REVIEW

Revisiting the Th1/Th2 Paradigm

E. MURAILLE & O. LEO

Laboratoire de Physiologie Animale, Département de Biologie Moléculaire, Université Libre de Bruxelles, Rhode-St-Genèse, Belgium

(Received 26 July 1997; Accepted 21 August 1997)

Muraillle E, Leo O. Revisiting the Th1/Th2 Paradigm. Scand J Immunol 1998;47:1–9

The identification of subsets of $CD4^+$ helper cells producing distinct pattern of cytokines has provided a valuable framework for understanding how different effector populations of immune cells can be recruited *in vivo* during infection. In the view of most investigators, Th1 and Th2 cells produce factors that serve as their own autocrine factors and cytokines exerting suppressive activities on each other's development and activity. This concept intuitively explains the natural tendency of immune responses to become progressively polarized. However, several experimental observations appear difficult to rationalize with a simple, 'symmetrical' Th1/Th2 paradigm including those that Th1 cells do not produce their own growth factor; that both Th1 and Th2 cells can promote inflammatory responses; that interleukin-10 (IL-10) inhibits inflammatory responses in a Th1/Th2-independent fashion; that IL-10 promotes the development of Th1-type effector cells; and that IL-12 can amplify pre-established Th2 responses. The purpose of the present analysis is to provide a revised model for better understanding how cytokines regulate immune responses *in vivo*.

O. Leo, Laboratoire de Physiologie Animale, Université Libre de Bruxelles, 67 rue des Chevaux, B-1640 Rhode-Saint-Genèse, Belgium

INTRODUCTION

The most important function of the immune system is probably to protect the organism against infectious diseases. Since no single effector response can effectively deal with all forms of pathogenic aggressions, the diversity of effector mechanisms and their regulation are absolutely essential for host survival, as demonstrated in many experimental infectious animal models [1–3]. The identification of functionally distinct $CD4^+$ T helper subpopulations (termed Th1 and Th2) [4], producing distinct patterns of cytokines, has provided an important insight into the mechanisms by which polarized immune responses occur *in vivo* in response to several pathogens. Indeed, Th1 cells secreting interferon-γ (IFN-γ) are involved in monocyte/macrophage-mediated inflammatory responses, while Th2-derived cytokines [interleukin-4 (IL-4), IL-5 and IL-10] encourage antibody production (including IgE responses) and promote mast cell and eosinophil proliferation and function (reviewed in refs 1–3). Observations performed using T-cell receptor (TCR)-transgenic mouse models have clearly demonstrated that the cytokine environment present during early T-cell activation determines the dominant cytokine profile of the subsequent T-cell response. Thus, antigen-presenting cell-derived IL-12 has been shown to promote the differentiation of naïve T cells toward the Th1 response phenotype, while IL-4, produced early in the response by mast cells, basophils and/or subpopulations of $CD4^+$ $NK1.1^+$ and/or $CD4^+$ $NK1.1^-$ [5] cells favours the development of the Th2 response phenotype (reviewed in refs 1–3). According to this scheme, the differentiation of helper cells from uncommitted precursors is regulated by the balance of IL-4 and IL-12 present during the onset of the response (Fig. 1A).

A natural tendency toward polarization of immune effector *in vivo* has been inferred from the observation that cytokines produced by Th2-like cells (IL-4, IL-10) inhibit the induction of Th1 responses, while Th1-derived cytokines (notably IFN-γ) are thought to counteract Th2 cell development (reviewed in refs 1–3). Collectively, these studies have led to the classical model of Th1/Th2 cross-regulation, as depicted in Fig. 1B, implying the existence of two 'competing helper subsets', each producing their own autocrine growth factor and cytokines able to counteract each other's development and activity.

The purpose of the present study is to discuss recent experimental observations which suggest that regulation of an immune response does not rely on the 'symmetrical' cross-regulatory activity of Th1- and Th2-like cells. In particular, cytokines that have long been considered to act in a helper subset-specific manner (such as IL-10 and IL-12) can exert their regulatory functions in a Th1/Th2-unrestricted fashion, a finding that led us

© 1998 Blackwell Science Ltd

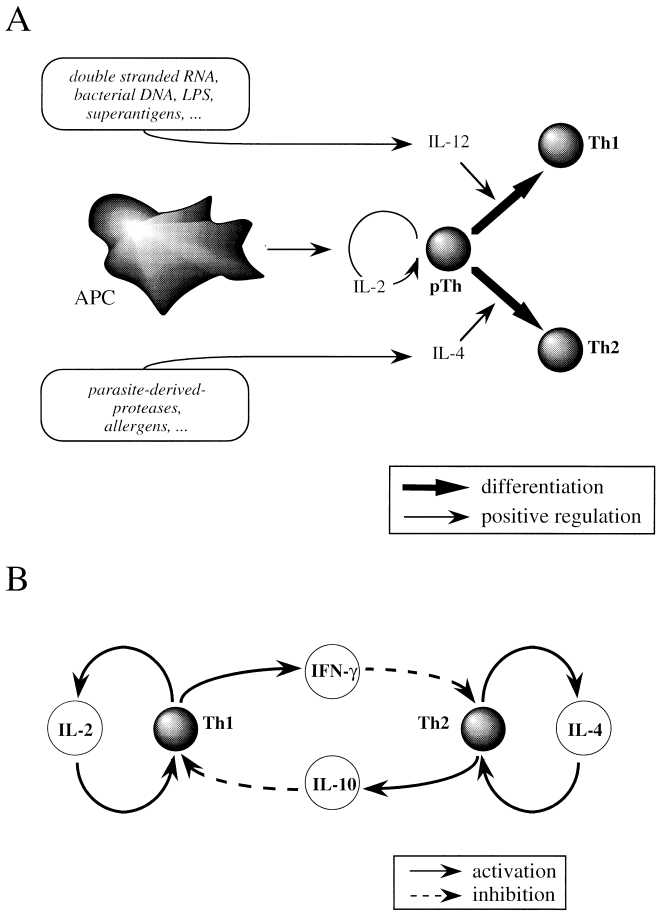

Fig. 1. (A) Differentiation of $CD4^+$ T helper subsets. Following interaction with appropriate costimulatory antigen-presenting cells, uncommitted T helper precursor cells (pTh) undergo IL-2-dependent clonal expansion and differentiate into helper subsets (Th1, Th2) producing a limited set of cytokines. Both environmental (including pathogen-derived) and genetic factors determine the early predominance of IL-12 and IL-4, the most potent factors inducing selective Th1 and Th2 differentiation. (B) The classical (symmetric) model of Th1/Th2 cross-regulation.

to propose a revised version of the Th1/Th2 regulatory pathway. This study also offers an alternative explanation to the the frequently observed dichotomy between cellular and humoral responses, a finding difficult to rationalize with the current concept of Th1/Th2 cross-regulation.

PARACRINE AND AUTOCRINE GROWTH OF HELPER SUBSETS

Studies performed on differentiated helper cells have suggested that IL-12 represents not only an important differentiation cytokine promoting the development of Th1 responses, but also acts as an important growth factor for Th1-like lymphocytes. In a particular study, the proliferation induced by antigen/antigen-presenting cell (APC) stimulation of several Th1 clones was not significantly affected by anti-IL-2 antibodies but was markedly inhibited by anti-IL-12 antibodies [6]. These findings confirm previously reported observations suggesting that, in contrast to naive T cells, proliferation of terminally differentiated Th1 clones, relies on IL-12 rather than on an IL-2-driven autocrine mechanism [6–8]. IL-2 production by established Th1 clones may therefore result from *in vitro* selection, and may not represent a physiologically relevant autocrine growth mechanism. In support of these findings, it has been recently demonstrated that Th1 and Th2 cells derived from TCR transgenic mice rapidly lose the ability to produce IL-2 while acquiring the capacity to produce, respectively, IFN-γ and IL-4 [9, 10].

It has been recently recognized that exogenous IL-12 does not exert an inhibitory effect on IL-4-producing cells [11], and sometimes even enhances the production of IL-4 when present during restimulation of differentiated Th2 cells [12–14]. Similarly, IL-12 has been recently shown to induce IL-4 mRNA in naive murine T cells [15]. These *in vitro* observations have been corroborated by *in vivo* studies, which showed that although IL-12 is a potent inducer of Th1-like responses when administered during antigen-priming of naive animals, it supports the development of a Th2 recall response when injected into previously immunized animals [16]. In particular, IL-12 given with inoculation of *Leishmania* parasites in susceptible BALB/c mice induced IFN-γ and suppressed IL-4 production, while when it was injected 14 days following infection, it led to increased IL-4 production [17]. Thus, and although the cellular targets and molecular basis of this phenomenon have not been established yet, IL-12 appears to exert a positive regulatory role on established Th2 responses. It should be noted that although Th2 cells selectively lose expression of the β2 subunit of the IL-12 receptor required for STAT-mediated signalling, they express high levels of the IL-12 receptor β1 subunit mRNA and protein, indicating that they may retain some responsiveness to IL-12 [18].

IL-12-mediated suppression of IL-4 producing cells observed in several *in vivo* models may therefore reflect the Th2-inhibitory properties of IFN-γ, rather than a direct antagonistic effect of IL-12 on IL-4 production [19], as evidenced by the finding that IL-12 administered to IFN-γ knockout mice exacerbated schistosome-induced Th2-dependent granuloma formation and caused a dramatic increase in serum IgE levels [19]. Note that in a similar fashion, IL-12-induced IFN-γ has been shown to down-regulate the stimulatory activity of IL-12 on haematopoiesis [20]. Suppression of Th2 responses by IL-12 in several models may therefore reflect the positive selection of non-IL-4-producing cells rather than the suppression of IL-4 secretion.

Collectively, these data suggest that APC-derived IL-12 and not IL-2, plays a major role in the development and clonal expansion of Th1-like cells, while Th2-like cells produce their own growth factor (IL-4) in an IL-12-independent fashion [21]. Moreover, although IL-12 clearly represents a Th1-promoting factor when present early during the response, it does not counteract, and may even amplify, an existing Th2 response. Thus clonal expansion of Th1-like cells depends on the paracrine secretion of growth factors, while Th2 cells appear to grow in an autocrine fashion.

IL-10 DOWN-REGULATES BOTH Th1 AND Th2 INFLAMMATORY RESPONSES

The observation that murine Th2 clones produce large amounts of IL-10, a cytokine known to block the production of pro-inflammatory cytokines such as IFN-γ and IL-12 (see refs 2, 3 for review and references below) has led to the conclusion that Th2 cells act as natural regulators of Th1-mediated inflammatory responses. However, although inflammatory responses have been generally associated with macrophages and natural killer (NK) cell activation (Th1-mediated inflammation), recent observations have clearly established that both Th1 and Th2 cells can mediate inflammatory responses, i.e. a local increase in blood flow and permeability of the microvascular wall, leading to accumulation of leucocytes in the inflamed tissue. Collectively, these responses have been referred to as hypersensitivity reactions, ranging from type I (immediate hypersensitivity reaction, mediated by IgE and mast cells) to type IV (delayed-type hypersensitivity reaction, mediated by IFN-γ-regulated effectors).

The airway inflammation observed during asthma is characterized by infiltration of the airway wall by Th2-like cells, eosinophils and mast cells [22]. Subcutaneous injection of activated Th2 cells in normal mice results in rapid interstitial oedema associated with a cellular infiltrate of neutrophils and mononuclear cells at the site of injection [23]. Th2 cells are also responsible for granuloma formation and hepatic fibrosis observed in mice infected with *Schistosoma mansoni* [24]. It has been recently demonstrated that polarized Th2-like cells can promote acute allograft rejection [25], an effector mechanism generally attributed to Th1-like responses. Finally, a detailed kinetic study performed in an *in vivo* model of delayed-type hypersensitivity (DTH) has recently shown that both IFN-γ- and IL-5-producing lymphocytes are recruited at the site of antigen injection [26]. Collectively, these data suggest that both Th1 and Th2 responses can mediate acute inflammatory responses characterized by local recruitment of antigen-non-specific effectors (such as macrophages, mast cells, eosinophils), causing tissue damage.

IL-10 was originally described as a Th2-derived factor inhibiting the synthesis of cytokines, particularly IFN-γ, by Th1 cells. Although IL-10 may have some direct effect on T cells [27], most of its inhibitory activity has been associated with an effect on macrophages and APC functions [28].

Several studies, mostly performed on human T cells, have recently suggested that IL-10 may down-regulate immune responses in a Th subset-unrestricted fashion. Indeed, IL-10 down-regulates APC-dependent cytokine synthesis by Th0, Th1 and Th2 human cells. In particular, synthesis of the Th2-type cytokines IL-4 and IL-5 was clearly inhibited [29]. Similarly, human IL-10 has been found to inhibit IL-5 production from both human T and natural killer (NK) cells [30, 31]. Down-modulation of both constitutive and IFN-γ- induced expression of major histocompatibility complex (MHC) class II by monocyte/macrophage may at least in part explain the lack of helper subset restriction for the inhibitory properties of human IL-10. It should be noted, however, that human IL-10 also inhibits T-cell growth triggered by immobilized anti-CD3 antibodies in the absence of monocytes [32].

In murine models, IL-10 has been generally shown to inhibit Th1 but not Th2 cell growth. IL-10 inhibits the development of Th1 responses by down-regulating IL-12 production by APC [33–35] including dendritic cells [36]. Accordingly, IL-10 has been shown to play an important regulatory role during Th1-type bacterial superantigen and endotoxin-induced septic shock. Indeed, antibody-mediated neutralization of endogenous IL-10 significantly enhanced SEB and lipopolysaccharide (LPS)-induced IFN-γ secretion and lethality in murine models [37, 38]. However, it has been recently demonstrated that IL-10 can down-modulate IL-5 production by murine T lymphocytes. Treatment of ovalbumin-sensitized animals with recombinant murine IL-10 (rmIL-10) down-regulates antigen-induced allergic eosinophilia and *in vivo* IL-5 production [39]. In agreement with these findings, it has been reported that mice rendered genetically deficient for IL-10 expression and infected by *Nippostrongylus* produced higher levels of both IL-5 and IFN-γ in response to lectin-mediated *in vitro* stimulation [40]. Notably, these animals also displayed increased levels of serum IgG2a and IgA (immunoglobulin subclasses positively regulated by, respectively, IFN-γ and IL-5) before immunization. Of note, murine IL-10 has been recently shown to prevent CD86 up-regulation on macrophages [41] and Langerhans' cells [42]. As priming of Th2 cells appears to require CD86 expression [43–45], IL-10 may also inhibit Th2-cell activation by interfering with the delivery of appropriate costimulatory signal(s).

Although observations made with human cells suggest a broader regulatory role for IL-10 (including inhibition of Th2-cell proliferation and IL-4-induced IgE synthesis, see ref. 46), the available data demonstrate that, in murine models, IL-10 is at least able to down-regulate the production of IL-5, a typical Th2-derived cytokine. Thus IL-10 appears to inhibit pro-inflammatory cytokine production by both Th1-like [tumour necrosis factor (TNF-α), IFN-γ] and Th2-like (TNF-α, IL-5) effectors. We therefore propose that, in contrast to the classical 'symmetrical' view of Th1/Th2 cross-regulation, IL-10 represents an anti-inflammatory agent acting in a Th-subset independent fashion (see Fig. 2).

THE POSITIVE REGULATORY PROPERTIES OF IL-10

Although originally described as cytokine synthesis inhibitory factor, IL-10 expresses proliferative, activating and chemotoactic properties upon various subsets of lymphocytes. In particular, IL-10 acts as a chemoattractant for $CD8^+$ T cells [47], and enhances the differentiation, growth and cytotoxic activity of $CD8^+$ cytotoxic T lymphocytes [48] and NK cells [49, 50]. IL-10 extends the viability of mouse mast cell lines *in vitro* and synergizes with IL-3 and IL-4 to stimulate their proliferation. Recent studies have also disclosed the positive effects of IL-10 on B-cell responses, including induction of class II expression,

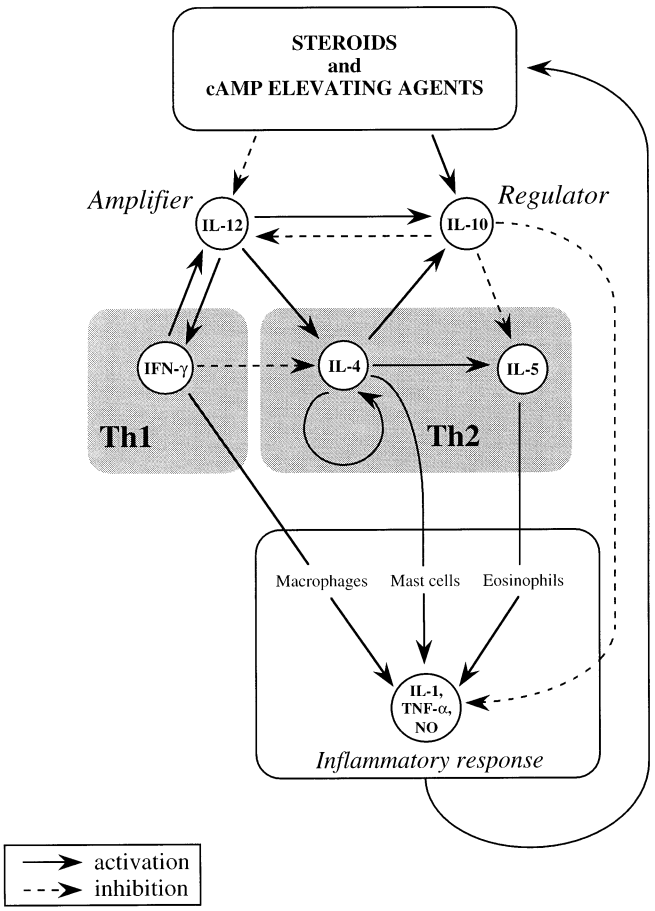

Fig. 2. The relationship between key cytokines during a T-helper-dependent immune response. Both Th1 and Th2 subsets can promote an inflammatory response, characterized by the activation of antigen-non specific effectors causing tissue injury. The inflammatory response is regulated in a T helper-unrestricted fashion by IL-12 (acting as an amplifier) and IL-10 (acting as a negative regulator). Steroids and cAMP-elevating agents exert their influence by affecting the IL-12/IL-10 balance.

enhanced growth and differentiation [51] and rescue from apoptosis in germinal centres [52].

These observations have been corroborated by several *in vivo* models showing that IL-10 (administered systemically or produced by engineered tumour cell lines) enhances tumour rejection and elicits a strong cytotoxic and antibody-dependent immune memory response [53, 54]. Similarly, the use of transgenic mice expressing IL-10 under a tissue-specific promoter has led to the conclusion that IL-10 can augment *in vivo* immune responses to self antigens [55]. Collectively these observations indicate that IL-10 does not simply act as an anti-inflammatory agent, but also promotes and sustains the development of immune effectors, some of which (cytotoxic cells) are generally thought to represent typical Th1-like effectors.

A MODEL OF Th1/Th2 CROSS-REGULATION

It has been recently reported that exogenous murine IL-12 induces a large increase in IL-10 secretion *in vivo* [17, 56]. This finding correlates with earlier reports describing the ability of bacterial products to induce both IL-10 and IL-12 [57, 58]. Recently, numerous investigators have confirmed these observations and have shown that IL-12 strongly enhances IL-10 production by human T cells [59–62]. As IL-10 in turn strongly inhibits APC-derived IL-12 production [33–36], these studies suggest that IL-12 itself initiates a negative feedback loop that limits its pro-inflammatory properties.

Thus, several observations are in agreement with the idea that IL-10 represents a key regulatory molecule whose natural function is the down-modulation of immune inflammatory responses. First, as previously discussed, IL-10 can down-regulate both Th1- and Th2-driven inflammatory responses; second, IL-10 can be produced by a very diverse set of lymphoid (including most T-cell subsets [1], $CD5^+$ B cells [63], mast cells [64], eosinophils [65], macrophages/monocytes [66, 67]) and non-lymphoid (keratinocytes [68], placental cytotrophoblasts [69]) cells. Finally, many anti-inflammatory compounds exert their immunosuppressive activity by inducing IL-10 production. These include agents able to increase intracellular cAMP such as prostaglandins [70–72], β-adrenergic agonists [73], synthetic peptides representing a conserved domain of the transmembrane envelope of several retrovirus [74], and phosphodiesterase inhibitors [75]. Similarly, glucocorticoids [76] and ultraviolet radiation [77] have been shown to promote IL-10 production. Consistent with the hypothesis that the IL-10/IL-12 balance plays a predominant role in the regulation of an immune response, most anti-inflammatory agents known to promote IL-10 synthesis inhibit IL-12 production (prostaglandin E_2 [72, 78], β-adrenergic agonists [79], dexamethasone [80], phosphodiesterase inhibitors [81] or induce a selective loss of IL-12-producing cells (ultraviolet light [82], dexamethasone [83]).

A model representing the previously discussed regulatory interactions between selected cytokines is presented in Fig. 2. As shown, a predominant role in the control of an ongoing immune response has been attributed to IL-12, considered as an amplifier of the immune response, and to IL-10, which acts as a negative regulator of all inflammatory responses. As we have focused our discussion on the cross-regulatory properties of cytokines produced by differentiated helper/effector cells, the role of IL-2, known to affect clonal expansion and differentiation of Th0 cells, has not been considered. Similarly, and for the sake of simplicity, the role of several other important cytokines (including IL-1, IL-6, IL-13, Il-15, IGIF or transforming growth factor-β) and the effect of genetic background differences will not be discussed.

It should be noted that IL-10 has not been considered as a typical Th2 cytokine, as it may be produced by virtually all immune effectors under appropriate experimental settings. In contrast to the 'classical' view of Th1/Th2 cross-regulation, the present model postulates a predominant anti-inflammatory role for IL-10 (including suppression of IL-5) and highlights the fact that only Th2 cells behave in an autocatalytic manner (IL-4 enhances its own production). Consequently, a Th2 response

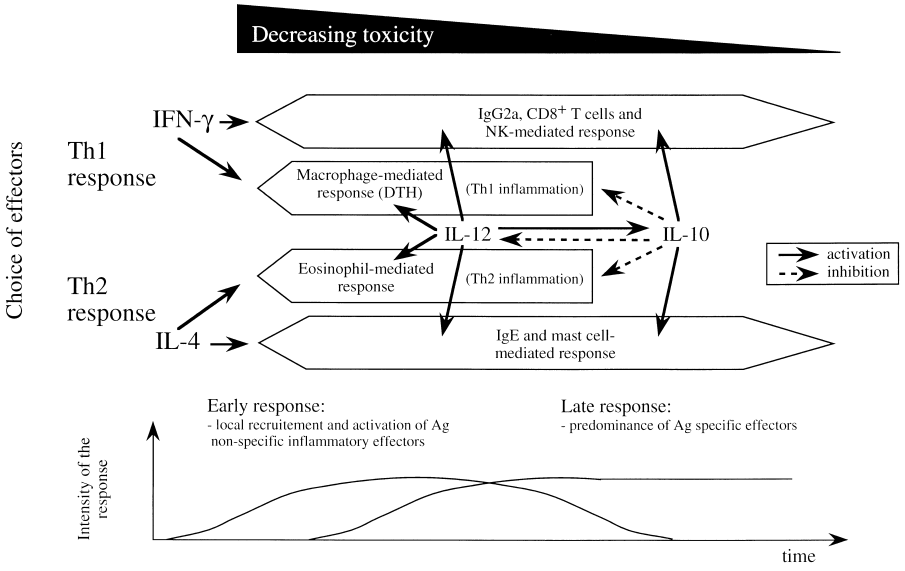

Fig. 3. Regulation of the immune response. IL-10 appears to down-regulate inflammatory, noxious responses, while favouring the 'switch' to less aggressive forms of immune defence characterized by antigen-specific effector and memory response.

characterized by IL-4 and IL-10 production but lacking IL-5 secretion can be envisioned, in agreement with recent observations performed in both human [84] and murine [85] models. Importantly, Th1 responses lack a cell autonomous autocatalytic loop, as expansion of Th1 clones is assumed to require APC-derived IL-12, and are consequently particularly sensitive to IL-10-mediated negative regulation. By contrast, the idea that some Th2 responses (in particular the production of regulatory cytokines such as IL-4 and IL-10) are less stringently regulated than Th1 responses may at least partially explain the high incidence of allergen-specific IgE antibodies in human populations.

IMMUNE DEVIATION AND HELPER SUBSETS DICHOTOMY

It has been recently recognized that in addition to the self/non-self discrimination problem and the selection of an adequate effector response, the immune system must cope with the inherent toxicity of its effectors. A need for regulation of potentially harmful responses specific for non-self constituents has been clearly inferred from studies performed in animal models of experimental septic shock [86] and infectious diseases [87–89]. These studies suggested that regulatory mechanisms must operate to counteract immune-mediated tissue damage. It is therefore tempting to speculate that suppressor mechanisms down-regulating the inflammatory response must have appeared before the development of an adaptive immune response. IL-10-like cytokines may have evolved as natural feedback mechanisms for containing the potentially self-destructive properties of primitive inflammatory cells. To this cell autonomous, early regulatory circuit a more sophisticated process of down-regulation of noxious (inflammatory) cells by other cells (IL-10-producing, regulatory) with shared specificity may have been added during vertebrate evolution.

Notably, it has been demonstrated that anti-inflammatory cytokines, including IL-10, play an important role in the survival to term of the fetal allograft in mammals [90, 91], suggesting that the development of a separate set of regulatory/suppressive cells may have been an important evolutionary step towards the development of viviparity. In agreement with the current idea that cellular immunity predates humoral immunity, primordial regulatory cells may have later acquired the ability to promote antibody production (by conferring to immunosuppressive cytokines novel stimulating properties and through the development of novel cytokines such as IL-4), possibly a less autoaggressive form of immune response. The complete set of Th2-like effectors (comprising eosinophils, mast cells and IgE production) may represent a further evolutionary adaptation in response to parasite infection.

Based on these evolutionary considerations and taking into account the experimental evidence accumulated to date, we propose the following model in order to understand the development and regulation of an immune response.

(1) Following interaction with an antigen that is both foreign and sensed by the immune system as a potential threat, uncommitted helper cells undergo clonal expansion and differentiate into cells expressing a limited set of cytokines. IL-4 and IL-12 clearly represent important polarizing factors directing the choice of effectors during the onset of the response (Fig. 1A).

(2) The inflammatory properties of the antigen-specific immune response are controlled by the relative balance between IL-12 and IL-10, acting in a Th1/Th2-unrestricted fashion. During this early phase, the immune system attempts to eliminate and control infection using the appropriate inflammatory effector cells, which can belong to both the Th1 (macrophage, NK cells), or the Th2 (eosinophils, mastocytes) subset and are characterized by lack of antigen-specificity and host toxicity (Fig. 3). The choice of effectors depends on signals provided by members of the innate immune system (APC or other sentinel-like cells such as the $NK1.1^+ CD4^+$ subset) able to detect microbial-derived molecular signatures.

(3) In a later phase, the immune response appears to be

dominated by effector cells displaying a high level of antigen specificity associated with minimal host toxicity (such as antibody and cytotoxic T-lymphocyte responses). This response, which can be well tolerated by the organisms, may represent both an attempt to contain infectious agents that were not eliminated by the early, noxious inflammatory response and/or a long-term protection against further re-infection. IL-10 (and possibly IL-4), may represent a key factor regulating this particular transition in the immune response (Fig. 3). Again it is important to note that memory cells belonging to both the Th1 (cytotoxic T lymphocytes) and the Th2 (B cells) subset can coexist in immune animals [92], a finding difficult to rationalize with the classical view of 'competing immune responses'.

In this context, it is interesting to note that the phenomenon of 'immune deviation' whereby an inflammatory response is gradually shifted towards a less aggressive form of immune response does not fit a simple Th1/Th2 paradigm. According to our hypothesis, immune deviation is characterized by a shift from an immune response characterized by the presence in the extravascular compartment of effectors lacking antigen-specificity to an immune response displaying high levels of antigen-specificity and minimal toxicity. Thus, the reciprocal relationship between cellular and humoral responses, often referred to as an *in vivo* illustration of the competing nature of Th1 and Th2 responses, probably reflects the inflammatory nature of the cellular response studied (such as delayed type hypersensitivity) versus the non-inflammatory properties of the humoral response and does not simply match a Th1/Th2 dichotomy.

CONCLUDING REMARKS

By analogy with ideas developed by C. Janeway [93] and later P. Matzinger [94] concerning the importance for the immune system to detect 'potential threat to the organisms', we would argue that negative regulation of an immune response evolved mainly to prevent self destruction caused by excessive inflammatory responses. In this perspective, IL-10 may represent a key regulatory cytokine acting in a Th-unrestricted fashion to down-regulate dangerous inflammatory responses (which can belong to both the Th1 and Th2 subsets) leaving other effector responses (again both Th1- and Th2-regulated, such as cytotoxic and antibody responses), available to protect against persistent or recurrent infections.

We hope that the present model will contribute to a better understanding of the positive and negative roles of cytokines in immune regulation and will provide information to develop cytokine-based therapies for the manipulation of immune responses in health and disease.

ACKNOWLEDGMENTS

The authors wish to thank J. Urbain, M. Moser and M. Goldman for helpful discussions. This work presents research results of the Belgian Program in Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister's office, Science Policy Programming. The authors wish to thank the Fonds Emile Defay for providing additional financial support. The scientific responsibility is assumed by its authors. E.M. is supported by a grant Télévie from the FNRS, Belgium.

REFERENCES

1 Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 1996; 17138–46.
2 Reiner SL, Seder RA. T helper cell differentiation in immune response. Curr Opin Immunol 1995;7:360–6.
3 Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996;383:787–93.
4 Mosmann TR, Cherwinski HM, Bond MW, Giedlin MA, Coofman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136:2348–57.
5 Nishimura T, Santa K, Yahata T *et al*. Vβ8.2$^+$ CD4$^+$ CD62L$^-$ CD45RB$^-$ T cells in non-MHC gene-controlled predisposition toward skewing into T helper type-2 immunity in BALB/c mice. J Immunol 1997;158:5698–706.
6 Maruo S, Toyo-oka K, Oh-hora M *et al*. IL-12 produced by antigen-presenting cells induces IL-2-independent proliferation of T helper cell clones. J Immunol 1996;156:1748–55.
7 Murphy EE, Terres G, Macatonia SE *et al*. B7 and interleukin 12 cooperate for proliferation and interferon gamma production by mouse T helper clones that are unresponsive to B7 costimulation. J Exp Med 1994;180:223–31.
8 Kennedy MK, Picha KS, Shanebeck KD *et al*. Interleukin-12 regulates the proliferation of Th1, but not Th2 or Th0 clones. Eur J Immunol 1994;24:2271–8.
9 Lederer JA, Perez VL, DesRoches L *et al*. Cytokine transcriptional events during helper helper T cell subset differentiation. J Exp Med 1996;184:397–406.
10 Bucy RP, Panoskaltsis-Mortari A, Huang G-Q *et al*. Heterogeneity of single cell cytokine gene expression in clonal T cell populations. J Exp Med 1994;180:1251–62.
11 Manetti R, Gerosa F, Giudizi MG *et al*. Interleukin 12 induces stable priming for interferon γ (IFN-γ) production during differentiation of human T helper (Th) cells and transient IFN-γ production in established Th2 cell clones. J Exp Med 1994;179:1273–1283.
12 Schmitt E, Hoehn P, Germann T, Rüde E. Differential effects of interleukin-12 on the development of naive mouse CD4$^+$ T cells. Eur J Immunol 1994;24:343–7.
13 Germann T, Guckes S, Bongartz M *et al*. Administration of IL-12 during ongoing immune responses fails to permanently suppress and can even enhance the synthesis of antigen-specific IgE. Int Immunol 1995;7:1649–57.
14 Jeannin P, Delneste Y, Life Y *et al*. Interleukin-12 increases interleukin-4 production by established human Th0 and Th2-like T cell clones. Eur J Immunol 1995;25:2247–52.
15 Nakamura T, Kamogawa Y, Bottomly K, Flavell RA. Polarization of IL-4- and IFN-γ-producing CD4$^+$ T cells following activation of naive CD4$^+$ T cells. J Immunol 1997;158:1085–94.
16 Bliss J, Van Cleave V, Murray K *et al*. IL-12, as an adjuvant, promotes a T helper 1 cell, but does not suppress a T helper 2 cell recall response. J Immunol 1996;156:887–94.
17 Wang ZE, Zheng S, Corry DB *et al*. Interferon gamma-independent

effects of interleukin 12 administered during acute or established infection due to Leishmania major. Proc Natl Acad Sci USA 1994;91:12932–6.
18 Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the interleukin (IL)-12R β2 subunit expression in developing T helper 1 (Th1) and Th2 cells. J Exp Med 1997;185:817–24.
19 Wynn TA, Jankovic D, Hieny S *et al*. IL-12 exacerbates rather than suppresses T helper 2-dependent pathology in the absence of endogenous IFN-γ. J Immunol 1995;154:3999–4009.
20 Eng VM, Car BD, Schnyder B *et al*. The stimulatory effects of interleukin (IL)-12 on hematopoiesis are antagonized by IL-12-induced interferon-γ *in vivo*. J Exp Med 1995;181:1893–8.
21 Hu-Li J, Huang H, Ryan J, Paul WE. In differentiated $CD4^+$ T cells, interleukin 4 production is cytokine-autonomous whereas interferon γ production is cytokine-dependent. Proc Natl Acad Sci USA 1997;94:3189–94.
22 Drazen JM, Arm JP, Austen KF. Sorting out the cytokines of asthma. J Exp Med 1996;183:1–5.
23 Muller KM, Jaunin F, Masouyé I, Saurat JH, Hauser C. TH2 cells mediate IL-4-dependent local tissue inflammation. J Immunol 1993;150:5576–84.
24 Wynn TA, Cheever AW. Cytokine regulation of granuloma formation in shistosomiasis. Curr Opin Immunol 1995;7:505–11.
25 VanBuskirk AM, Wakely ME, Orosz CG. Transfusion of polarized TH2-like cell populations into SCID mouse cardiac allograft recipients results in acute allograft rejection. Transplantation 1996;62:229–38.
26 Buchanan KL, Murphy JW. Kinetics of cellular infiltration and cytokine production during the efferent phase of a delayed-type hypersensitivity reaction. Immunology 1997;90:189–97.
27 Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse helper T cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989;170:2081–95.
28 Moore KV, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin–10. Annu Rev Immunol 1993;11:165–90.
29 Del Prete G, De Carli M, Almerigogna F *et al*. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993;150:353–60.
30 Schandené L, Alonso-Vega C, Willems F *et al*. B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. Immunology 1994;152:4368–74.
31 Warren HS, Kinnear BF, Phillips JH, Lanier LL. Production of IL-5 by human NK cells and regulation by IL-4, IL-10 and IL-12. Immunology 1995;1254:5144–52.
32 Taga K, Mostowski H, Tosato G. Human interleukin-10 can directly inhibit T-cell growth. Blood 1993;81:2964–71.
33 D'Andrea A, Aste-Amezaga M, Valiante NM. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993;178:1041–8.
34 Chensue SW, Ruth JH, Warmington K, Lincoln P, Kunkel SL. *In vivo* regulation of macrophage IL-12 production during type 1 and type 2 cytokine-mediated granuloma formation. J Immunol 1995;155:3546–51.
35 Hino A, Nariuchi H. Negative feedback mechanism suppress interleukin-12 production by antigen-presenting cells interacting with T helper 2 cells. Eur J Immunol 1996;26:623–8.
36 Koch F, Stanzl U, Jennewein P *et al*. High level IL-12 production by murine dendritic cells: up-regulation via MHC class II and CD40 molecules and down-regulation by IL-4 and IL-1. J Exp Med 1996;184:741–6.
37 Florkin S, Amraoui Z, Abramowicz D, Goldman M. Systemic release and protective role of IL-10 in staphylococcal enterotoxin B-induced shock in mice. J Immunol 1994;153:2618–23.
38 Gerard C, Bruyns C, Marchant A *et al*. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med 1993;177:547–50.
39 Zuany-Amorin C, Créminon C, Nevers MC *et al*. Modulation by IL-10 of antigen-induced IL-5 generation, and $CD4^+$ T lymphocyte and eosinophil infiltration into the mouse peritoneal cavity. J Immunol 1996;157:377–84.
40 Kühn R, Löhler J, Rennick D, Rajewski K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;75:263–74.
41 Flores-Villanueva PO, Reiser H, Stadecker MJ. Regulation of T helper cell responses in experimental murine schistosomiasis by IL-10. Effect on expression of B7 and B7-2 costimulatory molecules by macrophages. J Immunol 1994;153:5190–9.
42 Kawamura T, Furue M. Comparative analysis of B7.1 and B7.2 expression in Langerhans cells: differential regulation by T helper type 1 and T helper type 2 cytokines. Eur J Immunol 1995;25:1913–17.
43 Freman GJ, Boussiotis VA, Anumanthan A *et al*. B7-1 and B7-2 do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferentially co-stimulates the initial production of IL-4. Immunity 1995;2:523–32.
44 Morton PA, Fu X, Stewart JA *et al*. Differential effects of CTLA-4 substitutions on the binding of human CD80 (B7-1) and CD86 (B7-2). J Immunol 1996;156:1047–54.
45 Ranger AM, Das MP, Kuchroo VK, Glimcher LH. B7-2 (CD86) is essential for the development of IL-4-producing cells. Int Immunol 1996;8:1549–60.
46 Punnonen J, de Waal Malefyt R, van Vlasselaer P, Gauchat JF, de Vries JE. IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. J Immunol 1993;151:1280–9.
47 Jinquan T, Larsen CG, Gesser B, Matsushima K, Thestrup-Pedersen K. Human IL-10 is a chemoattractant for $CD8^+$ T lymphocytes and an inhibitor of IL-8-induced $CD4^+$ T lymphocyte migration. J Immunol 1993;151:4545–51.
48 Chen W-F, Zlotnik A. IL-10: a novel cytotoxic T cell differentiation factor. J Immunol 1991;147:528–34.
49 Schwarz MA, Hamilton LD, Tardelli L, Narula SK, Sullivan LM. Stimulation of cytotoxic activity by interleukin-10. J Immunother Emphasis Tumor Imm 1994;16:95–104.
50 Carson WE, Lindemann MJ, Baiocchi R *et al*. The functional characterization of interleukin-10 receptor expression on human natural killer cells. Blood 1995;12:3577–85.
51 Rousset F, Garcia E, Defrance T *et al*. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 1992;89:1890–3.
52 Levy Y, Brouet J-C. Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the Bcl-2 protein. J Clin Invest 1994;93:424–8.
53 Giovarelli M, Musiani P, Modesti A *et al*. Local release of IL-10 by transfected mouse mammary adenocarcinoma cells does not suppress but enhances antitumor reaction and elicits a strong cytotoxic lymphocyte and antibody-dependent immune memory. J Immunol 1995;155:3112–23.

54 Berman RM, Suzuki T, Tahara H *et al*. Systemic administration of cellular IL-10 induces an effective, specific, and long-lived immune response against established tumors in mice. J Immunol 1996;157:231–8.
55 Lee MS, Mueller R, Wicker LS, Peterson LB, Sarvetnick N. IL-10 is necessary and sufficient for autoimmune diabetes in conjunction with NOD MHC homozygosity. J Exp Med 1996;183:2663–8.
56 Morris SC, Madden KB, Adamovicz JJ *et al*. Effects of IL-12 on *in vivo* cytokine gene expression and Ig isotype selection. J Immunol 1994;152:1047–56.
57 Tripp CS, Wolf SF, Unanue ER. Interleukin 12 and tumor necrosis factor α are costimulators of interferon γ production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc Natl Acad Sci USA 1993;90:3725–9.
58 Svetic A, Jian YC, Lu P, Finkelman FD, Gause WC. Brucella abortus induces a novel cytokine gene expression pattern characterized by elevated IL-12 and IFN-γ in $CD4^+$ T cells. Int Immunol 1993;5:877–83.
59 Windhagen A, Anderson DE, Carrizosa A, Williams RE, Hafler DA. IL-12 induces human T cells secreting IL-10 with IFN-gamma. J Immunol 1996;157:1127–31.
60 Daftarian PM, Kumar A, Kryworuchko M, Diaz-Mitoma F. IL-10 production is enhanced in human T cells by IL-12 and IL-6 and in monocytes by tumor necrosis factor-alpha. J Immunol 1996;157:12–20.
61 Gerosa F, Paganin C, Peritt D *et al*. Interleukin-12 primes human CD4 and CD8 T cell clones for high production of both interferon-gamma and interleukin-10. J Exp Med 1996;183:2559–69.
62 Jeannin P, Delneste Y, Seveso M, Life P, Bonnefoy JY. IL-12 synergizes with IL-2 and other stimuli in inducing IL-10 production by human T cells. J Immunol 1996;156:3159–65.
63 Finke J, Ternes P, Lange W, Mertelsmann R, Dolken G. Expression of interleukin 10 in B lymphocytes of different origin. Leukemia 1993;7:1852–7.
64 Marietta EV, Chen Y, Weis JH. Modulation of expression of the anti-inflammatory cytokines interleukin-13 and interleukin-10 by interleukin-3. Eur J Immunol 1996;26:49–56.
65 Nakajima H, Gleich GJ, Kita H. Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. J Immunol 1996;156:4859–66.
66 Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991;147:3815–22.
67 Ghanekar S, Zheng L, Logar A *et al*. Cytokine expression by human peripheral blood dendritic cells stimulated *in vitro* with HIV-1 and herpes simplex virus. J Immunol 1996;157:4028–36.
68 Enk AH, Katz SI. Identification and induction of keratinocyte-derived IL-10. Immunology 1992;149:92–5.
69 Roth I, Corry DB, Locksley RM *et al*. Human placental cytotrophoblasts produce the immunosuppressive cytokine interleukin 10. J Exp Med 1996;184:539–48.
70 Platzer C, Meisel C, Vogt K, Platzer M, Volk HD. Up-regulation of monocytic IL-10 by tumor necrosis factor-α and cAMP elevating drugs. Int Immunol 1995;7:517–23.
71 Strassmann G, Patil-Koota V, Finkelman F, Fong M, Kambayashi T. Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E. J Exp Med 1994;180:2365–70.
72 Kalinski P, Hilkens CMU, Snijders A, Snijdewint FGM, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturating human naive T helper cells. J Immunol 1997;159:28–35.
73 Suberville S, Bellocq A, Fouqueray B *et al*. Regulation of interleukin-10 production by β-adrenergic agonists. Eur J Immunol 1996;26:2601–5.
74 Haraguchi S, Good RA, Day NK. Immunosuppressive retroviral peptides: cAMP and cytokine patters. Immunol Today 1995;16:595–9.
75 Kambayashi T, Jacob CO, Zhou D *et al*. Cyclic nucleotide phosphodiesterase type IV participates in the regulation of IL-10 and in the subsequent inhibition of TNF-alpha and IL-6 release by endotoxin-stimulated macrophages. J Immunol 1995;155:4909–16.
76 Brinkmann V, Kristofic C. Regulation by corticosteroids of Th1 and Th2 cytokine production in human $CD4^+$ effector T cells generated from $CD45RO^-$ and $CD45RO^+$ subsets. J Immunol 1995;155:3322–8.
77 Ullrich SE. Mechanism involved in the systemic suppression of antigen-presenting cell function by UV irradiation. J Immunol 1994;152:3410–16.
78 van der Pouw-Kraan TC, Boeije LC, Smeenk RJ, Wijdenes J, Aarden LA. Prostaglandin E2 is a potent inhibitor of human interleukin 12 production. J Exp Med 1995;181:775–9.
79 Bordignon PP, Di Lucia P, Self C *et al*. β2-adrenoreceptor agonists specifically inhibit IL-12 production. Abstract presented at Second Symposium of Pathogenesis and Immunotherapy of Autoimmune Diseases. 1996. Milan, Italy.
80 Blotta MH, DeKruyff RH, Umetsu DT. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in $CD4^+$ lymphocytes. J Immunol 1997;158:5589–95.
81 Moller DR, Wysocka M, Greenlee BM *et al*. Inhibition of human interleukin-12 production by pentoxyfilline. Immunology 1997;91:197–203.
82 Cooper KD, Oberhelman L, Hamilton TA *et al*. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, CD1a-DR^+ epidermal macrophage induction, and Langerhans cell depletion. Proc Natl Acad Sci USA 1992;89:8497–501.
83 Grabbe S, Steinbrink K, Steinert M, Luger TA, Schwartz T. Removal of the majority of epidermal Langerhans cells by topical or systemic steroid application enhances the effector phase of murine contact hypersensitivity. J Immunol 1995;155:4207–17.
84 Jung T, Schauer U, Rieger C. Interleukin-4 and interleukin-5 are rarely co-expressed by human T cells. Eur J Immunol 1995;25:2413–16.
85 Garlisi CG, Pennline KJ, Smith SR, Siegel MI, Umland SP. Cytokine gene expression in mice undergoing chronic graft-vs.-host disease. Mol Immunol 1993;30:669–77.
86 Miethke T, Wahl C, Heeg K *et al*. T cell-mediated lethal shock triggered in mice by the superantigen *Staphylococcus aureus* enterotoxin B: critical role of tumor necrosis factor. J Exp Med 1992;175:91–8.
87 de Kossodo S, Grau GE. Profiles of cytokine production in relation with susceptibility to cerebral malaria. J Immunol 1993;151:4811–20.
88 Ando K, Moriyama T, Guidotti LG *et al*. Mechanism of class I restricted immunopathology. A transgenic mouse model of fulminant hepatitis. J Exp Med 1993;178:1541–54.
89 Fleicher B, Fleicher S, Maier K *et al*. Clonal analysis of infiltrating T

lymphocytes in liver tissue in viral hepatitis A. Immunology 1990;69:14–19.

90 Chaouat G, Assal-Meliani A, Martal J *et al*. IL-10 prevents naturally occurring fetal loss in the CBA × DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by *in vivo* injection of IFN-tau. J Immunol 1995;154:4261–8.

91 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993;14:353–6.

92 PLanz O, Ehl S, Furrer E, Brundler M-A, Hengartner H, Zinkernagel RM. A critical role for neutralizing-antibody-producing B cells, $CD4^+$ T cells, and interferons in persistent and acute infections of mice with lymphocytic choriomeningitis virus: implication for adoptive immunotherapy of virus carrier. Proc Natl Acad Sci USA 1997;94:6874–9.

93 Janeway CA. The immune system evolved to discriminate infectious nonself from noninfectious self. Immunol Today 1992;13:11–15.

94 Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991–1045.