Immunological decision-making: how does the immune system decide to mount a helper T-cell response?

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Summary

Aberrant T-cell responses underpin a range of diseases, including asthma and allergy and autoimmune diseases. Pivotal immune elements of these diseases are the development of antigen-specific effector T-helper type 2 (Th2) cells, Th1 cells, or the recently defined Th17 cells that are associated with the clinical features and disease progression. In order to identify crucial processes in the pathogenesis of these diseases it is critical to understand how the development of these T cells occurs. The phenotype of a polarized T-cell that differentiates from a naïve precursor is determined by the complex interaction of antigen-presenting cells with naïve T cells and involves a multitude of factors, including the dominant cytokine environment, costimulatory molecules, type and load of antigen presented and a plethora of signaling cascades. The decision to take the immune response in a certain direction is not made by one signal alone, instead many different elements act synergistically, antagonistically and through positive feedback loops to activate a Th1, Th2, or Th17 immune response. The elucidation of the mechanisms of selection of T-cell phenotype will facilitate the development of therapeutic strategies to intervene in the development of deleterious T-cell responses. This review will focus on the pathways and key factors responsible for the differentiation of the various subsets of effector CD4 T cells. We will primarily discuss what is known of the Th1 and Th2 differentiation pathways, while also reviewing the emerging research on Th17 differentiation.

Keywords: dendritic cell; polarization; T-cell; Th1; Th2; Th17

Introduction: T-helper cell subsets

Naïve T helper (Th) cells are activated by recognition of a peptide antigen–class II histocompatibility complex (MHC) presented on antigen-presenting cells (APCs) through the interaction with the T-cell receptor (TCR). After activation, Th cells begin to divide and/or give rise to a clone of effector cells, each specific for the same antigen–class II MHC complex.1 These effector Th cells are CD4+ and can be divided into three main types, with

Abbreviations: AP-1, activator protein-1; APC, antigen-presenting cell; BCR, B-cell receptor; CTLA-4, cytotoxic T lymphocyte antigen-4; DAG, diacylglycerol; DC, dendritic cell; DC1, Th1-inducing DC; DC2, Th2-inducing DC; ICAM-1, intercellular adhesion molecule-1; ICOS, inducible costimulator; ICOS-L, inducible costimulator ligand; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IP3, inositol-1,4,5-trisphosphate; ITAM, immuno-receptor tyrosine-based activation motif; JAK, Janus kinase; LAT, linker for activated T cells; MAPK, mitogen-activated protein kinase; LFA, lymphocyte function-associated antigen; MCP-1, monocytoic chemotactic protein-1; MHC, major histocompatibility complex; NFAT, nuclear factor of activated T cells; PAMP, pathogen-associated molecular pattern; pDCs, DCs derived from plasmacytoid cells; PIP2, phosphatidylinositol-bis-phosphate; PKC, protein kinase C; PLC, phospholipase C; PRR, pattern recognition receptor; RORγt, receptor-related orphan receptor γ-t; STAT, signal transducers and activators of transcription; TCR, T-cell receptor; TGF, transforming growth factor; Th, T helper; Th1, Th type-1; Th2, Th type-2; Th17, Th type-17; TLR, Toll-like receptor; TNF, tumour necrosis factor; Treg, regulatory T-cell; tyk, tyrosine kinase.
distinct cytokine-secretion phenotypes eliciting unique functional characteristics for each type. These cells are referred to as Th type-1 (Th1), Th type-2 (Th2) or Th type-17 (Th17) cells, depending on their phenotype. Th1 cells secrete the cytokines interferon (IFN)-γ and tumour necrosis factor (TNF)-β, which allow these cells to be particularly effective in protecting against intracellular infections by viruses and bacteria and micro-organisms that grow in macrophages, as well as eliminating cancerous cells.\(^3\) Th2 cells secrete interleukin (IL)-4, -5, -10 and -13, which up-regulate antibody production and target parasitic organisms. Th2 cells activate B cells, which are adapted for defence against parasites that are vulnerable to IL-4-switched immunoglobulin (Ig)E production, IL-5-induced eosinophilia, and IL-3- and IL-4-stimulated mast cell proliferation and degranulation. Th2 cells are predominately responsible for the development of asthma. Until recently the latter two subsets were considered to be the only types of CD4 effector responses; however, studies over the last few years have revolutionized this area of immunology with the discovery of a third subset known as Th17 cells. These cells secrete IL-17, IL-17F, IL-6, IL-22 and TNF-α and appear to play an integral role in both tissue inflammation and activation of neutrophils to combat extracellular bacteria. The inhibition of dendritic cell (DC) maturation and/or their secretion of T-cell inhibitory molecules leads to the development of a non-effector cell (which is not the focus of this review) known as the effector cell (which is not the focus of this review) known as the effector cell (which is not the focus of this review). This review focuses on the pathways involved in the development of these effector Th cells. Specifically, we will overview the major aspects of the APC–T-cell interactions and associated molecular determinants, before discussing the various cytokines and pathways that lead to the development of Th1, Th2 or Th17 cells.

Mechanisms of T-cell polarization

Differentiation of naive Th cells into Th1, Th2 or Th17 effector cells occurs within a few days of direct contact with APCs.\(^4\) A number of factors are involved in determining the nature of the effector phenotype that develops, and these include the nature and affinity of the antigen, the type of TCR signaling, the nature of the coreceptor signals that are involved and the primary factor, which is the predominant cytokine environment. Th cells respond to the products of many signaling cascades from a wide range of membrane-bound receptors, including cytokine receptors, and undergo four defined stages of development.

1. Activation of particular cytokine genes.
2. Commitment to a certain effector phenotype (Th1, Th2, or Th17).
3. Suppression of the opposing cytokine genes.
4. Stabilization and potentiation of the phenotype.\(^5\)

The developmental stages are mediated by an array of different molecular mechanisms, including control of gene expression by intracellular signaling cascades from cell-surface receptors, chromatin remodelling and epigenetic factors, such as acetylation, phosphorylation and methylation of DNA.

Role of Toll-like receptors

The generation of a specific immune response (Fig. 1) begins with the interaction of cells of the innate immune system with an antigen. APCs initiate the first step in the development of adaptive immunity and mould the T-cell response in accordance with the nature of the invading pathogen. Pathogens express pathogen-associated molecular patterns (PAMPs) (conserved regions of pathogens) that activate APCs, in particular DCs, directly through ligation of pattern recognition receptors (PRRs) on the APCs.\(^6,7\) The most common receptors involved are the Toll-like receptors (TLRs), which discriminate between different types of pathogens. TLRs are members of the IL-1 receptor superfamily.\(^8\) In addition to these mechanisms, pathogens induce the release of certain tissue factors by activating neighbouring cells in the infected tissue by the same PAMP ligating to PRR principle. Receptors on DCs bind these inflammation-associated tissue-specific factors, which are characteristic for the type of tissue and the pathogen-specific response pattern of this tissue. The binding of tissue factors also activates DCs and constitutes an indirect method of pathogen-induced maturation.\(^7\) Tissue factors include, but are not limited to, cytokines, chemokines, eicosanoids, heat-shock proteins and necrotic cell lipids.\(^9\) DCs develop in the bone marrow and migrate as immature cells to sites of potential pathogen invasion. Pathogens activate immature DCs in these peripheral tissues, which then mature into immunostimulatory effector cells and relinquish their endocytic capability and responsiveness to the environment (Fig. 2). The mature cells migrate into lymphoid organs and provide naive T cells with: antigen that stimulates specific TCRs; costimulatory molecules that prevent the development of tolerance; and polarizing factors that determine which
phenotype of effector T-cell is produced. DC maturation results in the production of functionally different effector DC subsets that release polarizing signals (the most important of which are cytokines), which selectively promote the development of Th1, Th2, or Th17 cell responses.10

Role of DCs

In the past it has been suggested that in mice and humans there exists a distinct division between the origin of DCs that induce a Th1 vs. a Th2 phenotype.11,12 Human monocyte-derived DCs were thought to induce Th1 differentiation, whereas DCs derived from plasma-cytoïd cells (pDCs) favoured Th2 development.13 This concept has been largely revoked, however, as it has been more recently demonstrated in many studies that both of these DC subsets can induce either type of immune response, depending on the nature of the DC. Th1, T helper type-1; Th2, T helper type-2; Treg, regulatory T-cell.

Figure 1. Initiation of the immune response and determination of phenotype. Pathogen-associated molecular patterns (PAMPs) are recognized by antigen-presenting cells (APCs), which present antigenic components bound to major histocompatibility complex II (MHC II) and a variety of costimulatory molecules and cytokines are released, which are determined by the nature of the pathogen. Tissue factors are released by infected tissues and influence APC activity. The nature of cytokines and costimulatory factors determines the phenotype of T-cell development and the effects of these signals are reinforced by cytokine release from the T cells themselves. T cells develop in stages, which are controlled by the interaction of a variety of controlling mechanisms. Negative-feedback loops ensure the predominance of a single phenotype, and tolerant mechanisms exist to prevent excessive responses to innocuous or self-antigens. Polarizing factors: T helper type-1 (Th1); interleukin (IL)-12, interferon (IFN)-α, β, or γ, IL-18, IL-27, CD80, intercellular adhesion molecule 1 (ICAM1), T helper type-2 (Th2); IL-4, IL-6, IL-11, CD2 and CD86. Tissue factors: cytokines; chemokines; eicosanoids; heat shock proteins; necrotic cell lipids. Phenotype determinants: nature and load of antigen; T-cell receptor (TCR); coreceptor signals; cytokine environment. Stages of development: 1, activation of cytokine genes; 2, Th cell commitment to phenotype; 3, repression of opposing Th cells; and 4, stabilization of phenotype. The molecular mechanisms that control the stages of development include intracellular signaling cascades, chromatin remodeling and epigenetic factors. CTLA-4, cytotoxic T lymphocyte antigen-4; ICOS, inducible costimulator; ICOS-L, inducible costimulator ligand; PRR, pattern recognition receptor; TGF-β, transforming growth factor-β; TLR, Toll-like receptor; TNF-β, tumour necrosis factor-β; Treg, regulatory T-cell.

Figure 2. Role of dendritic cells (DCs) in the development of the T-cell phenotype. DCs develop from progenitor cells in the bone marrow. DCs enter the circulation as immature cells and traffic to peripheral tissues and areas of potential pathogen exposure, where they perform continual surveillance for pathogens. Immature DCs are activated by pathogen-associated molecular patterns (PAMPs) and tissue factors, which induce the maturation of DCs into specific subsets characterized by the types of cytokines and costimulatory factors that they express. Mature DCs then move into lymphoid tissues where they induce the development of different phenotypes of T cells, depending on the nature of the DC. Th1, T helper type-1; Th2, T helper type-2; Treg, regulatory T-cell.

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remain to be elucidated. Recently, however, Reddike et al. demonstrated, using synthetic TLR ligands, that the binding of the ligand Pam3Cys to TLR2 can elicit a Th2-inducing phenotype in DCs. DCs stimulated directly or indirectly by PRRs from pathogens, mature into a specific form and are able to activate a single specific immune response that is appropriate for the elimination of the pathogen. In this way, the DCs determine the nature of the foreign antigen and the intensity and phenotype of immune response generated.

The next stage in the development of a Th1 or a Th2 immune response is the physical interaction between the activated forms of the DC and the naive Th cell. One proposed mechanism is that an immunological synapse forms between T lymphocytes and APCs, and this induces molecular rearrangements in both of these cell types. This is a dynamic process and one that relies heavily on the nature of the cytoskeleton of the cells, which enables movement of molecules to precise locations on the cell membrane. A critical part of this process may be for tubulin fibres to deliver MHC class II molecules loaded with antigen peptides into the site of contact in the centre of the synapse. The process is initiated when a T-cell comes into physical contact with the DC. When a specific T-cell encounters a specific peptide–MHC class II complex, which is presented by a DC, the DC also provides additional costimulatory signals (cytokines and coreceptors) that direct the T-cell to produce the type of immune response required. The naive Th cell then matures into a cell capable of performing a Th1 or a Th2 immune function. Whether a Th1 or a Th2 response is induced is determined when TCRs recognize the specific antigen peptide and induce the release of intracellular signals [such as protein kinase C (PKC), calcium ions, nuclear factor-κB] that help generate the appropriate immune response. In addition to those of the TCR, other signals (costimulation) are critical for the activation of Th cells and these are dependent on cell interactions involving the association of coreceptor molecules on the DC and the T-cell. For example, CD40, CD80 and CD86, and the more recently identified programmed death-1 ligand (PD-L1), programmed death-2 ligand (PD-L2) and inducible costimulator ligand (ICOS-L) are expressed on APCs and their respective binding partners CD40L, CD28/cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death-receptor 1 (PD-1), and ICOS on T cells. In addition, two other coreceptors have been identified on APCs – B7-H3 and B7-H4; however, their T-cell receptors remain to be elucidated. Most importantly, in order to achieve proper differentiation of the activated naive cells, T-cell polarizing cytokines are required. For secretion of these factors to occur at the optimal time, that is, when the DC comes into contact with the T-cell, the DC must be restimulated by the binding of CD40 to its ligand (CD40L) on the T-cell. This occurs when the TCR and coreceptors (CD80 and CD86) on the lymphocyte are activated, thereby inducing rapid expression of CD40L. Polarizing signals are widely considered to be primarily cytokines; however, as will be discussed later, evidence has shown that both the TCR signals and coreceptors are also integral in directing the immune response towards either the Th1 or the Th2 lineage. Furthermore, it has recently been proposed that polarization signals are dependent on contributions from other innate immune cells (natural killer, natural killer T and γδ T cells and basophils, eosinophils and mast cells).

**Role of signaling cascades**

In order to encapsulate fully the process of naive Th cell decision making, it is important to consider briefly the various signaling cascades and molecular mechanisms that underpin the induction of the Th phenotype. The development of a T-cell phenotype is differentially controlled by transcription factors that are activated by the predominance of one type of signaling cascade over the other. Primarily cytokines, but also the nature of T-cell receptors and coreceptors, can alter the balance between the signaling pathways and thereby each of these factors can favor or inhibit a certain immune response. TCR activation in response to a recognized antigen is followed by tyrosine phosphorylation of specific domains on the TCR, termed immuno-receptor tyrosine-based activation motifs (ITAMs) (Fig. 3a). ITAMs occur in the CD3 molecule associated with the TCR. A cascade of tyrosine kinase activation events then induces the movement of adapter molecules to the membrane of the T cells with GTPase activation. Such adapter molecules include the linker for activated T cells (LAT), which has been shown to positively regulate TCR signals leading to lymphocyte activation. These molecules mediate the activation of a cascade of serine and threonine kinases, known as mitogen-activated protein kinases (MAPKs), which lead to the up-regulation of the transcription factor activator protein-1 (AP-1), a proponent of cell proliferation. Within the cell membrane other signaling cascades originate with the induction of phospholipase C (PLC), which acts on membrane lipids, such as phosphatidylinositol-bis-phosphate (PIP2), to generate the second messengers inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 diffuses into the cytoplasm and acts on its receptor located on the endoplasmic reticulum to induce internal calcium release. Fluxes of calcium lead to the translocation to the nucleus of the transcription factor, nuclear factor of activated T cells (NFAT), which is important for the expression of a number of cytokine genes for both Th1 and Th2 cells. DAG regulates PKC, which is another important mediator of T-cell activation, once again through the induction of the MAPK pathway.
Cytokines are the most influential factors that modulate T-cell phenotype, and their mechanism of action involves intracellular signals transmitted through cytokine receptors expressed on the surface of T cells. When cytokines bind to their receptors on naïve Th cells, the receptor subunits move closer together and Janus kinase and signal transducers and activators of transcription (JAK-STAT) pathways are activated. The receptor subunits contain tyrosine kinase activity on their cytoplasmic tails, known as JAKs. There are four mammalian JAKs: JAK1; JAK2; JAK3; and tyrosine kinase 2 (tyk2). When the subunits condense, the JAKs phosphorylate and activate each other and induce tyrosine phosphorylation of the cytokine receptor. The STAT proteins, which occur as monomers in the cytoplasm, bind to the phosphorylated tyrosines on the receptor and also become phosphorylated. Phosphorylation induces the STAT proteins to dimerize and translocate to the nucleus where they act as DNA transcription factors. Certain combinations of JAKs and STATs, alternatively spliced STATs, and STATs interacting with other signaling molecules and transcription factors, are all responsible for the specificity of cytokine signaling (Fig. 3b,c). Notably, IFN-γ activates JAK1 and JAK2, leading to activation of STAT1, whereas following the interaction of IL-4 with its receptor, JAK1 and JAK3 are activated, which culminates in the activation of STAT6. Stimulation of the IL-12 receptor by its ligand induces the activation of JAK2 and tyk2, which in turn mediate the recruitment of STAT3 and STAT4. The interaction elicited by the cytokine, IL-10, upon binding its receptor involves activation of JAK1 and tyk2 with subsequent movement of STAT1 and STAT3 to the nucleus.

**Epigenetic mechanisms**

Epigenetic mechanisms, such as chromatin remodelling, result in increased accessibility of the cytokine genes responsible for differentiation to transcription factors. Furthermore, chromatin remodelling can induce the silencing of cytokine genes of the opposing phenotype by restricting access to transcription factors and through methylation of DNA. By assessment of T cells removed from one set of polarizing conditions and placed in conditions to induce the alternative cytokine program, Grogan et al. demonstrated that this plasticity to change was lost after three or four cell divisions. Further studies revealed that increased cytokine allele colocalization with heterochromatin was responsible for restricting access to the cytokine genes. This indicates how the CD4 T-cell phenotypes become stabilized. TCRs and cytokine signals initiate these chromatin rearrangements. The specific expression of appropriate subsets of genes is attributable to changes in gene structure, such as the repositioning of nucleosomes or de-compaction of condensed chromatin fibers, increased nuclease sensitivity and increased histone acetylation. Studies by Bird et al. showed that the process of Th differentiation can be instructed by enforced epigenetic modifications in place of cytokine signaling. They demonstrated that used alone either the demethylating agent, azadeoxycytidine, or the histone hyperacetylating agent, trichostatin A, could yield Th2 differentiation upon IL-4 restimulation in Th2 transcription factor-deficient cells, whereas for non-treated IL-4 restimulated cells this was not apparent. Furthermore, the combination of the two drugs synergistically interacted to prime the cells for even greater Th2 differentiation. This demonstrates that cytokine signaling may act through epigenetic changes to drive T-cell phenotypes and that the state of differentiation and epigenetic modifications is heritable from parent to daughter cell. Thus, at a certain point, the process of chromatin rearrangement becomes irreversible.
and this involves inactivation of genes. At this stage of stabilization, or fourth stage of differentiation, Th cells are unable to revert to any other phenotype.\textsuperscript{5,35}

**Level of stimulation**

TCRs specifically recognize a given antigen and this triggers intracellular TCR-mediated signals. The amount of antigen and the affinity of antigen recognition have an impact on the strength of the TCR signals and affect the intensity of response of the Th cell. Rogers et al.\textsuperscript{21} were able to demonstrate in vitro that the initial level (amount and affinity of antigen in TCR ligation) and duration of stimulation can also play a minor role in determining the phenotype of effector cells.\textsuperscript{19,21} Moreover, mixtures of stimulation can also play a minor role in determining the phenotype of effector cells.\textsuperscript{19,21} Moreover, mixtures of Th1 and Th2 cells can be generated in the same exogenous cytokine environment.\textsuperscript{20} Over a long period of time, a low level of stimulation favors the growth of uncommitted T cells, whereas moderate stimulation leads to the differentiation of cells expressing the Th2 cytokine phenotype and a high level of stimulation induces differentiation into cells secreting Th1 cytokines.\textsuperscript{21} This ligation of the MHC and the TCR during T-cell–APC interaction induces three major signaling pathways within the T-cell cytoplasm: PKC activation; calcium signalling; and p21 activation. Stimulation with different antigens involves different doses and levels of receptor/ligand affinities, resulting in the production of different ratios of PKC and calcium signals. This has an influence on the decision to differentiate into a type 1 or a type 2 T-cell.\textsuperscript{20} Studies by Noble et al.\textsuperscript{20} utilized selective intracellular signaling stimulators or partial inhibitors to demonstrate that calcium mobilization or inhibition of PKC favors Th1 polarization, whereas the converse biases differentiation towards activation of the Th2 pathway. These studies were performed in an environment of excess IL-12 and IL-4.\textsuperscript{20} These authors showed that two altered peptides induced two different effector T-cell phenotypes in transgenic T cells. They then demonstrated that different ratios of selective inhibitors of calcium and PKC signaling were required to prevent proliferation in these peptide-stimulated T cells.\textsuperscript{20} The exact mechanism by which calcium and PKC signaling leads to Th1 or Th2 differentiation remains to be elucidated. One potential hypothesis is that signals downstream of each of these pathways (namely members of the NFAT/AP-1 family of transcription factors) preferentially activate various Th1 or Th2 cytokine gene clusters.

**Novel hypothesis for the involvement of T cells and B cells in T-cell polarization**

Recently, a novel mechanism for pathogen-mediated selection of immune phenotype was proposed by Kalinski & Moser.\textsuperscript{37} They suggest that the development of either Th1 or Th2 responses is driven by successful eradication of one or other cell type, which propagates the expansion of the successful cell type. In this hypothesis the effector cells (T cells and B cells) have an inherent ability to distinguish between different types of pathogens through activation of different receptors [TCRs and B-cell receptors (BCRs)] by PAMPs. As a result, particular DC functions are specifically induced, which act as a positive feedback loop and promote the maturation and persistence of the appropriate T-cell subset. TCRs and BCRs are not germline but are hypervariable receptors and function adaptively to differentiate between different types of pathogens. Previous exposure of the immune system to pathogens would educate these effector mechanisms to promote selectively the appropriate phenotype of Th-cell response for a specific type of pathogen in a specific tissue.

**Th1 polarizing cytokine signals**

Th1-cell development begins with the secretion of IL-12 and type 1 IFNs (IFN-\(\alpha\) and IFN-\(\beta\)). These cytokines are released by macrophages and DCs upon activation by intracellular pathogens.\textsuperscript{38} IL-12 induces the production of IFN-\(\gamma\) by these same cells, which then acts in an autocrine manner to generate a positive feedback loop, producing further IL-12. The production of IL-12 can also activate natural killer cells to release IFN-\(\gamma\), which then also reinforces the macrophage and DC production of IL-12 in another amplifying positive feedback loop. While IFN-\(\gamma\), IL-12 and type 1 IFNs directly induce T cells to differentiate into Th1 cells, it is exclusively the IFN-\(\gamma\) from APCs and natural killer cells that also acts as an inhibitor of the Th2 pathway by preventing Th2 cell proliferation.\textsuperscript{4,39} Furthermore, it has recently been demonstrated that only microbial stimuli characterized by the induction of TLR activation and Th1 responses can enable DCs to activate natural killer cells, which then assist in the initiation of this type-1 response. Those stimuli that are TLR-independent or promote Th2 responses do not have the same effects.\textsuperscript{40} IFN-\(\gamma\) attachment to naive Th cells leads to the JAK1- and JAK2-mediated activation of the transcription factor STAT1, which then induces the expression of T-bet. T-bet is a member of the TATAAA-box family of transcription factors. T-bet production initiates the remodeling of the IFN-\(\gamma\) gene locus, the production of IFN-\(\gamma\), expression of the IL-12 receptor and stabilization of its own expression through the autocrine activity of IFN-\(\gamma\).\textsuperscript{41} Once the IL-12 receptor is expressed, this cytokine is then able to bind its receptor and further reinforce the differentiation of Th1 cells. IL-12 signalling activates the transcription factors STAT3, STAT4 and nuclear factor-\(\kappa\)B to promote the production of cytokines associated with the Th1 phenotype and chromatin remodelling. The latter process also allows the
access of NFAT to its target genes and further amplifies expression of IFN-γ utilizing this pathway. The IFN-γ secreted by Th1 cells as they develop stimulates surrounding naive Th cells to begin polarization into more Th1 cells, in a self-renewing paracrine loop. IL-12 also acts to up-regulate IL-18 receptor expression. DC-derived IL-18 then acts to potentiate the functions of the IL-12 at a later stage in the development of the Th1 phenotype. Models of intracellular bacterial infection have demonstrated that the function of IL-18, in promoting Th1 cell development, is less crucial than that of IL-12 because of partial redundancy. A protective Th1 response against Chlamydia species is generated in the absence of IL-12, but not in the absence of IL-18. Interestingly, prototypical type-1 and type-2 cytokines are not always autonomous, especially when they are not directed at T cells; in fact, paradoxically, Th1-inducing cytokines can stimulate Th2 cytokines from innate immune cells. It was recently shown that IL-18 may act synergistically with IL-12 to induce the development of natural killer-like cells that release the Th2 cytokine, IL-13.

In order for activation and differentiation of a Th cell to be complete, both the cytokine environment and the signals from the coreceptors, which are generated by direct contact between DCs and T cells, are important. Following the activation of DCs, several membrane-associated coreceptors are expressed and are involved in the priming of Th cells. TCRs are clustered in the centre of the synapse, together with CD2, CD28 and CD4. The constitutively expressed CD28 on the naive Th cell is the most important coreceptor in positively driving the development of this cell. Early studies suggested that the CD28 ligand, CD80, which is constitutively expressed on the surface at low levels in unstimulated DCs, was up-regulated early after activation of DC1. There is now much conflicting evidence, however, to suggest that CD80 is not exclusively involved in stimulating Th1 differentiation, but rather contributes to the general activation of Th cells. Endocytosis of double-stranded viral RNA promotes the up-regulation of CD54 on the DC1 surface, and this also facilitates the differentiation of antiviral Th1 cells. Other proposed Th1 polarizing factors include IL-27, and intercellular adhesion molecule-1 (ICAM-1) binding its receptor lymphocyte function-associated antigen (LFA)-1.

**Th2 polarizing cytokine signals**

The production of Th2 effector cells primarily involves the action of the cytokines IL-4, -6, -10 and -11. Interestingly, the origin of the IL-4 that leads to this phenotype has not been elucidated and as DCs are incapable of its expression, it may therefore arise from natural killer T cells, eosinophils or mast cells. IL-4 induces the production of STAT6 in naive T cells, which in turn activates the expression of the zinc finger transcription factor GATA-3. GATA-3 and T-bet are mutually antagonistic. When IFN-γ, IL-12 and T-bet levels are high, GATA-3 production is inhibited, and when IL-4 and GATA-3 levels increase, T-bet release is repressed. Both IL-4 and TCR signaling are required to up-regulate GATA-3 transcription, which can induce epigenetic remodeling of the Th2 cytokine cluster of genes. Specifically, GATA-3 augments promoter activity or reverses chromatin structure-based suppression of regions that are responsible for controlling Th2 cytokine gene expression. This results in the release of cytokines characteristic of the Th2 phenotype (IL-4, -5, -9, -10 and -13) and inhibits the expression of the IL-12 receptor and therefore Th1 development. Another transcription factor that is specific to Th2 cells is c-MAF; this protein is also responsible for regulating IL-4 synthesis through the activation of the IL-4 promoter. Further signaling through these pathways causes NFAT and AP-1 (which are non-lineage specific) to induce acute IL-4 transcription. Once GATA-3 production reaches a certain threshold, its own gene expression is auto-activated, hence stabilizing the Th2 phenotype through an intrinsic positive-feedback loop. Through this process, GATA-3 enables the fourth stage of development of the Th2 cell to take place.

As Th2 cells mature they produce increasing levels of IL-4, which generates a paracrine loop and induces neighboring naive T cells to develop into Th2 cells. IL-6 is another cytokine that is released during the early stages of a Th2 immune response. IL-6 release induces the Th2 phenotype through the up-regulation of IL-4 and inhibition of STAT1 phosphorylation, thereby preventing IFN-γ gene expression. The IL-6 at this early stage of differentiation arises from macrophages, mast cells and pulmonary DCs. IL-6 also plays an integral role in Th17 differentiation. In humans, IL-11 that is released by myeloid cells acts directly on T cells to stimulate IL-4 and IL-5 synthesis while also simultaneously inhibiting IFN-γ production. Furthermore, IL-11 suppresses IL-12 secretion from macrophages and therefore also contributes to Th2 differentiation through this indirect mechanism. In contrast to the Th1 phenotype, a soluble factor released by activated DC2 that is responsible for Th2 differentiation is yet to be identified. IL-6 is one potential candidate; however, activated DC2 may induce Th2 differentiation indirectly via the secretion of IL-10, which then inhibits IL-12 synthesis at the mRNA level and thus the Th1 pathway. At least one study has shown that IL-10 also down-regulates IL-12β2 receptor expression. This suggests that the development of the Th2 phenotype is the default pathway, occurring spontaneously in the absence of IL-12, which is a point of continuing controversy. Whether DC2 secrete other soluble factors that promote the development of Th2 cells remains unknown.
Recently, the induction of mast cell degranulation and the release of histamine in vivo have been demonstrated to polarize the function of DCs and Th cells towards a Th2 phenotype. Degranulation reduced the capacity of DCs to induce Th1 cells and instead promoted the development of increased numbers of IL-4-secreting T cells. This indicates that mast cells may have a crucial function in the development of the antigen-specific Th2 cell phenotype in mast cell-mediated diseases, such as asthma.

Several coreceptors are implicated in the activation of the T-cell and reinforcement of the Th2 phenotype. The CD28 ligand, CD86, is not expressed constitutively but is thought to be induced late in the DC2 postactivation response. The signals elicited by the ligation of these two surface molecules are considered to initiate the up-regulation of the GATA-3 transcription factor. This strong costimulation would provide a STAT-6 (IL-4)-responsive reinforcement of effector functions, depending on the density of surface expression and tissue localization of the immune response. Fortunately, the up-regulation of IL-4 receptor signaling following ICOS stimulation is not entirely exclusive to Th2 cells. It appears that ICOS is capable of costimulating distinct effector functions, depending on the density of surface expression and tissue localization of the immune response. Interestingly, there appears to be a relationship between ICOS cell-surface density and the type of cytokines produced. There is a strong association between intermediate expression of ICOS (the bulk of the Th cell population) and secretion of Th2 cytokines, and high levels of ICOS expression and release of the regulatory cytokine IL-10.

CD28 functions as a costimulatory receptor that rescues T cells from anergy and promotes T-cell proliferation; however, there are other coreceptors that have opposing effects, inducing the negative regulation of T-cell responses. The best characterized of these is CTLA-4, which is a homologue of CD28 and binds to the same ligands (CD80 and CD86) but with a greater affinity. The up-regulation and stimulation of CTLA-4, after T-cell activation, inhibits responses, including proliferation and cytokine production, by both Th1 and Th2 subsets. Originally it was suggested that CTLA-4 achieved its inhibitory effects by antagonizing TCR-mediated signals.

It has now been demonstrated to be more likely that CTLA-4 blocks the expression of genes involved in CD28 costimulation rather than interdicting TCR signalling.

**Th17 lineage**

Over the past few years it has become increasingly apparent that the diversity of CD4 effector T-cell responses may have been underestimated. Indeed, in retrospect, the Th1–Th2 paradigm was insufficient to convey protection against all types of foreign pathogens. A lineage of IL-17-producing CD4 T helper (Th17) cells, which are distinct from Th1 and Th2 cells, was recently discovered and shown to be crucial in autoimmune diseases and defence against extracellular bacteria. Th17 cells produce IL-17, IL-17F, IL-6, IL-22 and TNF-α, which, in turn, act on fibroblasts, macrophages, and endothelial and epithelial cells to elicit inflammatory mediator and chemokine release (Fig. 4). The resultant environment recruits granulocytes (in particular neutrophils) and creates a general state of tissue inflammation.

The Th17 lineage was originally discovered when the cytokine IL-23 was identified as a member of the IL-12 family with which it shared the homology of the p40 subunit. Earlier antibody-neutralization studies that had claimed to target IL-12p40 to alleviate autoimmune disease were later shown also to block IL-23p40. To elucidate further the precise role of these cytokines it was found that IL-23p19−/− (IL-23-deficient) mice were incapable of developing autoimmune disease, whereas IL-12p35−/− (IL-12-deficient) mice were susceptible. IL-17 was found to be the downstream effector cytokine of this IL-23-mediated process. The seminal findings of these studies revealed that a new distinct type of CD4 helper T-cell that was reliant upon IL-23 and IL-17 was critical to the development of autoimmunity, and thus the Th17 lineage was identified.
**Th17 polarizing cytokine signals**

Th17 cells represent a subset of CD4 effector T cells that is both distinct from and antagonised by cells of the Th1 and Th2 lineages. Although observed in lymphoid tissues throughout the body, Th17 cells are predominately found in the lung and digestive mucosa, suggesting a homeostatic role in these tissues. Th17 cells appear not to be critical to Th17 cell differentiation has remained the focus in vitro, but is instead instead induced by a combination of the polarizing cytokine, IL-6, and the regulatory cytokine, TGF-β (Fig. 4). The combination of these cytokines in vitro induces the predominant generation of Th17 cells with minimal numbers of Tregs in a mutually exclusive pattern. As TGF-β is involved in the development of both Tregs and Th17 cells, which may occur through the inhibition of IL-4- and IFN-γ-dependent pathways, it appears that IL-6, a known inhibitor of Treg development, plays an integral role in switching between these inflammatory and suppressive cell types. IL-6 acts on naive T cells to induce the downstream expression of IL-21, which initiates an autocrine loop that results in self-induced expression. TGF-β then acts in synergy with IL-21 to induce the expression of RORγt via a STAT3-dependent mechanism. The action of RORγt induces transcription of the genes encoding IL-17 and IL-17F.

Th17 cell development is not completely dependent on IL-6 because mice deficient in this cytokine are capable of producing Th17 responses in the absence of Tregs through an IL-21-dependent mechanism. Interestingly, neutralizing IL-17 in cultures of Th17 cells alters the balance in favour of the generation of Tregs, suggesting an important inhibitory action of IL-17 on Treg cells.

The precise manner in which IL-23 functions in the Th17 response remains to be elucidated. Studies in vitro have shown that IL-23 alone, or in combination with anti-IFN-γ or anti-IL-4, or TGF-β, or IL-6, does not elicit Th17 differentiation from naïve precursors. Instead, IL-23 appears to be essential to the survival and activation of memory and/or effector Th17 cells. Although not expressed on naïve cells, following Th17 differentiation TGF-β up-regulates IL-23R such that its expression is restricted to memory and activated Th17 cells. Other studies have shown that IL-23 is capable of acting on a pool of memory cells to induce IL-17 secretion. This suggests that the function of IL-23 may be targeted to effector sites. Taken together, the evidence to date emphasizes that IL-23 is more, IL-2, IL-25 and IL-27 appear to play additional roles in abrogating Th17 cell development and in the suppression of inflammation in murine models of autoimmune disease; however, their mechanisms of action remain to be delineated.
required for Th17-mediated protection and immunopathology, but not for Th17 differentiation.

Conclusion

The development of a polarized Th cell from a naïve T-cell is a complex process involving stimulation of TLRs, and activation and maturation of DCs, which leads to TCR engagement and cytokine release that triggers distinct signaling cascades and epigenetic mechanisms. The phenotype of the T-cell that is generated is influenced by the tissue microenvironment, which is generated by the dominant type of cytokines, costimulatory molecules and nature and dose of antigen presented. These factors, in turn, influence the activation of intracellular signaling pathways and subset-specific genes. Th1 cell development is promoted by IL-12, IL-18, type 1 IFNs and IFN-γ, and primarily involves STAT1, -3, -4 and T-bet signaling. The production of Th2 cells, while less well defined, is probably induced by IL-4, IL-6, IL-11 and ICOS costimulation, which induce signaling through STAT6, GATA-3 and c-MAF. The recently identified Th17 lineage is induced by the secretion of IL-6, IL-21 and TGF-β; however, these cells are dependent on IL-23 to achieve full effector and memory functions. A more complete understanding of this phenotype, including its influence on the differentiation of other effector T-cell subsets, is the subject of ongoing research. In disease states, such as multiple sclerosis, asthma and inflammatory bowel disease, aberrant CD4 T-cell responses play a crucial role in pathogenesis. Understanding the mechanisms of polarization of these aberrant T cells is pivotal for the development of effective prevention and treatment of these diseases.

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