $\alpha\beta$ TcR	CD4 $\alpha\beta$ TcR	CD8 $\alpha\beta$ TcR	CD8 $\alpha\beta$ TcR	CD8 $\alpha\beta$ TcR	CD4 CD45RB ^{low}	CD4 $\alpha\beta$ TcR	CD4 $\gamma\delta$ TcR	CD4 $\gamma\delta$ TcR	CD8 $\gamma\delta$ TcR
Cytokine secretion										
IFN- γ	+++	-	+++	-	-	-	+	-	+++	+++
IL-2	+++	-	+++	-	-	-	-	-	+++	+++
IL-4	-	+++	-	+++	-	+	-	+++	-	-
IL-5	-	+++	-	+++	-	-	++	-	-	-
IL-6	-	+++	-	+++	-	-	-	-	-	-
IL-10	-	+++	-	+++	-	+	+++	+++	-	-
IL-13	-	+++	-	+++	-	-	-	-	-	-
TGF- β	++	++	++	++	+++	+++	+	-	-	-
Function										
	DTH	B-cell help	Immunity	Immune	Suppression	Induces	Suppression	Early	Tolerance of	Tolerance of
	CMI	Allergy	to viruses	suppression	of EAE in	tolerance in	of Th1	source of	respiratory	respiratory
		Asthma			rat through	mouse	responses;	IL-4	antigens	antigens
					TGF- β	models of	induces		through	through
						EAE and	tolerance in		secretion of	secretion of
						colitis	mouse		IFN- γ ;	IFN- γ
						through	models of		early	
						TGF- β	EAE and		source of	
							colitis		IFN- γ	
							through IL-10			
References	1, 2	1, 2	7-10, 118, 120	7-10, 118, 120	62-64	68-70	75, 76	95, 96	96	89, 90

cellular milieu of IL-12, which can be derived from antigen-presenting cells (APC) such as macrophages and dendritic cells, promotes the Th1 or Tc1 phenotype. The IL-12 present, as well as the type 1 subsets generated, will in turn inhibit the generation of Tc1 and Tc2 cells (type 2 subsets) by producing IFN- γ (3, 22, 23). Additional cofactors such as IL-1 α , TNF- α , and IL-18 further support the generation of type 1 responses (24, 25). This process has been termed reciprocal or cross-regulation (14) (Fig. 1).

Established T-cell subset function

CD4 T cells generally respond to exogenous antigens internalized and processed by APCs in endosomes which are then presented as peptides by major histocompatibility complex (MHC) class II molecules on the cell surface. Endogenous antigens are degraded in the cytosol by the proteasome and are then transported to the lumen of the endoplasmic reticulum (ER). Inside the ER, peptides are bound to MHC class I molecules by a chaperone-assisted assembly mechanism involving peptide-transporter proteins (TAP), and are then presented at the cell surface to CD8 T cells (26-29). There is, however, increasing evidence of a degree of degeneracy between these two pathways, and several groups have demonstrated that exogenous antigen can stimulate specific MHC class I restricted CD8 T cells (30).

Th1 cells

Th1 cells are principally involved in delayed-type hypersensitivity (DTH) reactions and cooperate with other immune cells (such as macrophages) to rid the host of infectious organisms. Professional APCs, such as macrophages and dendritic cells, after contact with nonself products, migrate to lymphoid organs, where they present antigens to naive Th precursors and produce proinflammatory cytokines such as IL-12, thereby promoting the Th1 phenotype (22, 31). Production of IFN- γ by these Th1 cells will in turn activate surrounding macrophages and fuel the momentum of the type 1 response (31). Activated Th1 cells can help B cells to facilitate class switching to lytic IgG isotypes (32). Thus, the default pathway of the immune system appears to be Th1.

Th1 cells are an important source of IL-2 to CD8 T cells during an antiviral or antitumour immune response (33). Endogenously produced viral proteins are presented by MHC class I molecules of an infected host cell to specific Tc precursors, which compete for sites on the host cell on the basis of epitope specificity (34). This activation stimulates differentiation to a mature Tc1 phenotype, which clonally expands to mount a specific cytotoxic response (33). To help this clonal expansion, Th1 cells can provide IL-2, although Tc1 cells can produce sufficient IL-2 themselves when antigen is abundant (34). Recent studies have shown that the

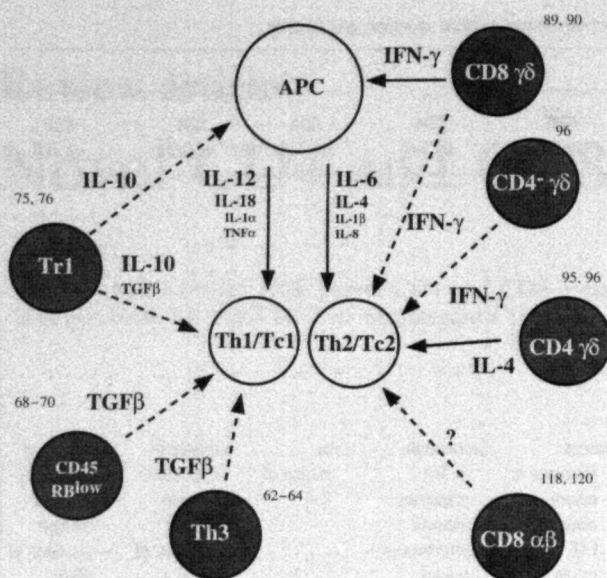


Fig. 1. Regulation of classic T-cell network by novel T-cell subsets. Small numbers indicate publications from which data were obtained.

presence of IL-4 during a CTL response may not immediately affect Tc1 cells effector function, but does compromise their long-term capabilities (35). The cytotoxic nature of Tc1 cells is reflected in their use of both Fas- and perforin-mediated killing pathways (36). Despite their cytotoxic nature, Tc1 cells have also shown the ability to activate macrophages and elicit DTH reactions (37).

Th2 cells

Th2 cells are principally involved in the humoral arm of the immune system, which mediates immunity to helminth infections. Helminth-derived or soluble protein antigens are presented to Th precursors in the context of MHC class II, where, upon activation, the precursor cell differentiates into Th2 effectors. The influence of the chemical nature of the antigen, combined with that of the type of APC, on T helper cell differentiation remains unclear. However, it is clear from studies by several groups that the presence of IL-4 and the absence of IFN-γ in the initial stages are vital (14, 38, 39). Once activated, Th2 cells provide help to B cells in class switching to IgE and neutralizing IgG subclasses (40). Th2 cells play a major role in the underlying lung inflammation that causes asthma (41). Indeed, in a rat model of asthma, we have shown that we can adoptively transfer lung hypersensitivity and eosinophil infiltration with CD4 T cells alone (42).

The mechanisms of IgG1 and IgE switching differ depending on the type of parasite/antigen involved. IL-4 signalling via the IL-4 receptor α-

chain, leading to STAT6 and IRS-2 activation, in combination with CD40/CD40L interaction, is thought to be the most important pathway (43). However, in a model of *Nippostrongylus brasiliensis* expulsion, in IL-4 knockout mice, Urban et al. have demonstrated that IL-4 is not an absolute requirement for defence against this parasite. An IL-4-dependent, STAT6-independent pathway of murine IgG1 B-cell switching has also been demonstrated, suggesting a function of IL-4 via IRS-2 alone, and a role for STAT6 in the IgG1 response (44). IL-4 and IL-9 from activated Th2 cells have been shown to stimulate the growth of mast cells, which release inflammatory mediators upon ligation of FcεR1 with IgE (45). IL-5 is essential for the differentiation and activation of eosinophils, whose antiparasitic effects are mediated by an antibody-dependent, cell-mediated cytotoxic reaction.

Tc2 cells are a relatively recent discovery, and less is known about how these cells fit into the classic type 1/type 2 network. It has been demonstrated with perforin knockout mice that Tc2, like Tc1 cells, are cytotoxic but preferentially use a perforin-mediated mechanism (7, 46). Noncytotoxic Tc2 cells have also been identified in a model of graft versus host disease (GVHD) and in acute type 2 antiparasite responses (40, 47). It has been shown by several groups that Tc2-like cells can coexpress the CD40 ligand and CD30, which, together with the production of IL-4, can provide B-cell help *in vitro* for switching to IgG1 and IgE synthesis (11, 40, 48). Tc2 cells are producers of IL-5, and in common with Th2 cells may effect the growth and infiltration of eosinophils into sites of inflammation. This Tc2-mediated effect has been described during immune responses to inhalant viruses and allergic eosinophilic gastroenteritis, resulting in increased type 2 disorder (49, 50).

Novel T-cell subsets

Classic Th1/Th2 and Tc1/Tc2 cells collectively exhibit various functions to protect the host from attack by foreign organisms. However, this network of effector functions has to be tightly regulated in order to avoid disorder as the consequence of an uncontrolled or inappropriate response. The classic T-cell subset network is generally self-regulating; however, in recent years, a number of novel, nonclassical CD4 and CD8 subsets have come to light which may be vital to the regulation of Th1/Th2 and Tc1/Tc2 responses, and which may explain many of the phenomena observed.

Perhaps the most important decision made once an antigen is encountered and presented to cells of the immune system is the discrimination between

self and non-self. The vast majority of self-peptides are encountered during early development in the thymus, leading to the deletion of randomly generated T cells specific for such peptides (51). However mechanisms other than thymic education must exist for the regulation of T cells in the periphery. Collectively, these can be defined as forms of immune tolerance. Three models of peripheral tolerance have been proposed; deletion by apoptosis, induction of anergy, and active immune suppression (52). We shall focus here on active immune suppression. Investigation into the mechanism of immunologic tolerance has mainly focused on the suppression of classical cell-mediated type 1 responses. It is now clear that immunologic tolerance also exists in type 2 responses. For example, IgE (type 2) responses are readily suppressed after administration of antigen via the oral (53), respiratory (54), and nasal routes (55). The mechanism for this is not known, but type 2 tolerance can be induced in IL-4 knockout mice (56) and mice treated with anti-IL-10 (57), indicating that other molecules may be involved.

T helper 3 (Th3) cells

T helper 3 (Th3) cells were first identified by Weiner et al., who were studying oral tolerance of myelin basic protein (MBP) in models of experimental autoimmune encephalomyelitis (EAE). Mowat et al. had previously demonstrated that orally administered antigen could induce tolerance via active suppression (53). Friedman & Weiner then demonstrated that high doses of antigen preferentially induced clonal deletion or anergy, whereas low doses favoured T-cell-dependent active suppression (58). In a Lewis rat EAE model, these actively suppressing T cells were shown to be predominantly CD8 cells whose effects were mediated by transforming growth factor-beta (TGF- β) (59–61).

In an SJL mouse EAE model, oral administration of MBP tolerized both CD4 and CD8 type 1 T cells but also activated type 2 and Th3 CD4 and CD8 T cells in mice fed either MBP or the irrelevant antigen hen egg lysozyme (HEL) in serial doses at low concentrations before immunization with MBP. Repeating this protocol in CD8 knockout mice followed by adoptive transfer experiments demonstrated that both classic type 2 and TGF- β -secreting CD4 subsets could mediate active suppression in the absence of CD8 T cells (62). Whether TGF- β was acting independently or synergistically with IL-4/IL-10 is unclear. Several CD4 T-cell clones have been generated from MBP-fed SJL mice, which produced mainly TGF- β with

varying amounts of IL-4/IL-10. Adoptive transfer of these cells suppressed EAE in recipient mice. This effect could be abrogated by administration of anti-TGF- β before disease induction (63), further supporting the role of TGF- β . The suppressive clones generated were identical to encephalitogenic Th1 clones with regard to TcR usage, MHC class II restriction, and epitope recognition, indicating that this novel subset has a common precursor shared with pathologic Th1 cells. This was confirmed in a study using mice expressing a TcR specific for the acetylated N-terminal peptide of MBP. When these mice were given low oral doses of MBP, TGF- β -producing cells were predominantly generated, whereas if MBP was administered subcutaneously, the resulting phenotype was an encephalitogenic, IFN- γ -secreting Th1 cell (64).

To investigate the relative importance of antigen dose as a factor in deciding the mechanism of tolerance employed, anti-IL-12 was administered *in vivo* in transgenic mice that possessed only functional CD4 T cells expressing a class II-restricted ovalbumin peptide-specific TcR. These studies showed that high dose clonal deletion is IL-12 dependent, and suggested that low dose tolerance is a consequence of immune stimulation too weak to induce IL-12, and thus allowing TGF- β -mediated suppression to dominate the pathway of tolerance induction (65). However, other studies in similar mice showed that low doses of ovalbumin administered orally induced both IL-4/IL-10-producing Th2 cells and TGF- β -producing cells. In contrast, high doses resulted in the clonal deletion of most T cells by apoptosis after 3–4 feeds, except those T cells producing TGF- β , thus suggesting a TGF- β -mediated pathway of tolerance quite separate from that generated in response to high doses of antigen (66).

Another experimental system in which TGF- β -producing CD4 T cells seem to be important is Th1-mediated murine colitis. Coffman and others found that progressive colon inflammation could be induced in immunodeficient C.B-17 severely combined immunodeficient (SCID) mice by transfer of CD45RB^{high} CD4 T cells from normal C.B-17 mice (67), and that the resulting inflammation was caused by an uncontrolled Th1 response. When CD45RB^{low} CD4 T cells were transferred, no inflammation was observed, but when they were transferred in addition to CD45RB^{high} cells, the disease was completely inhibited (68, 69). Studies using IL-4 knockout mice showed that the differentiation and effector function of the suppressive CD45RB^{low} cells was IL-4 independent. Further work using neutralizing antibodies *in vivo* identified TGF- β as the cytokine through which these cells suppress (70), thus providing more evidence

that this CD45RB^{low} subpopulation of CD4 T cells is identical to the TGF- β -producing T-cell subset described by Miller et al. (60).

It is now clear that a novel subset of CD4, and perhaps CD8, T cells exists which differentiates from a common T-cell precursor. The generation of these cells is dependent on the route of antigen entry. Exposure to small amounts of antigen generates cells which produce varying amounts of type 2 cytokines and display a regulatory function via TGF- β . This novel subset of T cells has been referred to as T helper 3 (Th3) cells (Fig. 1). An important property of Th3 cells is their potential for bystander suppression. This is illustrated in a human trial for multiple sclerosis in which patients were fed MBP, and which resulted in an increase in frequency of MBP-specific Th3 cells. These cells are generated in the gut but then move to the target organ, where the TGF- β secreted suppresses local inflammation caused by the MBP-specific and non-MBP-specific pathogenic T cells (71).

Tr1 cells

Murphy et al. have described another suppressor T-cell subset to Th3 called T regulator 1 (Tr1). Ovalbumin (OVA)-specific naive CD4 T cells were isolated from $\alpha\beta$ TcR DO11-10 transgenic mice. These cells were cultured with splenic APCs and OVA peptide in the presence of IL-4, IL-10, or a combination of both. T cells cultured with IL-4 alone differentiated into the classical Th2 phenotype, producing high levels of IL-4, IL-5, and IL-10 (72). However, a large proportion of the cells cultured with IL-10 alone or IL-4/IL-10 differentiated into a distinct and stable subset of T cells characterized by the production of high levels of IL-10 and IL-5, with levels of TGF- β and IFN- β comparable to those of the Th1 and Th0 subsets, respectively, and undetectable levels of IL-4 and IL-2. These cells also displayed low levels of proliferation after antigenic stimulation, a possible reason for their previously being missed. Allo-geneic and nonspecific human Tr1 clones have also been generated from peripheral blood T cells cultured in the presence of IL-10 alone. These clones display a very similar cytokine profile of extremely high IL-10 production, Th0-comparable levels of IFN- γ , TGF- β , and IL-5, and very low or undetectable levels of IL-2 and IL-4. The kinetics of proliferation of these cells was comparable to murine Tr1 cells. The low proliferative capabilities of Tr1 cells have been attributed to the unusual speed at which IL-10 is released in response to TcR/CD3 stimulation in comparison to classical T-cell subsets (73). The addition of both anti-IL-10 and anti-TGF- β augmented the proliferative response

of Tr1 cells to normal levels, confirming that the slow growth of these cells was due to their production of these cytokines whose immunosuppressive effects are well documented (74). The antigen nonspecific suppressive effects of both murine and human Tr1 cells on autologous naive CD4 cells was demonstrated in a transwell coculture system, resulting in reduced proliferation and secretion of IL-2, IL-4, IL-5, and IFN- γ after restimulation (75). The effects on the naive CD4 T cells could be reversed by addition of anti-IL-10 and anti-TGF- β antibodies to the coculture system (76). Furthermore, there is considerable evidence of IL-10 in the downregulation of

- 1) dendritic cell-driven IFN- γ production by T cells (77)
- 2) antigen-specific T-cell responses via inhibition of IL-2 and IL-2R α expression (75, 78)
- 3) IL-4-induced IgE production (79).

There have also been several studies suggesting a role for IL-10 in the suppression of APC costimulation of both CD4 and CD8 T cells (77, 80). This was tested *in vivo* in a model of Th1-mediated colitis induced by CD4CD45RB^{high} T cells in SCID mice. Providing the donor mice were fed OVA to stimulate the specific Tr1 cells, the adoptive transfer of as few as 2×10^5 cells could prevent disease induced by cotransferred CD45RB^{high} T cells (76). The suppressive effects of T-cell-derived IL-10 have been found in another experimental model in which T cells, genetically altered to secrete large amounts of IL-10, were able to delay the onset of murine EAE when adoptively transferred (81). Furthermore, double transgenic mice expressing both haemagglutinin (HA) under the control of the Ig- κ promoter, and a class II-restricted TcR $\alpha\beta$ specific for influenza HA showed that despite the vast majority of self-reactive T cells being deleted in the thymus, a proportion of self-reactive T cells remained in the periphery (82). Such cells are anergic and are unable to proliferate in response to antigenic stimulation or exogenous IL-2.

Naive CD4 T cells from single HA-specific TcR transgenic mice may cause the onset of diabetes when adoptively transferred to transgenic recipients expressing HA under the control of the insulin promoter. In contrast, adoptive transfer of the anergic cells does not cause diabetes. The anergic cells were found to produce unusually large amounts of IL-10 and little else, suggesting that a possible response of a self-reactive T cell to specific stimulation could be differentiation into an IL-10-producing Tr1-like anergic cell which could promote the generation of other nonresponsive cells via the secretion of IL-10 (83, 84) (Fig. 1).

$\gamma\delta$ T cells

There is increasing evidence to suggest that effector and regulator functions are not restricted to T cells expressing the $\alpha\beta$ chains of the TcR. T cells expressing the $\gamma\delta$ TcR share many characteristics with their $\alpha\beta$ counterparts, such as cytotoxicity (85) and association of the TcR with CD3 (86). However, their ontogeny, distribution, and functional roles are significantly different (87, 88). One proposed role for $\gamma\delta$ T cells is in the induction and maintenance of tolerance to nasal, inhaled, and oral antigen. McMenamin and colleagues have shown, in both rats and mice, that IgE-specific tolerance could be induced in animals by treatment with aerosolized OVA. This reduction in the IgE response was attributed to OVA-specific CD8 TcR $\gamma\delta$ T cells. When adoptively transferred to recipient animals responding to OVA, as few as 1000 $\gamma\delta$ T cells were reportedly able to suppress the OVA-specific IgE response. It is proposed that the mechanism by which this subset is suppressive is via the production of high levels of IFN- γ , which alters the balance of type 1 and type 2 CD4 T cells (54, 89, 90). TcR $\gamma\delta$ knockout mice are resistant to oral tolerance (91), which can also be blocked with anti-TcR $\gamma\delta$ antibodies *in vivo* (92). In addition, non- $\gamma\delta$ CD4 T-cell populations may also contribute to oral tolerance (55, 56, 93). Reduction of specific IgE by a system like that of Holt has also been recently described by Seymour et al. (94). However, repetition of the experiments with β 2m knockout mice which lack CD8 T cells, anti-CD8 antibody-treated mice, $\gamma\delta$ knockout mice, and IFN- γ knockout mice has consistently shown that $\gamma\delta$ CD8 T cells are not essential for aerosol or nasal tolerance (94). In combination with data from Marth et al. showing the suppressive capabilities of TGF- β in a system lacking IL-12/IFN- γ -induced tolerance (65), it has been proposed that the suppression seen in this system of aerosolized, antigen-induced tolerance is independent of CD8 TcR $\gamma\delta$ T cells producing IFN- γ , and may be due to TGF- β -producing Th3 cells (94).

Several other different roles have recently been proposed for TcR $\gamma\delta$ T cells. TcR α knockout mice have been instrumental in the study of the ability of $\gamma\delta$ T cells to replace functions of TcR $\alpha\beta$ T cells during an immune response. TcR $\gamma\delta$ T cells have been found to provide B-cell help in antibody class switching and germinal centre formation in the absence of TcR $\alpha\beta$ T cells (95). TcR $\gamma\delta$ T-cell clones have been generated by several groups. Most of the clones described were CD8⁺ CD4⁻, which upon analysis seemed to display a type 1 phenotype with high levels of IFN- γ and IL-2 production. By contrast, some rare CD4 TcR $\gamma\delta$ clones were also

generated which conformed to a classical type 2 phenotype. In coculture experiments with B cells from TcR δ knockout mice, type 2 $\gamma\delta$ T cells were found to promote IgG1 immunoglobulin production by B cells, and type 2 $\gamma\delta$ T cells to promote IgG2a, a pattern identical to that seen with type 1 and type 2 TcR $\alpha\beta$ T cells (96, 97). The relationship between CD4 expression and type 2 differentiation was investigated by comparing RNA levels in freshly isolated CD4⁺ and CD4⁻ T-cell TcR $\gamma\delta$ populations. These findings showed that CD4⁺ $\gamma\delta$ T cells expressed greater than 10-fold more IL-4 and IL-10 than CD4⁻ cells, which expressed significantly more IFN- γ , an observation which was also true for inactivated cells, suggesting that $\gamma\delta$ T cells may differentiate into type 1 and type 2 phenotypes before stimulation (96).

Many lines of research have proposed $\gamma\delta$ T cells as a first line of defence during infection (98–100). Combined with evidence of the existence of type 1 and type 2 TcR $\gamma\delta$ T cells displaying comparable function to their $\alpha\beta$ counterparts, this raises the question of what roles such novel T-cell subsets play in the initiation of a primary immune response. TcR $\gamma\delta$ and TcR $\alpha\beta$ T cells in mice infected with either the intracellular bacterium *Listeria monocytogenes*, which promotes Th1 responses, or the extracellular parasite *N. brasiliensis*, which promotes Th2 responses, exhibited increased IFN- γ in both $\gamma\delta$ and $\alpha\beta$ T cells during *L. monocytogenes* infection, although $\gamma\delta$ T cells were responsible for the early release of the type 1 cytokine. An early and sustained increase in IL-4 production was seen during *N. brasiliensis* infection almost solely from $\gamma\delta$ T cells, thus suggesting a role for type 1 and type 2 $\gamma\delta$ T cells in determining the direction of an immune response (97). Further evidence has come from a study using $\gamma\delta$ knockout mice back-crossed onto the type 2 biased BALB/c background. Repeated intraperitoneal injections of OVA resulted in high titres of both OVA-specific IgE and IgG1 in wild-type mice, whereas mice deficient in $\gamma\delta$ T cells produced no IgE and 100-fold less IgG1. Upon intranasal challenge after intraperitoneal immunization, $\gamma\delta$ knockout mice did not produce IL-5 in bronchoalveolar lavage (BAL) fluid, an effect which subsequently reduced airway eosinophilia, as compared to wild-type mice. These effects were reversed in the knockout mice by the *in vivo* administration of IL-4 during the immunization period, thus suggesting an essential role for a subset of $\gamma\delta$ T cells in initial IL-4 production, early IgE and IgG1 synthesis, and the development of a type 2 response in the airways (101).

The source of IL-4 during the initial stages of a primary response which would induce type 2 T cells has been an area of considerable controversy.

Several candidates have been proposed, including naive CD4 T cells (102), mast cells and eosinophils (103), and a novel T-cell subset expressing NK1.1 (104). Bendelac has provided much of the data on NK1.1 T cells, having shown them to express CD4 or be CD4⁸, and to be restricted to two nonclassical MHC class I molecules: thymic leukaemia (TL) antigen and CD1. NK1.1 cells can produce large amounts of IL-4 very rapidly in response to anti-CD3 both *in vivo* and *in vitro*, and have been shown to play a role in polyclonal anti-IgD-mediated IgE production (105). However, studies using CD1 and TL knockout mice, and anti-NK1.1 neutralizing antibody *in vivo* have shown that normal IL-4-dependent Th2 responses may occur in the absence of NK1.1 T cells (106).

Other studies have identified $\gamma\delta$ T cells that can enhance activation of APCs such as macrophages by increasing nitric oxide production (107), as well as regulate natural-killer (NK) cell and classical TcR $\alpha\beta$ T-cell activity *in vivo* (108, 109). Clearly, TcR $\gamma\delta$ T cells can display a type 1 or type 2 phenotype with regard to cytokine profile before stimulation, and this differentiation could be based largely on the expression of CD4. *In vivo* models of infection have demonstrated the ability of $\gamma\delta$ T cells to produce IFN- γ and IL-4 very rapidly and appropriately for type 1 and type 2 pathogens, respectively. Therefore, this novel T-cell subset could be responsible for releasing cytokines that are able to enhance or suppress the activity of APCs, together with providing the appropriate influence on surrounding classical TcR $\alpha\beta$ T cells, thereby starting a cascade of differentiation toward type 1 or type 2 that is dependent on the nature of the antigen encountered (Fig. 1).

$\alpha\beta$ CD8 T cells

CD8 T cells that inhibit type 2 immune responses have been known for many years (110–112). Exposure to castor-bean dust induces lasting IgE responses both in those with an atopic and a nonatopic background (113). After parenteral immunization of rats with castor-bean ricin, IgE responses to OVA were enhanced (114). Ricin depleted an early activated population of CD8 T cells found in the spleen (115). Adoptive transfer of ricin-sensitive CD8 T cells suppressed IgE in an antigen nonspecific, isotype-specific fashion (115, 116). MHC class-I restricted OVA-specific clones which also suppress IgE have been generated from these animals (118). Depletion of CD8 T cells in immunized rats enhances antigen-specific IL-4 and suppresses IFN- β synthesis (119). Elimination of IgE regulatory CD8 T cells *in vivo* with anti-CD8 monoclonal antibody differentially enhances synth-

esis of OVA-stimulated CD4 T-cell IL-4 and inhibits IFN- β production (120), suggesting that CD8 T cells switch the CD4 T-cell response from a Th2 to a Th1 phenotype.

Secretion of IFN- β by CD8 T cells is the most obvious explanation of their ability to inhibit IgE production. The presence of IFN- β during the formative stages of a CD4 T-cell response could inhibit the growth of Th2 cells directly, while favouring the expansion of Th1 cells. CD8 T-cell-derived IFN- β could act directly on B cells to inhibit immunoglobulin synthesis (121); alternatively, cytolytic CD8 T cells could selectively target antigen-specific IgE B cells or APC. However, the ability of OVA-specific CD8 T-cell clones to inhibit IgE is unrelated to the levels of IFN- β they produce or to their *in vitro* cytolytic activity for OVA-pulsed targets (118). Thus, IgE suppressor cells are distinct from the Tc1, IFN- γ -producing, subset and may produce other cytokines that suppress IgE responses. It seems likely that the mechanisms underlying the immunoregulatory activities of CD8 T cells are complex and that several factors, including specific cytokines, limited cytotoxicity, and cell motility and survival could combine to mediate the effects of these cells *in vivo*.

Conclusions

As more research is undertaken into classical type 1/type 2 responses – how they are initiated and regulated – the T-cell network becomes more complex. As each novel T-cell subset is described, a multitude of new characteristics, cell-cell interactions, and functions are proposed. Many of these functions may be bystander effects that have evolved as a safeguard against immunologic defects in other cell populations, or may even be unused mediators of an obsolete mechanism, but all of these factors help to obscure the primary function of the cell. Far more work is required in all aspects of the T-cell network to clarify the role of classical and nonclassical T-cell subsets in many forms of immunity. Investigations into the interactions between these subsets and their activation, recruitment, and function are currently being undertaken by several groups worldwide.

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