

# VIP: a very important peptide in T helper differentiation

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**Vasoactive intestinal peptide (VIP), a neuropeptide mainly produced by Th2 cells following antigenic stimulation, has a general anti-inflammatory effect, both in innate and adaptive immunity. Recent studies indicate that lymphocyte-derived VIP acts as an important T helper-differentiating factor, promoting Th2-type, and inhibiting Th1-type responses, through several mechanisms, including preferential survival of Th2 effectors and generation of memory Th2 cells. These effects appear to be responsible for the beneficial effect of VIP in models of Th1-type autoimmune diseases, such as rheumatoid arthritis and Crohn's disease.**

Two recent studies have pointed to lymphocyte-derived vasoactive intestinal peptide (VIP) as an important T helper-differentiating factor, promoting Th2-type and inhibiting Th1-type responses through several mechanisms, including preferential survival of Th2 effectors and generation of memory Th2 cells [1,2]. VIP is a 28 amino-acid peptide originally described by Said and Mutt in lung and small intestine in its capacity as vasodilator [3]. VIP was later identified in the nervous and endocrine systems, acting as a multifunctional neurotransmitter and neuro-modulator. Recently, this pleiotropic neuropeptide has been 'rediscovered' in the immune system, and it has been shown to have a key role in the maintenance of neuroendocrine-immune communication. In addition to VIP-containing nerve fibers found in proximity to immune cells [4], immune cells themselves, especially lymphocytes, are the main sources of VIP in lymphoid organs, which express and secrete VIP on activation by several stimuli [5,6]. Originally considered a negative regulator of several T-cell and macrophage functions and recently identified as a potent anti-inflammatory factor (reviewed in Refs [7,8]), VIP appears to have a more complex role in immune homeostasis.

## VIP promotes Th2-type immune responses

The adaptive immune response against a specific antigen is initiated by the cellular contact between an antigen-presenting cell (APC) and a naïve T cell bearing the appropriate T-cell receptor (TCR). Both stimulatory (MHC class II-antigen on the APC and TCR on the T cell), and costimulatory (B7-1 or B7-2 and CD40 on the APC and CD28 and CD40L on the T cell) interactions are required for the activation of naïve T cells. On activation, antigen-specific T cells express high-affinity interleukin-2 (IL-2) receptors and proliferate in response to autocrine or

paracrine IL-2. Following antigenic stimulation and proliferation, CD4 T cells differentiate into Th1 and Th2 effector cells, characterized by specific cytokine profiles and functions. Th1 cells secrete cytokines [IL-2, interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\beta$  (TNF $\beta$ )] crucial for the generation of a cellular immune response, thereby activating macrophages, inducing delayed-type hypersensitivity (DTH) responses and stimulating IgG2a antibody production by B cells in mice. Th2 cells produce IL-4, IL-5 and IL-10, which are crucial for IgG1 and IgE synthesis and immunity against helminthic parasites, thereby increasing allergic reactions and inhibiting macrophage activation and antigen presentation. Determining factors for differentiation into Th1 or Th2 effectors include the nature of the APCs, the nature and amount of antigen, the genetic background of the host and particularly the cytokine microenvironment; IL-12 is the major Th1- and IL-4 the major Th2-promoting cytokine (reviewed in Ref. [9]). Recently, other endogenous factors, such as progesterone, glucocorticoids and norepinephrine, have also been reported to favor Th2 differentiation (reviewed in Refs [10,11]).

The question of whether neuropeptides, such as VIP, affect Th1 and Th2 differentiation has only recently been addressed. Initial studies of the effects of VIP on T-cell cytokines showed apparent preferential inhibition of some that are predominantly Th1-type cytokines, such as IL-2, and enhancement of others derived from Th2 cells, such as IL-5 (reviewed in Ref. [7]). However, more complex and sometimes conflicting results were obtained regarding VIP effects on other T-cell cytokines, also considered markers for the Th1 and Th2 subsets, exemplified by IFN- $\gamma$  and IL-4, respectively [7,12]. Recent studies have shed some light on the matter, clearly demonstrating that VIP acts as an important endogenous T helper-differentiating factor, promoting Th2-type, and inhibiting Th1-type responses *in vivo* and *in vitro*, through several mechanisms.

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We reported previously that macrophages treated *in vitro* with VIP gain the ability to induce Th2-type cytokines (IL-4, IL-5) and inhibit Th1-type cytokines (IFN- $\gamma$ , IL-2) in antigen-primed CD4 T cells [13]. Similar results were obtained recently with bone-marrow-derived dendritic cells (DCs) [14]. In agreement with the *in vitro* results, *in vivo* administration of VIP in antigen-immunized mice results in a decreased number of IFN- $\gamma$ -producing cells (Th1) and an increased number of IL-4-secreting cells (Th2) [13].

Two recent studies from Voice *et al.* [15] and Goetzl *et al.* [16], using transgenic and deficient mice for the type II G protein coupled VIP receptor (VPAC2), have been crucial in confirming and extending the concept that VIP affects the Th1 and Th2 balance *in vivo*. Both type I G protein-coupled VIP receptor (VPAC1) and its homologous VPAC2 have been reported in different populations of T cells [7]. The expression of the two receptors appears to be differentially regulated; VPAC1 is highly expressed constitutively on T cells, especially Th cells, and VPAC2 is marginally expressed on unstimulated Th cells. VPAC2 is upregulated and VPAC1 downregulated during Th-cell stimulation (reviewed in Ref. [7]). This suggests that VPAC2 is the essential transducer of effects of VIP on activated Th cells. Transgenic mice with Th cells over-expressing VPAC2 showed a preferential deviation from normal CD4 T-cell cytokine secretion profiles toward Th2, with elevated blood IgG1, IgE and eosinophilia, and consequent decreased DTH and increased cutaneous allergic reactions [15]. By contrast, VPAC2-null mice exhibited increased Th1-type responses, characterized by increased DTH and decreased cutaneous anaphylaxis compared to the wild-type controls [16].

### Lymphocyte-derived VIP drives Th-cell differentiation

Research to date supports a relationship between VIP and Th2 cells, involving a positive feedback loop. Recently, we reported that Th2, but not Th1 effectors, express and secrete VIP following antigenic stimulation [17]. The physiological relevance of VIP production by Th2 effectors might reside in the fact that both endogenous and exogenous VIP promote Th2-type responses *in vivo*, implicating VIP in the Th2 amplification loop. The role of lymphocyte-derived VIP in the Th1–Th2 balance has been indirectly shown by two independent studies. Vassiliou and colleagues [18] reported the secretion of functional VIP by antigen-stimulated Th2 cells by determining the cAMP induction (the major VIP second messenger in T cells) in stable VPAC1 transfectants. Furthermore, VPAC2-null mice not only demonstrate the involvement of VPAC2 in the effect of VIP in Th2-type responses but they also indirectly show that endogenous VIP is implicated in such *in vivo* responses [16]. However, the definitive confirmation of the role of endogenous Th2-derived VIP in maintaining the Th2 bias has come recently from studies in which elimination of VIP from TCR-stimulated T cells from VPAC2 transgenic mice with VIPase IgG resulted in the readjustment of the Th1–Th2 balance, decreasing the secretion of IL-4 and IL-10, and increasing the production of IFN- $\gamma$  [1]. Although the differential contribution of co-existing

nerve- and lymphocyte-derived VIP during a local inflammatory process and during specific immune responses is difficult to evaluate, probably both VIP sources have a complementary role under such conditions. However, a negative loop has been described in murine schistosomiasis, in which IL-4 released by Th2 effectors of chronic inflammatory granulomas inhibits VIP expression in macrophages [19] and reduces VPAC2 expression in Th2 cells [20].

### Mechanisms by which VIP promotes Th2-type immune responses

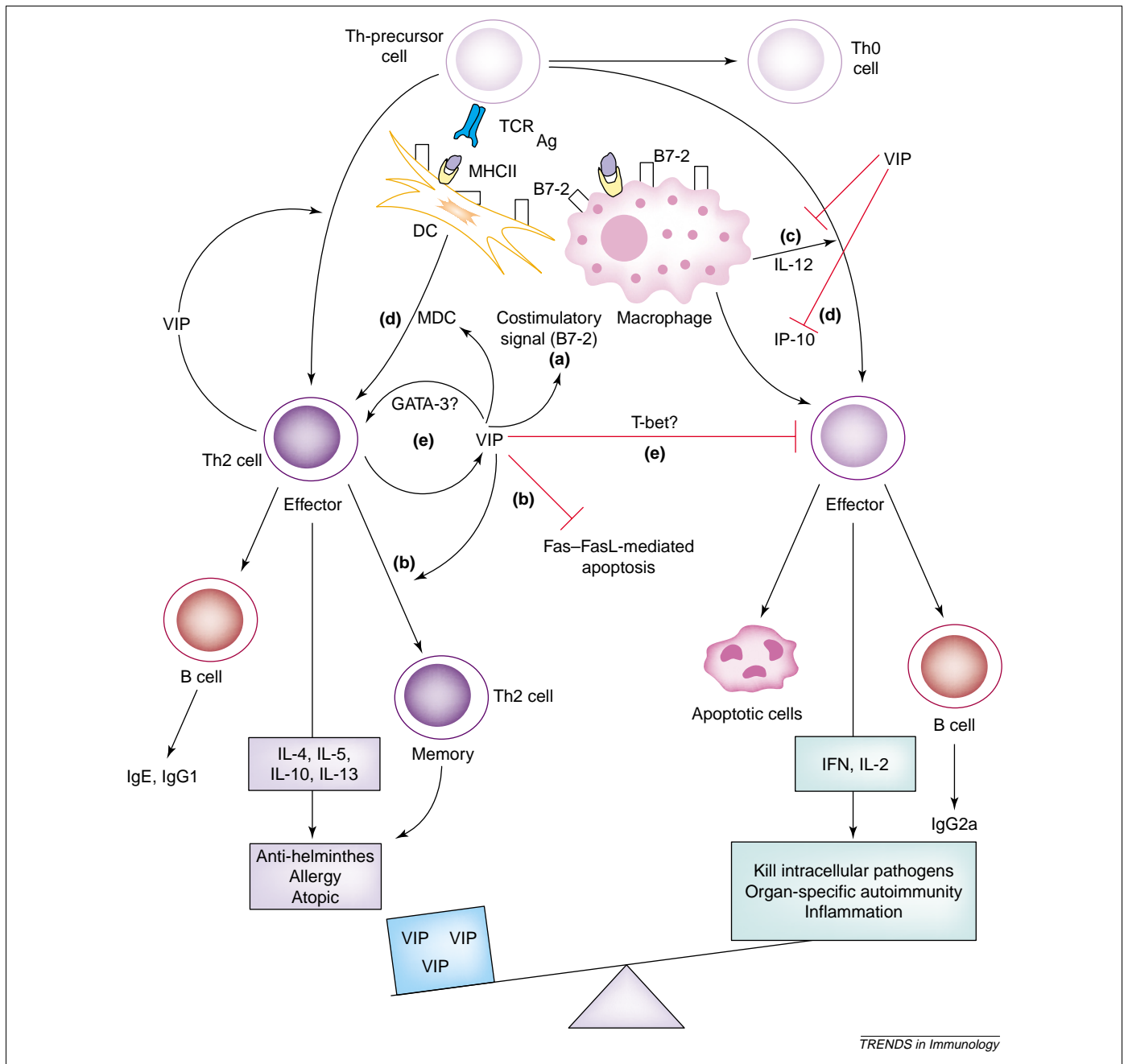
Several non-excluding mechanisms (Fig. 1) could contribute to the Th2 bias by VIP. The spectrum of VIP effects leading to Th2-cell predominance includes action at the level of Th1 and Th2 generation, either directly or through actions on APCs, and/or at the level of the already generated effectors, by selectively promoting Th2 proliferation, survival or accumulation.

There is evidence that VIP affects APCs in at least two ways that are relevant to the generation of Th1 and Th2 effectors. First, VIP inhibits IL-12 production from activated macrophages [21]. Because differentiation into Th1 cells is driven primarily by the availability of IL-12, the Th1–Th2 balance will be altered in favor of Th2 in the presence of sub-optimal levels of IL-12. Second, the presence of membrane-bound costimulatory factors, such as B7-1 and B7-2, on APCs seems to be significantly more important for the development of Th2, compared to Th1 cells [22]. We have recently demonstrated that VIP-induced B7-2 expression in resting macrophages and immature DCs is partially related to the preferential promotion of Th2-type responses [13,14].

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VIP could also promote Th2 development directly by acting on the differentiating CD4 T cells because VIP addition to TCR-transgenic T cells cultured with irradiated APCs and antigenic peptide, in the presence or absence of polarizing cytokines, leads to a Th2-type cytokine profile [14]. Whether VIP affects the expression of the master-switch transcription factors T-bet and GATA-3 (a zinc-finger protein), required for Th1 and Th2 differentiation, respectively, between others (i.e. c-maf or JunB), remains to be established.

In addition, VIP could act on the already generated Th1 and Th2 effectors. Recent *in vitro* and *in vivo* experiments indicate that VIP supports the survival, and possibly the proliferation of Th2, but not Th1 effectors [2]. The majority of Th1 and Th2 effectors suffer clonal deletion through apoptosis, mediated primarily through Fas ligand–Fas interactions, following a relatively short period of intense activity. The relatively few surviving T cells become antigen-specific long-lived memory cells, which differ



TRENDS in Immunology

**Fig. 1.** Preferential induction of Th2 responses by vasoactive intestinal peptide (VIP). The same Th-precursor cell can differentiate into Th0, Th1 or Th2 cells depending primarily on the cytokine microenvironment provided by dendritic cells (DCs) and other antigen-presenting cells (APCs). Interleukin-12 (IL-12) induces Th1 cells and IL-4 promotes Th2 cells. In addition to these cytokines, VIP released from antigen (Ag)-stimulated Th2 cells drives Th differentiation and generation acting on both CD4 T cells and APCs. (a) Action on APCs. VIP induces B7-2 expression on resting macrophages and DCs and promotes Th2-type responses. (b) Action on Th1–Th2 effectors. VIP promotes the preferential survival of Th2 versus Th1 effectors, by regulating Fas–FasL-mediated apoptosis, and generating Th2 memory cells. (c) Action on activated macrophages. VIP inhibits IL-12 production by activated macrophages, reducing differentiation into Th1 effectors. (d) Action on Th1- and Th2-specific chemokines: VIP inhibits and stimulates interferon- $\gamma$  (IFN- $\gamma$ )-induced protein-10 (IP-10) and macrophage-derived chemokine (MDC) production, respectively, and it favors Th2- versus Th1-chemoattraction to the site where antigen is being presented. (e) Direct effect on primed CD4 T cells: the possibility exists (indicated by a question mark) that VIP also affects Th1 and Th2 differentiation directly by acting on transcription factors, for example, GATA-3 and T-bet. Abbreviations: Ag, antigen; TCR, T-cell receptor.

from naïve T cells in terms of homing patterns and activation requirements. The phenotype of the CD4 T-cell population, whose survival is promoted by VIP, is typical of memory Th2-type (CD44<sup>high</sup>, L-selectin<sup>low</sup>, CD45RB<sup>low</sup> and IL-4 and IL-5 but not IFN- $\gamma$  or IL-2 producers) [2]. This effect is probably mediated through a prevention of apoptosis by VIP in activated Th2 but not Th1 effectors [2]. This is supported by the previously described protective effect of VIP against antigen-induced cell death (AICD) of activated

CD4 T cells both *in vitro* and *in vivo*, through the inhibition of Fas-ligand expression [23]. The reasons why VIP affects specifically Th2 cells are not clear. Whereas studies using VPAC2 transgenic T cells [13] and a murine schistosomiasis model [24] have suggested that differential expression of this receptor could be a plausible explanation, no differences between Th1 and Th2 effector cells in VIP receptor density have been found [2]. Therefore, the nature of transcriptional factors affecting anti-apoptotic molecules might be the answer.

## Recent *in vitro* and *in vivo* experiments indicate that VIP supports the survival, and possibly the proliferation of Th2, but not Th1 effectors.

Finally, VIP could affect the selective recruitment of Th effectors to an inflammatory or antigenic activation site, which is controlled mainly by the expression of chemokines and their receptors. Th1 and Th2 cells were reported to express different chemokine receptors, and therefore home differently [25]. Thus, Th1 and Th2 cells migrate in response to the chemokines IP-10 (IFN- $\gamma$ -induced protein-10, CXCL10) and MDC (macrophage-derived chemokine, CCL22), respectively. Both *in vitro* and *in vivo* studies have demonstrated recently that VIP stimulates MDC and inhibits IP-10 production by splenocytes and DCs, promoting specific recruitment of Th2 cells [14,26]. These results add a new dimension to VIP participation in the Th2 autoregulatory loop.

### Concluding remarks

These findings indicate that VIP, produced and secreted by Th2 cells following antigen stimulation, participates in a Th2 autoregulatory loop, in which it promotes Th2-type responses, through multiple non-excluding and probably interrelated mechanisms, including direct effects on differentiating CD4 T cells, indirect effects on APCs and modulation of proliferation, survival and recruitment of already generated T-cell effectors. From the point of a normal physiological role, the fact that VIP promotes the generation and long-term survival of Th2 cells is particularly relevant in view of the concept that immune privilege in organs, such as brain and eye, is an active process of immune deviation mediated by regulatory T cells generated in the presence of Th2-derived cytokines. From a pathological point of view, these recent studies open the possibility of using VIP and its analogs in the treatment of autoimmune diseases with a prevalent Th1 background, and partially explain the beneficial effect shown by VIP in models of rheumatoid arthritis and Crohn's disease [27,28].

Finally, I would like to introduce a question. If we define the term 'cytokine' as a protein secreted by leukocytes (and many other cells in numerous types of tissues) in response to tissue disease, injury or repair, which predominantly acts locally, in a paracrine or autocrine manner, with pleiotropic actions, including several effects in immune cells and modulation of inflammatory response, could VIP be considered a Th2-type cytokine in view of its characteristics? In fact, if Said and Mutt had initially described VIP in the immune system, instead of the small intestine, we would probably be talking about VIP as a cytokine (perhaps IL-X) instead of a neuropeptide.

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### References

- 1 Voice, J.K. *et al.* (2003) Role of vasoactive intestinal peptide (VIP) in the expression of different immune phenotypes by wild-type mice and T-cell-targeted type II VIP receptor transgenic mice. *J. Immunol.* 170, 308–314
- 2 Delgado, M. *et al.* (2002) VIP and PACAP promote *in vivo* generation of memory Th2 cells. *FASEB J.* 16, 1844–1846
- 3 Said, S.I. and Mutt, V. (1970) Polypeptide with broad biological activity: isolation from small intestine. *Science* 169, 1217–1218
- 4 Bellinger, D.L. *et al.* (1990) Neuropeptide innervation of lymphoid organs. *Ann. N. Y. Acad. Sci.* 594, 17–33
- 5 Gomariz, R.P. *et al.* (1994) Vasoactive intestinal peptide (VIP) mRNA expression in rat T and B lymphocytes. *Regul. Pept.* 50, 177–184
- 6 Martinez, C. *et al.* (1999) Regulation of VIP production and secretion by murine lymphocytes. *J. Neuroimmunol.* 93, 126–138
- 7 Gomariz, R.P. *et al.* (2001) Immunology of VIP: a review and therapeutic perspectives. *Curr. Pharm. Des.* 7, 89–111
- 8 Delgado, M. *et al.* (2002) Vasoactive intestinal peptide in the immune system: potential therapeutic role in inflammatory and autoimmune diseases. *J. Mol. Med.* 80, 16–24
- 9 Liew, F.Y. (2002) Th1 and Th2 cells: a historical perspective. *Nat. Rev. Immunol.* 2, 55–60
- 10 Elenkov, I.J. *et al.* (2000) The sympathetic nerve: an integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.* 52, 595–638
- 11 Miyaura, H. and Iwata, M. (2002) Direct and indirect inhibition of Th1 development by progesterone and glucocorticoids. *J. Immunol.* 168, 1087–1094
- 12 Jabrane-Ferrat, N. *et al.* (1999) Enhancement by vasoactive intestinal peptide of  $\gamma$ -interferon production by antigen-stimulated type 1 helper T cells. *FASEB J.* 13, 347–352
- 13 Delgado, M. *et al.* (1999) VIP and PACAP stimulate the induction of Th2 responses by upregulating B7.2 expression. *J. Immunol.* 163, 3629–3635
- 14 Ganea, D. *et al.* VIP and PACAP modulate innate and adaptive immunity. *Cell Mol. Biol.* (in press)
- 15 Voice, J.K. *et al.* (2001) Allergic diathesis in transgenic mice with constitutive T-cell expression of inducible vasoactive intestinal peptide receptor. *FASEB J.* 15, 2489–2496
- 16 Goetzl, E.J. *et al.* (2001) Enhanced delayed-type hypersensitivity and diminished immediate-type hypersensitivity in mice lacking the inducible VPAC2 receptor for vasoactive intestinal peptide. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13854–13859
- 17 Delgado, M. and Ganea, D. (2001) Is vasoactive intestinal peptide a type 2 cytokine? *J. Immunol.* 166, 2907–2912
- 18 Vassiliou, E. *et al.* (2001) Th2 lymphocytes secrete functional VIP upon antigen stimulation. *Arch. Phys. Biochem.* 109, 365–368
- 19 Metwali, A. *et al.* (2002) IL4 inhibits vasoactive intestinal peptide (VIP) production by macrophages in murine schistosomiasis. *Am. J. Physiol.* 283, G115–G121
- 20 Metwali, A. *et al.* (2000) IL-4 regulates VIP receptor subtype 2 mRNA expression in T cells in murine schistosomiasis. *FASEB J.* 14, 948–954
- 21 Delgado, M. *et al.* (1999) VIP and PACAP inhibit IL-12 production in LPS-stimulated macrophages. Subsequent effect on IFN $\gamma$  synthesis by T cells. *J. Neuroimmunol.* 96, 167–181
- 22 Ranger, A.M. *et al.* (1996) B7-2 (CD86) is essential for the development of IL-4 producing T cells. *Int. Immunol.* 8, 1549–1560
- 23 Delgado, M. and Ganea, D. (2000) VIP and PACAP inhibit antigen-induced apoptosis of mature T lymphocytes by inhibiting FasL expression. *J. Immunol.* 164, 1200–1210
- 24 Metwali, A. *et al.* (1996) T-cell receptor subtype expression differs between granulomas and spleen of schistosome infected mice. *J. Immunol.* 157, 265–270
- 25 Sallusto, F. *et al.* (1998) Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. *Immunol. Today* 19, 568–574
- 26 Jiang, X. *et al.* (2002) VIP and PACAP downregulate CXCL10 (IP-10) and upregulate CCL22 (MDC) in spleen cells. *J. Neuroimmunol.* 133, 81–94
- 27 Delgado, M. *et al.* (2001) Vasoactive intestinal peptide prevents experimental arthritis by downregulating both autoimmune and inflammatory components of the disease. *Nat. Med.* 7, 563–568
- 28 Abad, C. *et al.* Therapeutic effects of VIP in the trinitrobenzene sulfonic acid mice model of Crohn's disease. *Gastroenterology* (in press)