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A neuropeptide in immune-mediated inflammation, Y?

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Disturbances in crosstalk between the immune system and the sympathetic nervous system (SNS) can contribute to the pathogenesis of Th1-mediated autoimmunity. Recent studies indicate that neuropeptide Y (NPY) has a major role in the regulation of Th1 responses. The precise role of NPY has been an enigma, but a recent study provides a breakthrough, demonstrating that NPY has a bimodal role as a negative regulator of T cells and an activator of antigen-presenting cell function.

Neuropeptide Y – an interface between nervous and immune systems

Despite decades of evidence for bidirectional communication between the nervous system and the immune system [1,2], the fields of neuroscience and immunology have evolved separately. Alterations in the sympathetic nervous system (SNS) are associated with certain inflammatory autoimmune diseases, such as multiple sclerosis (MS) [3] and rheumatoid arthritis (RA) [4]. Catecholamines, the major category of sympathetic neurotransmitter that have been shown to modulate some T-cell responses, have been the focus of research in communication between the nervous system and the immune system. Postganglionic SNS nerve fibers terminate in primary and secondary lymphoid organs, and 'synapse-like' contacts between sympathetic nerve terminals and T or B cells have been identified. However, postganglionic nerves also secrete neuropeptides including neuropeptide Y (NPY), a 36-amino-acid peptide, which co-localizes with catecholamines. NPY participates in the regulation of multiple physiological processes, including vasoconstriction, energy balance, feeding and anxiety, all of which are mediated through five different NPY G-protein-coupled

receptors (Y1, Y2, Y4, Y5 and y6). These NPY receptors co-localize with NPY-producing nerve cells and are widely distributed in the central nervous system (CNS). NPY receptors are also expressed by many non-CNS cell types. Y1 receptors are detected on immune cells, including T cells, B cells, macrophages, dendritic cells, mast cells and natural killer cells, and signaling through the Y1 receptor is involved in the modulation of some T-cell responses (Figure 1) [5], chemotaxis and endothelial adhesion [6]. However, the role of NPY-Y1 signaling in inflammation has been controversial. Treatment with NPY and NPY agonists ameliorates murine experimental autoimmune encephalomyelitis (EAE) [7], a Th1-mediated model for MS [8], and is associated with the suppression of myelin-specific Th1 responses. By contrast, deficiency of the NPY-Y1 receptor protects mice against colitis [9], suggesting a pro-inflammatory role for signaling through the NPY receptor. Wheway *et al.*'s [10] observation that NPY has a bimodal role, acting on both T cells and antigen-presenting cells (APCs) in an opposing manner, accounts for these apparently contradictory results.

NPY-deficiency promotes Th1-mediated responses

Several immunomodulatory properties are attributed to Y1 signaling [7]. Wheway *et al.* [10] observed that Y1-deficient mice had reduced numbers of B cells, a defect in isotype switching to IgG2a (normally associated with Th1 responses) and increased numbers of naïve T cells, confirming the importance of Y1 in immune functions. Indeed, they observed that Y1-deficient mice were resistant to dextran sulfate sodium (DSS)-induced colitis and developed reduced delayed-type hypersensitivity (DTH) to methylated bovine serum albumin (mBSA), which are two models of Th1-mediated inflammation. Although there was no modulation of interleukin (IL)-4 production, the altered phenotypes were associated with

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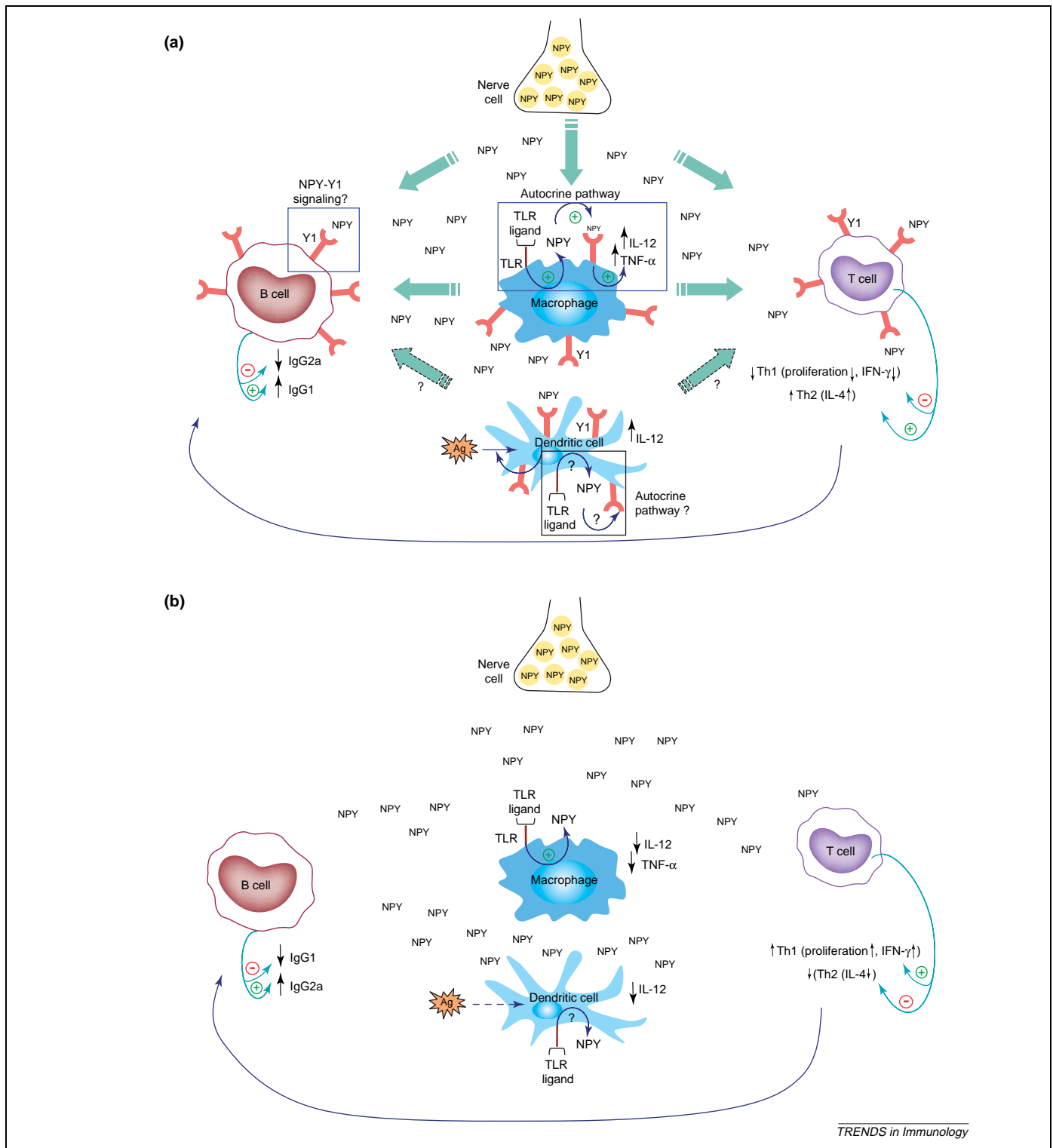


Figure 1. Hypothetical models of immune regulation in the presence or absence of Y1 signaling. **(a)** Under physiological conditions, NPY is secreted either by sympathetic postganglionic nerve fibers or by activated macrophages and binds to Y1 receptors expressed on B cells, T cells, DCs and macrophages. Although nerves secrete NPY, data indicate that immune cells (e.g. macrophages) can be the major source of NPY in inflammation. The NPY autocrine stimulation pathway on macrophages identified by Whewey *et al.* [10] provided new insights into the role of NPY in innate immunity. Stimulation of TLRs led to increased secretion of NPY that was responsible for the subsequent production of the pro-inflammatory molecules IL-12 and TNF- α . Interestingly, a role for TLRs has been described in diseases such as asthma, a Th2 allergic inflammatory condition in which NPY has been described to be upregulated [13]. Following TLR stimulation (e.g. by LPS, a component of the bacterial cell wall), macrophages secrete NPY, which binds to Y1 receptors expressed at their cell surfaces and stimulates the surrounding cells. As a consequence of this autocrine stimulation, Th1-polarizing cytokines (IL-12 and TNF- α) are secreted by normal (Y1-expressing) macrophages. Y1 signaling on DCs promotes increased antigen uptake and IL-12 secretion. TLR stimulation is involved in DC maturation. However, it is unknown whether NPY secretion occurs after such stimulation. Although NPY stimulates APCs to secrete pro-inflammatory cytokines, it seems paradoxical that Y1 signaling on T cells promotes Th2 responses by simultaneously increasing IL-4 production and inhibiting IFN- γ secretion. Consistent with this Th2 shift, B cells preferentially secrete IgG1 but not IgG2a. It is currently unclear whether this preferential secretion of IgG1 is a direct consequence of Y1 signaling on B cells or occurs in response to Th2 cytokines. Block arrows indicate cell-cell communication through NPY and Y1 signaling. **(b)** In Y1-deficient mice or in the absence of NPY signaling, T cells become preferentially Th1 polarized, potentiating isotype switching in B cells to IgG2a. APC activation is decreased, antigen uptake is impaired and secretion of IL-12 and TNF- α is reduced in APCs lacking Y1 receptors.

decreased levels of interferon (IFN)- γ , indicating a role for Y1 signaling in Th1 induction. However, these results apparently contradicted the previous studies that reported mice treated with NPY or other Y1 agonists were protected against EAE [7] and DSS-induced colitis [10]. Using their analysis of Y1-deficient mice, Wheway *et al.* were the first to resolve these discrepancies.

The bimodal role of NPY on T-cell regulation and APC activation

Wheway *et al.* [10] observed that Y1-deficient T cells *in vitro* showed hyperproliferation in response to anti-CD3 stimulation. In mixed leukocyte reactions (MLRs), NPY addition suppressed proliferation by wild-type T cells. Although previous data have indicated that Y1 is the principal receptor for NPY-mediated T-cell activity, testing NPY on Y1-deficient T cells (a negative control if NPY activity on T cells is mediated only through Y1) could have addressed whether NPY influences T-cell activation through other NPY receptors. In contrast to wild-type T cells, Y1-deficient T cells induced more rapid and severe colitis when adoptively transferred into mice deficient in mature T cells [recombination-activating-gene-1-deficient (RAG1^{-/-}) mice] and were associated with increased interferon (IFN)- γ secretion. These observations suggest that Y1-deficient T cells are not defective in Th1 polarization. Why were Y1-deficient mice resistant to Th1-mediated inflammatory conditions? Because APCs in RAG1-deficient mice expressed normal levels of Y1 and Y1-deficient T cells generated stronger responses when they were stimulated with wild-type APCs than with Y1-deficient APCs, the authors hypothesized that NPY has a stimulatory role on APCs. They showed that NPY signaling affected cytokine secretion and antigen presentation by APCs. Y1-deficient macrophages and dendritic cells (DCs) secreted less IL-12 and tumor necrosis factor α (TNF- α) than wild-type APCs, accounting for the defect in Th1 polarization observed in Y1-deficient mice. They showed that IL-12 production was dependent on NPY autocrine stimulation and potentiated Toll-like receptor (TLR)-dependent activation of APCs. When Y1-deficient T cells were transferred into RAG1-deficient hosts or RAG1-deficient mice lacking Y1 (RAG1^{-/-}, Y1^{-/-}), colitis was observed only in Y1-bearing recipients. In addition, antigen-pulsed wild-type DCs, but not antigen-pulsed Y1-deficient DCs, elicited antigen-specific T-cell responses when adoptively transferred into wild-type recipient mice. Finally, using reciprocal bone marrow chimeras constructed from wild-type and Y1-deficient mice, the authors demonstrated that these impaired Th1 responses were due solely to a defect in APC function in the immune system and not due to a lack of Y1-receptor expression in the peripheral or central nervous system.

Perspectives

NPY-Y1 is one of an increasing number of molecular pathways initially characterized in the nervous system that were subsequently discovered to interface with the immune system and participate in immune regulation [1,2]. Semaphorins, originally identified as guidance molecules for axonal development, are now recognized as

molecules involved in T-cell activation. The semaphorin Sema4A, expressed on APCs, participates in the immunological synapse, serving as a co-stimulatory molecule during Th1 differentiation [11]. As in Sema4A-deficient mice, T-cell responses are also impaired in Y1-deficient mice. Although the observation that NPY stimulates APCs is novel, further investigations are necessary to determine whether Y1-signaling on APCs influences the expression of molecules involved in the immunologic synapse, for example, MHC class II and co-stimulatory molecules.

The opposing activity of NPY on APCs and T-cell function seems problematic. However, endogenous NPY might have a key role in fine-tuning immune responses. By inhibiting T-cell activation, excess NPY might reduce immune surveillance and increase the risk of infections and cancer [12], effects that might be counterbalanced by increased APC activation. Conversely, the inhibition of T-cell activation by excess NPY might represent a means for dampening the adverse effects of overstimulated APCs in the maintenance of self-tolerance.

It is unclear whether immune cells or nervous system cells are the predominant source of NPY in inflammatory conditions. Wheway *et al.* demonstrated that Y1-receptor expression on immune cells has a key role in inflammation. Data suggest that immune cells are a major source of NPY secretion in certain chronic inflammatory conditions. Interestingly, the NPY autocrine stimulation pathway on macrophages identified by Wheway *et al.* provides insights into the role of NPY secretion in innate immune responses. Stimulation of APCs through TLRs promotes secretion of NPY (Figure 1). Innate stimuli acting through TLRs are thought to contribute to the pathogenesis of several chronic inflammatory diseases. In particular, elevated serum levels of NPY have been detected in the serum of patients with asthma and systemic lupus erythematosus [13,14], two inflammatory diseases associated with Th2 immunity. These findings raise the possibility that innate stimuli might promote NPY secretion by APCs in these and other inflammatory conditions but further investigation is required.

Concluding remarks

The identification of the proinflammatory effects of NPY on APCs might reduce interest in the potential application of NPY and NPY analogs in the treatment of Th1-mediated autoimmune conditions, for example, MS and RA. In this regard, although NPY prevented the induction of acute EAE by inhibiting Th1 responses, it could not reverse chronic EAE, a phase of the disease in which activated macrophages might predominate in CNS damage [15]. In addition, the findings that Y1-deficient mice (lacking Y1-receptor expression on both T cells and APCs) were resistant to inflammatory disease and exhibited reduced Th1 responses could indicate that the impact of Y1-deficiency on APCs is dominant, an aspect that will require further investigations in different inflammatory conditions.

Wheway *et al.* provide a novel insight into the bimodal role of NPY in immune responses: promoting APC activation and downregulating Th1 responses. Their

observations will undoubtedly inspire further investigations of the influence of NPY on APC function in neuroimmune communication and various inflammatory conditions.

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Letter

EAE: pitfalls outweigh virtues of screening potential treatments for multiple sclerosis

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I very much enjoyed the November, 2005 *TI* special issue ‘Disease models of autoimmunity’, which contained stimulating articles providing informed and useful scientific dialog. In the spirit of the Editor’s introduction [1], suggesting that the contributions could provide a ‘forum for debate’, I wish to address some issues raised by Steinman and Zamvil’s article that discussed the use of experimental autoimmune encephalomyelitis (EAE) for the development of therapies for multiple sclerosis (MS) [2]. The article provided a masterful introduction to the pathogenesis of EAE and constructive commentary about many aspects of MS drug toxicity that are not addressed by EAE experiments.

However, Steinman and Zamvil did not address the largely unrecognized pitfall of using EAE to screen for MS drugs. The authors described how new drugs can emerge from screening in EAE-affected animals but they omitted to mention the numerous potential treatments that were

rejected because they failed pharmaceutical company in-house- or contract EAE-screening and never reached clinical trials.

Steinman and Zamvil argue that ‘EAE has been a remarkably valuable model, despite its many drawbacks’ [2]. In science, as in political polling, the answer to a question is defined mainly by its detailed wording. If the question is ‘can EAE be used to explore mechanisms of autoimmunity and neuroinflammation, and will insights from this research contribute to effective treatment?’, the answer is unequivocally ‘yes’.

However, an equally important question is ‘does EAE form part of the critical path of drug development for MS?’ I suggest that the answer is ‘no’. In the process of drug development, relevant issues include: (i) whether it is necessary to conduct EAE-testing for a promising new compound that appears safe in humans, has favorable pharmacokinetics and targets a molecule likely to be important in MS, before proceeding to clinical trials; (ii) which EAE model should be used; and (iii) if the EAE test results are unpromising, should the drug program be abandoned?

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