IL-4: an important cytokine in determining the fate of T cells

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Received: 31 July 2013 / Accepted: 3 December 2013 / Published online: 9 January 2014
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Abstract The pleiotropic effect of cytokines has been well documented, but the effects triggered by unique cytokines in different T cell types are still under investigation. The most relevant findings on the influence of interleukin-4 (IL-4) on T cell activation, differentiation, proliferation, and survival of different T cell types are discussed in this review. The main aim of our study was to correlate the observed effect with the corresponding molecular mechanism induced on IL-4/IL-4R interaction, in an effort to understand how the same extracellular stimuli can trigger a wide spectrum of signaling pathways leading to different responses in each T cell type.

Keywords T cells · Interleukin-4 · Survival · Differentiation · Activation

Introduction

Interleukin-4 (IL-4), a short four-helix bundle peptide member of the γ -chain receptor cytokine family, is a pleiotropic cytokine produced mainly by Th2 lymphocytes, basophils, and

mast cells in response to a receptor-mediated activation (Rozwarski et al. 1994; Seder and Paul 1994). Other cell types that can produce IL-4 include NK T cells, a specialized subset of T cells that express NK1.1 and CD-1, eosinophils, and γ/δ T cells (Dubucquoi et al. 1994; Ferrick et al. 1995). Classically, IL-4 drives CD4⁺ T cell polarization in the Th2 phenotype together with suppression of interferon (IFN)- γ -producing Th1 cells (Nelms et al. 1999). IL-4 also supports the growth and differentiation of B lymphocytes (Howard and Paul 1982), controlling the specificity of the immunoglobulin G (IgG) class switching and the development of memory B cells. Other effects on different cells of the immune system and nonimmune cells have been described (Paul 1991; Nelms et al. 1999); however, in this review we focus on the role of IL-4 in the modulation of T cell responses during infection and immunity.

IL-4 receptor: a general view

The physiologic functions of IL-4 are mediated by the IL-4 receptor (IL-4R) complex. Several studies have demonstrated the existence of two types of IL-4 receptors: the type I receptor complex which comprises a 140-kDa chain (the IL-4R α chain) and the γ -common chain, which is also shared by IL-2, IL-7, IL-9, and IL-15 receptors (Sugamura et al. 1996). This is the prevailing heterodimer in hematopoietic cells and is activated exclusively on IL-4 binding. The IL-4R α chain may also heterodimerize with the IL-13R α 1 chain to form the type II receptor, which is able to transduce both IL-4 and IL-13 signals and is often the exclusive IL-4 receptor found in nonhematopoietic cells (Murata et al. 1998). The heterodimerization step is always necessary for the transduction of IL-4 signals (Russell et al. 1993). Although cytokine receptors belong to the tyrosine kinase receptor family, they do not possess intrinsic tyrosine

Special Issue Advances in Biophysics in Latin America

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kinase activity; instead, they require receptor-associated kinases, referred to as the Janus family (Jak) tyrosine kinases, which are critical in the initiation of signaling pathways (Paul 1991; Takeda et al. 1996; Nelms et al. 1999). Different members of the Jak family have been shown to be associated with IL-4R. In the type I receptor, the IL-4R α chain commonly associates with JAK1, whereas the γ c chain associates exclusively with JAK3 (Witthuhn et al. 1994; Yin et al. 1994; Chen et al. 1997). IL-4R α can also interact with JAK2 in other cell lines (Murata et al. 1996), and the corresponding JAK protein associated with the γ c chain varies and is different from that described for the type I receptor. IL-4R heterodimerization allows transphosphorylation and rapid activation of JAK proteins, which are responsible for the phosphorylation of tyrosine residues in the cytoplasmic domain of the IL-4R α chain (Smerzbartling and Duschl 1995; Takeda et al. 1996; Nelms et al. 1999). These phosphotyrosine residues serve as docking sites for downstream signaling proteins that contain the Src-homology 2 (SH2) domain, such as STAT6, or the phosphotyrosine-binding (PTB) domain, such as insulin receptor substrate 2 (IRS-2), IRS-1, downstream of kinase 1 (Dok-1), or Dok-2. STAT6 plays a role in gene regulation, differentiation of activated CD4⁺ T cells to the Th2 phenotype, inhibition of transforming growth factor (TGF)- α -induced Foxp3 expression (Dardalhon et al. 2008), development of the Th9 phenotype (Dardalhon et al. 2008), IL-4-induced GATA-3 expression, production of chemokines, and production of IgE and IL-4-inducible allergic responses. The PTB domain proteins are important for cell proliferation and cell survival, although the genes regulated by IL-4 through IRS-2 and other PTB domain adapters have not been fully elucidated (Zhu et al. 2001; Wurster et al. 2002). Furthermore, IL-13 is a cytokine related to IL-4 and also activates STAT6 after binding onto the IL-4R α chain (Urban et al. 1998). Due to its ability to induce signaling pathways through a similar receptor complex, some effects *in vivo* are shared by both cytokines. IL-13 induces Th2 responses against parasites, and both cytokines play an important role in the control of T cells functions in allergic diseases (Urban et al. 1998; Mohrs et al. 1999; Sivaprasad et al. 2010). In addition, IL-4 and IL-13 also regulate the quality of CD8⁺ T cell response during anti-viral immunity (Wijesundara et al. 2013).

Beyond the classic effects of IL-4 in CD4⁺ T cells, these molecular signaling pathways are also involved in IL-4-induced effects in other cell types. In B cells, IL-4 promotes a switch to the expression of IgE and the synthesis of specific IgG isotypes depending on the organism, including IgG4 in human B cells and IgG1 in mouse B cells (Vietta et al. 1985; Coffman et al. 1986; Gascan et al. 1991). In addition, IL-4 increases the expression of MHC class II molecules in B cells (Noelle et al. 1984; Takeda et al. 1996), upregulation of CD23 (Defrance et al. 1987; Takeda et al. 1996), IL-4 receptor (Ohara and Paul 1988), and expression of Thy-1 on B cells,

in association with lipopolysaccharide (Snapper et al. 1988). In the presence of tumor necrosis factor, IL-4 induces the expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells (Thornhill et al. 1991) and reduces E-selectin expression (Bennett et al. 1997). These effects are important to enhance the recruitment of T cells and eosinophils, rather than granulocytes, into the site of inflammation, in contrast to the concept that IL-4 acts only as an anti-inflammatory molecule. Moreover, as described later in this work, IL-4, together with TGF- β , is also involved in the development of a unique genetic program in CD4⁺ T cells, with inhibition of TGF- β -induced Foxp3 expression and IL-9 and IL-10 production (Dardalhon et al. 2008). It is now evident that many cell types, both hematopoietic and nonhematopoietic, contain the IL-4 receptor, which activates different signaling pathways in response to the pleiotropic effects of this cytokine. Here, these effects are reviewed specifically in T cells, highlighting the new functions of IL-4.

CD4⁺ T lymphocytes and Th2 differentiation

Interleukin-4-induced effects in T lymphocytes have been investigated since the 1980s, and these studies classically focused on the polarization of the T cell response. The first studies, using polyclonal stimuli *in vitro* and *in vivo*, described the requirement of IL-4 in the environment of primed T cells to stimulate them to produce IL-4 (Le Gros et al. 1990). These reports also launched the concept of a pattern of cytokines secreted by polarized populations of T cells after antigen stimulation, including the role of IL-4 in this differentiation (Kim et al. 1985; Mosmann et al. 1986).

The role of IL-4 in T cell differentiation has also been evaluated in studies using primed antigen-specific T cells with their cognate ligands presented by antigen-presenting cells (APCs). In this way, the evaluation of IL-producing populations was carried out under conditions closer to the physiologic process compared with polyclonal activation. Thus, these initial studies investigated the effect of IL-4 on the pattern of cytokines produced upon *in vitro* stimulation. During priming, the presence of IL-4 results in the production of large amounts of IL-4 and little or no detectable levels of IFN- γ during a secondary challenge (Le Gros et al. 1990). It was believed that the inhibition of IFN- γ production by IL-4 was mediated by IL-10, following reports that IL-10 was an inhibitor of IL-2 and IFN- γ production (Fiorentino et al. 1989). However, this possibility was ruled out because replacement of IL-4 by IL-10 in cultures did not inhibit IFN- γ production. Moreover, the treatment of these cultures with anti-IL-10 antibody did not influence IL-4-induced IFN- γ inhibition (Seder et al. 1992), indicating that IL-4 acts directly as an inhibitor of IFN- γ production when present during T cell priming. These initial reports contributed to the conclusion

that IL-4 is a regulator that plays a key role in the commitment of CD4⁺ T cells to the production of IL-4 concomitantly with the inhibition of IFN- γ and IL-2 production by themselves (Tanaka et al. 1993). It was later suggested that the effect of IL-4 in modulating the production of these two cytokines occurs at a specific stage of activation and differentiation, presumably while the cells are still in the naive state and that the response to stimuli depends on receptor engagement and the participation of APCs (Tanaka et al. 1993). These findings gave rise to the questions of how the specific pattern of cytokine production is regulated in vivo in activated T cells and what is the role of cytokine expression by precursor naive T cells.

Naive CD4⁺ T cells are able to produce IL-4. In this case, IL-4 controls the differentiation of T cells into IL-4-producing effector T cells. This phenomenon is probably involved in background differences between strains of mice in terms of the predominance of the Th1/Th2 response. For example, in Balb/c mice, it is possible to produce a significant population of naive CD4⁺ T cells secreting IL-4 after primary stimulation, a feature that is not observed in B10.D2 mice (Yagi et al. 2002). After T cell receptor engagement, the promoter region of the IL-4 gene is activated in both strains. However, the differences in IL-4 production and Th2 differentiation were correlated with the level of translocation to the nucleus of NF-AT2 and GATA-3, both transcriptional factors involved in IL-4 promoter activation and Th2 differentiation. Naive T cells from Balb/c mice were able to produce IL-4 independent of IL-4-signaling but dependent on NF-AT2 expression. Thus, NF-AT2 activity seems to be a key transcription factor in the generation of IL-4-producing naive T cells, and this could be responsible for significant GATA-3 expression after naive T cell activation, leading to Th2 differentiation (Yagi et al. 2002).

The molecular mechanisms involved in IL-4-induced T cell differentiation into the Th2 phenotype have been well elucidated. Stat6, a transcription factor activated in response to IL-4R signaling, is involved in a positive feedback loop for Th2 development because the IL-4 promoter and the IL-4 3' enhance both contain Stat6 binding sites (Kotaniides and Reich 1996; Zhu et al. 2001). Moreover, STAT6 induces the expression of other transcription factors, such as GATA-3 and c-maf, which have an important role in the generation of IL-4-producing Th2 cells. These developmentally regulated transcription factors lead to the transcription of other Th2 cytokine genes, such as IL-5, IL-9, and IL-13 cytokines (Kurata et al. 1999). In addition, GATA-3 inhibits the Th1 developmental genetic program by suppression of STAT4 and IL-12R β 2 expression in an IL-4-independent manner (Usui et al. 2003). The differentiation into Th2 cells also seems to be independent of STAT6, because it was demonstrated that Stat6^{-/-} Th cells are still capable of minimal Th2 cytokine production (Ouyang et al. 2000). The overexpression of

GATA-3 in STAT6-deficient cells was sufficient to induce and establish Th2 cytokine expression (Ouyang et al. 2000). However, a functional GATA-3 binding site at the proximal promoter region of the IL-4 gene is absent, which makes it unclear how GATA-3 really acts (Dong and Flavell 2000).

IL-4 responsive genes also include proteins that act as transcriptional repressors, which could be important to the downregulation of growth inhibitory genes in Th2 cells. One such protein is growth factor independent-1 (Gfi-1), a transcriptional repressor of these genes in Th2 cells, which is induced by IL-4 in a Stat6-dependent manner and is involved in Th2 cell proliferation (Zhu et al. 2002). On the other hand, another transcriptional repressor, Bcl-6, competes with Stat6 for DNA binding sites, thereby diminishing the levels of Th2 cytokines (Dent et al. 1997). Different studies have focused on the regulatory role of IL-4 in a STAT6-dependent manner during the early stages of T cell differentiation into Th2 phenotypes. It has been reported that IL-4 downmodulates IL-12R and IL-18R expression and inhibits IFN- α , IFN- β , and IL-18 production (Szabo et al. 1997; Smeltz et al. 2001). Although the molecular pathways behind these effects have not been characterized, previous reports have suggested a possible mechanism whereby STAT6 binds to a TTC-N₄-GAA motif, a specific DNA binding site that has been found in IL-4 and IL-4R promoters, but also to specific binding sites of STAT1 and STAT5, referred to as N3 (Ehret et al. 2001). Another possible mechanism involves competition between STAT6 and STAT1 for the binding site in the promoter regions (Chen et al. 2003) because the domain of STAT6 transcriptional activation is essential for the inhibition by IL-4 of IFN- γ -induced genes (Goenka et al. 1999).

CD4⁺ T lymphocytes and Th9/Treg differentiation

Another important effect of IL-4 described recently is a result of more detailed studies that emerged in the literature in the 1990s. These studies described the ability of CD4⁺ T cells to produce IL-9 and IL-10 in response to IL-4 combined with TGF- β . Under these conditions, IL-4 is able to inhibit the expression of Foxp3 induced by TGF- β , giving rise to a different phenotype not yet described that differs from that of the classic Treg cells (Schmitt et al. 1994; Dardalhon et al. 2008). This unique subset of CD4⁺ T cells produces a large amount of IL-9, and these cells are not only phenotypically but also functionally different from Tregs; they have been called Th9 cells. Although they are able to produce IL-10, they do not suppress T cell responses; they do potentiate an effector response, suggesting that Th9 cells represent a recently characterized population of inflammatory T cells that produce IL-10 (Dardalhon et al. 2008).

The molecular mechanisms behind CD4⁺ T cell differentiation into Th9 cells involve IL-4-induced STAT6 and GATA-3

transcription factors (Dardalhon et al. 2008; Wong et al. 2010). GATA-3 is upregulated in human Th9 cells (Wong et al. 2010), and the inhibition of Treg and Th2 cells by TGF- β and IL-4 seems to be regulated by the physical association of GATA-3 and Foxp3 (Dardalhon et al. 2008). Another essential mechanism to drive Th9 cell differentiation involves the activation of the IRF4 transcription factor, which could be upregulated by STAT6. However, the interaction between these transcription factors in the promotion of Th9 cell differentiation still needs to be investigated (Pernis et al. 2002; Staudt et al. 2010).

In regard to the Treg phenotype, IL-4 inhibits TGF- β -induced Tregs by STAT6 activation by at least two distinct mechanisms. Inside the Foxp3 promoter, there is an IL-4-responsive silencer region that serves as a binding site to STAT6 and a TGF- β -responsive enhancer region. It has been suggested that IL-4/STAT6-mediated repression involves the inhibition of Smad binding into the promoter region or the induction of closed chromatin conformation of the silencer region (Takaki et al. 2008). Thus, these mechanisms negatively affect the TGF- β 1-induced Foxp3 de novo expression; however, IL-4 had no effect on Foxp3 levels in CD4⁺CD25⁺ natural Tregs (Baron et al. 2007). Therefore, the establishment of an immune response or tolerance might be determined by Foxp3 levels regulated by TGF- β 1/IL-4 balance (Takaki et al. 2008). In the context of active inflammation, such as asthma, the handling of IL-4 signaling, with a stimulatory effect on the Th2 phenotype and an inhibitory effect on Tregs, would likely improve disease outcome.

CD8⁺ T lymphocytes

In CD8⁺ T cells, one report revealed that certain features of molecular events induced by IL-4 seem to be unique to this T-cell subset. Pinheiro et al. (2007) demonstrated that IL-4R engagement induces a wider spectrum of signaling mediators in CD8⁺ T cells than is often observed in CD4⁺ T cells stimulated with IL-4. This work further supports the relevance of IL-4 in the maintenance of CD8⁺ T cells during the liver stage of malaria parasites, a pathologic condition in which these cells are essential for a protective immune response (Carvalho et al. 2002). The investigators demonstrated that, in CD8⁺ T cells, IL-4 is able to induce the binding of IRS-2 to the activated receptor complex, which serves as a cytosolic docking site for downstream signaling molecules such as phosphoinositide 3-kinase (PI-3 K) (Pinheiro et al. 2007). The IL-4-induced IRS-2/PI-3 K/protein kinase B (PKB) and Jak/STAT signaling pathways seem to be functionally active in CD8⁺ T cells, such as in CD4⁺ T cells, and they are involved in CD8⁺ T cell rescue from apoptosis. In contrast to CD4⁺ T cells, STAT proteins other than STAT6, including STAT1, STAT3, and STAT5, are also activated in CD8⁺ T cells by IL-4 in a Jak3-dependent manner. The involvement of the

SOCS gene family in these diverse signaling mediators activated by IL-4 stimulation has also been demonstrated. In CD8⁺ T cells, the transcriptional levels of SOCS are comparable with those of CD4⁺ T cells, but these levels do not increase after IL-4 stimulation, suggesting diminished inhibitory activity on intracellular signaling by SOCS molecules in CD8⁺ T cells (Pinheiro et al. 2007). The physiologic consequence of these IL-4 effects on CD8⁺ T cells has yet to be fully elucidated. However, these phenomena could be involved in IL-4-mediated survival of memory CD8⁺ T cells, after these cells undergo an intense post-priming proliferation phase, which is followed by a contraction phase involving apoptosis. This wide-spectrum signaling pathway induces the generation of anti-apoptotic mediators, which is an efficient physiologic mechanism to ensure the development of memory populations (Pinheiro et al. 2007).

IL-4 has also been reported to provide protection from activation-induced cell death during the activation of CD8⁺ T cells. This process involves impairment of the upregulation of the Fas receptor at the time of T cell receptor stimulation, inducing protection from Fas-triggered apoptosis. It also involves the maintenance of the anti-apoptotic molecule Bcl-2, probably through activation of the PKB pathway, which is known to regulate the expression of Bcl-2 family proteins, including Bcl-2 (Nelms et al. 1999). Thus, IL-4 is able to control the survival of CD8⁺ T cells through the downregulation of Fas expression and stabilization of Bcl-2 expression during T cell activation, which could be important for the generation of long-term memory CD8⁺ T cells (Riou et al. 2006).

The influence of IL-4 on CD8⁺ T cell function seems to be paradoxical and remains poorly understood. It has been shown that IL-4 can act as a promoter or inhibitor or even that it has no effect on CD8⁺ T cell function for the antigen clearance as well as during naive CD8⁺ T cell differentiation in vitro (Kienzle et al. 2002, 2005; Marsland et al. 2005; Morris et al. 2009; Olver et al. 2006; Olver et al. 2013; Ranasinghe et al. 2007; Ranasinghe et al. 2009; Weinreich et al. 2010; Wijesundara et al. 2013).

During viral infection, some studies report that IL-4 dampens the response of human immunodeficiency virus-specific CD8⁺ T cells in pox-viral prime-boost-vaccinated mice (Ranasinghe et al. 2007, 2009). It was demonstrated that higher IL-4Ra expression reduces the polyfunctional capacity of anti-viral CD8⁺ T cells. Thus, IL-4 negatively regulates the quality of the CD8⁺ T cell response during viral infection, impairing the protective response (Wijesundara et al. 2013). IL-4 also upregulates its own expression and in parallel downregulates cytotoxic capacity, INF- γ production and CD8 expression during the primary activation of CD8⁺ T cells (Kienzle et al. 2005).

Using models of tumor immunity, it has been shown that IL-4-producing CD8⁺ T cells are less efficient than non-IL-4

producer cells for tumor clearance (Helmich et al. 2001; Kemp and Ronchese 2001). This effect could be due to the decreased INF- γ production and by the upregulation of antiapoptotic genes in tumor cells induced by IL-4 (Conticello et al. 2004). In addition, other studies have demonstrated that although high levels of IL-4 secretion during immune response induces the development of antigen-specific CD8⁺ T cells, these cells have diminished perforin/granzyme-mediated cytotoxicity (Villacres and Bergmann 1999). IL-4 also promotes the development and proliferation of a subpopulation of CD8⁺ T cells that do not upregulate CD25 and GrB expression (Riou et al. 2006). Moreover, T cell receptor stimulation of naive CD8⁺ T cells in the presence of IL-4 for several days promotes a subpopulation of CD8⁺ T cells expressing low levels of perforin and granzyme mRNA with limited cytolytic function (Kienzle et al. 2002). CD8⁺ T cells activated in the presence of IL-4 also fail to upregulate CD25, probably due to an inability to induce the pathway involved in CD25 gene transcription; however, these cells continue proliferating at levels comparable with those of CD25⁺CD8⁺ T cells.

On the other hand, all these effects of IL-4 described above have been brought in question by several studies showing that IL-4 could potentiate the cytotoxic activity of CD8⁺ T cells during tumor clearance, increasing tumor rejection *in vivo* (Santra et al. 1997). Moreover, IL-4 producing CD8⁺ T cell populations display strong cytotoxic-T-lymphocyte activity and CD8 expression (Carter and Dutton 1995; Sad et al. 1995; Cerwenka et al. 1998). Using short-term T cell culture, Oliver et al. (2012) verified that IL-4 is a potent stimulator of INF- γ synthesis, even in the absence of T cell receptor (TCR) stimulation, in a STAT6-independent manner. Interestingly, this effect is dependent on MAPK and PI3K and involves activation of two T-box transcription factors, namely, eomesodermin (Eomes) and T-bet. It was also observed that, in this experimental condition, INF- γ is not secreted but remains retained intracellularly (Oliver et al. 2012). With TCR stimulation, IL-4-induced INF- γ production increases and secretion occurs. These effects are due to a higher expression of Eomes and T-bet and, in this case, relies on STAT6 activity. In addition, the effect of IL-4 in stimulating both transcription factors, increased by T cell activation, is also involved with its ability to promote the cytotoxic activity of CD8⁺ T cells (Oliver et al. 2012).

The results of two studies further strengthen the notion of IL-4 as a stimulator of CD8⁺ T cell response. Marsland et al. (2005) demonstrated that CD8⁺ T cell expansion strongly relies on IL-4R α signaling, and (Morris et al. 2009) demonstrated that endogenously produced IL4 stimulates antigen-specific CD8 T cell proliferation in a MHC class-I dependent manner.

To date, it has been observed that the timing of IL-4 action is critical to the outcome of CD8⁺ T cell response. The production of IL-4 by CD8⁺ T cells right after antigen

recognition leads to dendritic cell maturation, which potentiates the differentiation of CD8⁺ T cells into effector cells (Schüler et al. 2001). On the other hand, IL-4 production could dampen the response of CD8⁺ T cells already differentiated into a phenotype committed to the production of type-I cytokines (Bot et al. 2000). In agreement with the latter, it has been suggested that IL-4 stimulates CD8⁺ T cell function during the priming phase but is dispensable during the effector phase (Schüler et al. 1999).

Conclusion

In conclusion, IL-4 drives an abundance of biological responses depending on the cell-specific molecular pathway triggered. IL-4 effects have been widely explored in Th2 cells, not less important is the action of IL-4 alone or combined with other cytokines in other T cell types, such as CD8⁺ T cells, Tregs, and Th9 cells. Cytokine-triggered signaling is not simple and may be multilayered, as observed by the influence of one cytokine on another. It would appear that the final picture is far from complete. An important challenge for further studies is to gain an understanding of the cross-talk between these signals at the molecular level to determine the final impact on the immune response.

Acknowledgments We thank Mr. Shanserley Leite do Espírito Santo and Mr. Mario Luiz da Silva Bandeira (Fundo de Amparo à Pesquisa do Estado do Rio de Janeiro–FAPERJ TCT fellowships) for excellent support.

Conflict of interest None.

Human and animal studies This article does not contain any studies with human or animal subjects performed by any of the authors.

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