

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article was published in an Elsevier journal. The attached copy is furnished to the author for non-commercial research and education use, including for instruction at the author's institution, sharing with colleagues and providing to institution administration.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



The emergence of neurotransmitters as immune modulators

Rafael Franco¹, Rodrigo Pacheco², Carmen Lluís¹, Gerard P. Ahern³ and Peta J. O'Connell⁴

¹ Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and Department of Biochemistry and Molecular Biology, Faculty of Biology, Diagonal 645, University of Barcelona, 08028 Barcelona, Spain

² Millennium Nucleus on Immunology and Immunotherapy, Department of Microbiology and Molecular Genetics, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Alameda 340, Santiago E-8331010, Chile

³ Georgetown University, 3900 Reservoir Rd, Washington DC 20007, USA

⁴ Robarts Research Institute and the Department of Anatomy and Cell Biology, University of Western Ontario, London, Ontario, Canada

Initially, the idea that neurotransmitters could serve as immunomodulators emerged with the discovery that their release and diffusion from nervous tissue could lead to signaling through lymphocyte cell-surface receptors and the modulation of immune function. It is now evident that neurotransmitters can also be released from leukocytes and act as autocrine or paracrine modulators. Here, we review the data indicating that leukocytes synthesize and release 'neurotransmitters' and we also discuss the diverse effects that these compounds exert in a variety of immune cells. The role of neurotransmitters in immune-related diseases is also reviewed succinctly. Current and future developments in understanding the cross-talk between the immune and nervous systems will probably identify new avenues for treating immune-mediated diseases using agonists or antagonists of neurotransmitter receptors.

Cross-talk between the nervous system and the immune system

Cytokines and other molecules released in the central nervous system (CNS) by activated cells of the immune system can influence neurotransmission [1]. It now seems that neurotransmitters also exert a considerable and reciprocal influence on the function of the immune system. Leukocytes express receptors for the main brain neurotransmitters, such as glutamate, dopamine, acetylcholine (ACh) and serotonin (5-HT), providing strong evidence for their role as immune modifiers. Exploring the direct mechanisms by which the immune and the nervous systems communicate holds great promise, not only for understanding how the entire organism operates in health, but also for developing novel therapeutic strategies for both neurological and immune-mediated diseases.

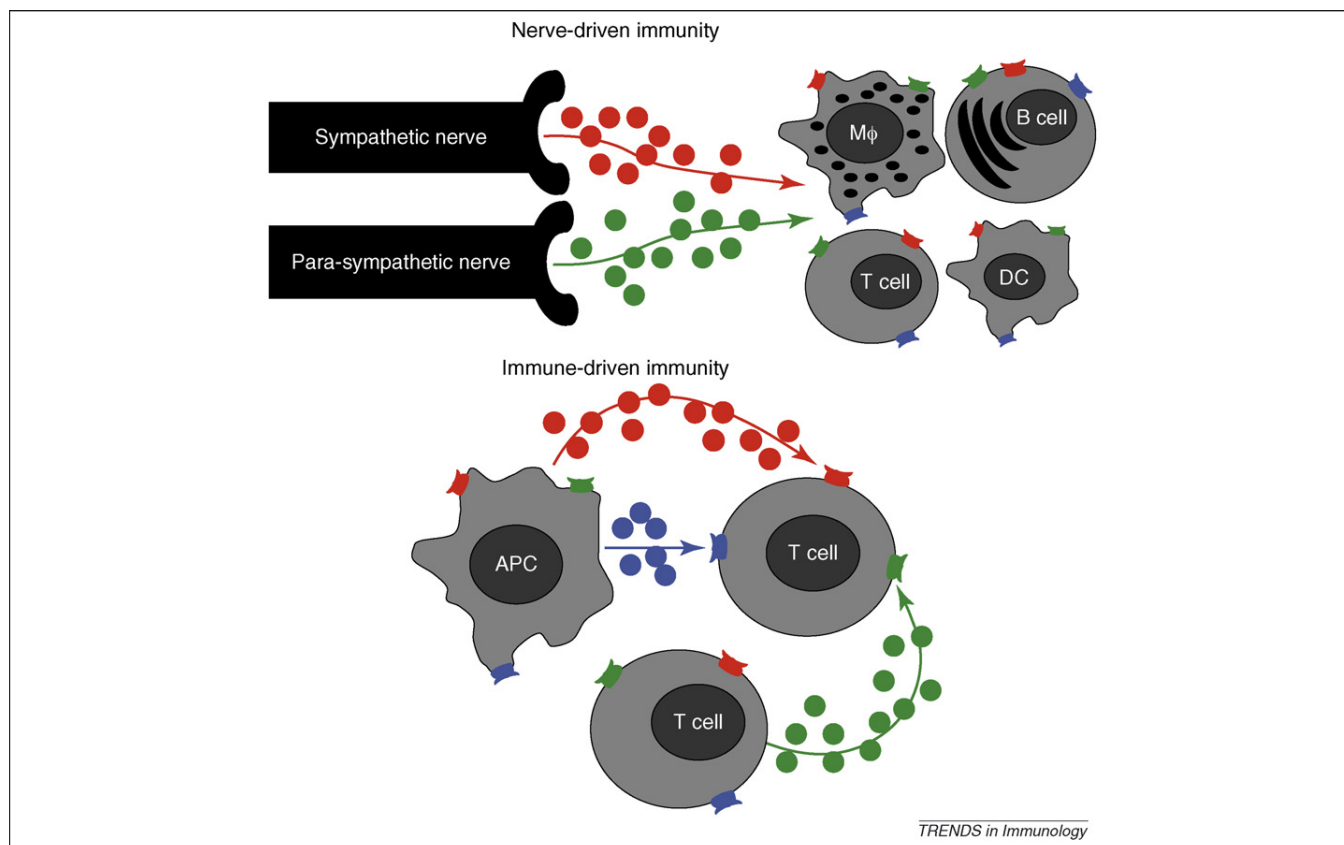
Neurotransmitters derived from sources outside of the immune system, for example, from gut enterochromaffin cells or autonomic nerves that innervate lymphoid organs, can initiate or modify leukocyte signal transduction directly (Figure 1). In addition, the brain can

influence immune function powerfully through the hypothalamic–pituitary–adrenal axis and the release of corticosteroids [2]. Consistent with this, immune competence is impaired in hypophysectomized (surgical removal of the pituitary gland) animals and restored by administration of growth hormone and prolactin, which both have potent immunoregulatory effects [3,4]. The neurotransmitters dopamine and glutamate interact directly with T-cell-expressed receptors, leading to the activation or suppression of various T-cell functions, including cytokine secretion, proliferation and integrin-mediated adhesion and migration (reviewed in [5–9]). Corticosteroids released from sympathetic nerves or the adrenal gland have profound immunosuppressive effects on the lymphoreticular system, inhibiting many effector functions of lymphocytes and macrophages and disrupting their trafficking patterns [10]. Elevated plasma norepinephrine and epinephrine concentrations that accompany stress produce changes in lymphocyte and monocyte function [11]. Interestingly, there are exceptions to this general trend and a good example is given by histamine; although a CNS neurotransmitter, histamine is released mainly by mast cells in the periphery (not by nerves) and has a fundamental role in innate immunity. In this review, we consider recent studies showing that classical neurotransmitters are synthesized and released by lymphocytes and antigen-presenting cells (APCs) and can signal changes in immune function (immune-driven immunity, Figure 1) directly.

A new paradigm: leukocytes produce and/or release neurotransmitters

Several recent studies have revealed that key players in the generation of adaptive immune responses, dendritic cells (DCs) and lymphocytes, are capable of synthesizing and/or releasing classical neurotransmitters, including ACh, dopamine, 5-HT and glutamate. Fujii *et al.* provided the first evidence for ACh synthesis in T cells [12]. CD4⁺ T cells contain substantially more ACh compared with CD8⁺ T cells or B cells [13,14] and mitogens increase both the synthesis and release of ACh from lymphocytes [13]. These studies demonstrate that lymphocytes possess the essential components for a non-neuronal cholinergic system and

Corresponding author: Franco, R. (rfranco@ub.edu).
Available online 6 August 2007.



TRENDS in Immunology

Figure 1. Nerve-driven and immune-driven immunity outside of the CNS. Colored solid circles (red and green) represent neurotransmitters diffusing to the same colored (cognate) receptors on the leukocytes. Green and red arrows represent volume transmission, that is, diffusion from the site of release to the receptor present in the target immune cell. In immune-driven immunity, the blue arrow represents wiring transmission, that is, quick activation of the receptor on the target cell located close to the site of release at the immunological synapse. Neurotransmitters discussed in this review can be divided largely into two groups on the basis of stability and mode of termination of their actions under physiological conditions. The first comprises enzymatically and physicochemically stable compounds, such as catecholamines, glutamate and 5-HT (red and green circles). Substantial amounts of these neurotransmitters can be detected in the extracellular fluids, including plasma, and their role as neurotransmitters is terminated mainly by re-uptake into nerve terminals and tissues. For these neurotransmitters, humoral transmission, as well as wiring and volume transmission, for modulating immune function would be suspected. The second group includes labile compounds, such as ACh (blue circles). Duration of ACh action on endplate potential at neuromuscular junctions lasts less than several milliseconds owing to its rapid enzymatic degradation. Very low ACh concentration is detected in plasma (pg/ml range) and it seems more likely that ACh acts primarily by wiring transmission (blue arrow) between closely interacting leukocytes, although this is as yet unproven.

that immune function is, at least in part, under the control of this pathway [14].

To many, 5-HT is primarily a mood-altering transmitter present in the brain, yet high concentrations of 5-HT are also found in platelets and its release regulates their aggregation [15]. Recent studies suggest that 5-HT might also be important for T-cell function [16]. Murine splenic T cells selectively express type 1 tryptophan hydroxylase (TPH), which is the enzyme that catalyses the conversion of L-tryptophan to 5-hydroxytryptophan, the immediate precursor of 5-HT. Accordingly, T cells can produce 5-HT and this capacity is increased considerably following activation. In naïve T cells, 5-HT appears to signal primarily through 5-HT₇ receptors, enhancing early events in T-cell activation and proliferation, including phosphorylation of ERK1/2 and NF- κ B [9]. By contrast, signaling through type 2 receptors can promote T-cell effector functions, including cytokine release and delayed-type hypersensitivity reactions [17,18]. These results suggest that 5-HT might act as an autocrine factor to enhance T-cell activation and function.

Professional APCs, such as DCs that can activate naïve T cells, provide another source of 5-HT. DCs do not synthesize 5-HT; rather, they express the 5-HT transporter (SERT) and

can therefore sequester microenvironmental 5-HT efficiently [19]. Sequestered 5-HT is released through Ca²⁺-dependent exocytosis. SERT expression by DCs is regulated dynamically [19]. DCs appear to maximize their uptake and storage capacity of 5-HT on maturation or activation, before productive encounters with naïve T cells. By contrast, ligation of B7 receptors on DCs by the inhibitory T-cell ligand CTLA-4 down-regulates SERT expression in DCs [19]. Thus, the differential 5-HT content of DCs might contribute to altered signaling at DC–T-cell synapses.

Another immune transmitter is glutamate. In contrast to 5-HT, DCs appear to synthesize glutamate and release it in a nonvesicular manner, through the cystine/glutamate antiporter (X_c⁻ system) [20]. Significantly, glutamate release from DCs is enhanced with DC maturation and activation; it is not clear, however, whether this reflects an increase in glutamate synthesis or in expression of transporters. Released glutamate might signal through metabotropic glutamate receptors (mGluR1 and mGluR5) expressed by T cells, which is consistent with the T-cell stimulatory function of DCs and observations that T cells produce minimal glutamate [20]. Indeed, the proliferation of T cells co-cultured with DCs is impaired when the X_c⁻

system is inhibited [20]. Interestingly, mGluRs have a low affinity for glutamate, suggesting that this form of communication will be optimized in the restricted space (~30 nm) at DC–T-cell synapses, which might enable high glutamate concentrations. These observations support the provocative hypothesis that glutamate, the major excitatory transmitter in the brain, also participates in communication at immune synapses.

Pioneering studies by Bergquist and colleagues demonstrated that lymphocytes synthesize dopamine and norepinephrine [21]. Single-cell capillary electrophoresis was used to quantify catecholamine and its regulation by mitogens or superantigen. More recently, Rajda *et al.* have reported the measurement of the intracellular concentration of these compounds in treated and untreated multiple sclerosis patients [22]. Catecholamines were reported generally as immunosuppressive and they down-regulate proliferation and differentiation but increase apoptosis. Additionally, Cosentino *et al.* have shown that regulatory T cells express the catecholergic synthetic enzyme tyrosine hydroxylase constitutively [23]. This might serve as an autocrine mechanism to control T regulatory-cell activity because the release of catecholamines inhibits their cytokine production and capacity to suppress effector T cells.

Signaling through neurotransmitter receptors in the immune system

In neuronal tissue, signal transduction is controlled by the spatial and temporal regulation of transmitter release into the synaptic cleft, and signaling is terminated by diffusion, rapid and selective re-uptake or by degradation. Many similarities are evident in the immune system. In addition to the synthesis and/or release of neurotransmitters, many leukocytes express a plethora of functional receptors. Thus, it seems likely that neurotransmitters can alter immune function by autocrine signaling. In addition, the synapse formed between APCs and cognate lymphocytes to concentrate signaling molecules and stabilize interactions [24] will facilitate paracrine communication. The release of neurotransmitters from sympathetic or parasympathetic innervation of secondary lymphoid tissues can also drive or modulate the physiology of leukocytes (Figures 1,2).

One paradigmatic difference in neurotransmitter signaling between neurons and leukocytes is owing to receptor coupling with alternate signaling pathways. For instance, mGluR5 is coupled to the stimulation of adenylate cyclase in T cells [25] whereas, in neurons, it is usually coupled to phospholipase C (PLC) [26]. Another example is provided by β_2 -adrenergic receptors (ARs), which are

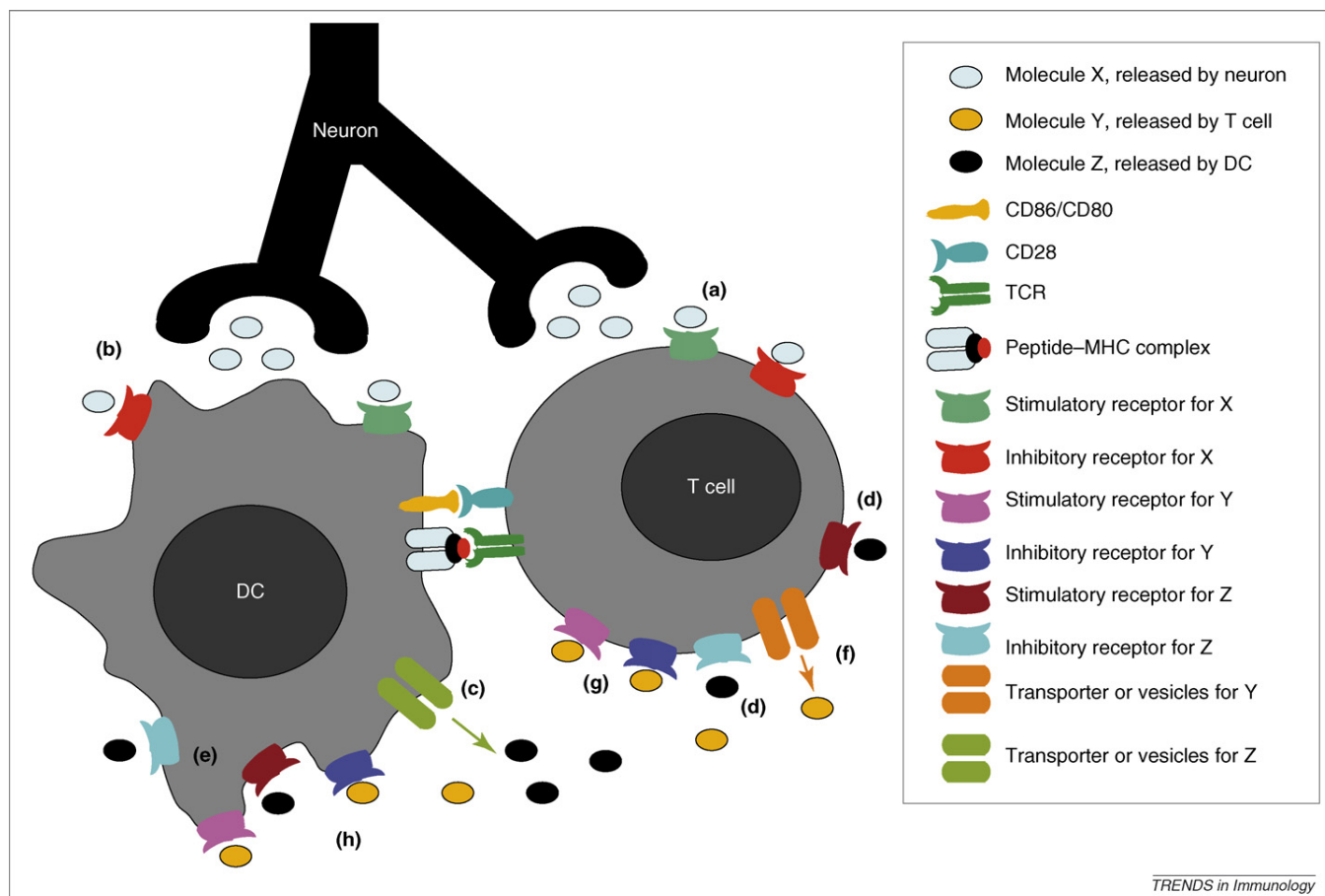


Figure 2. Proposed modulation of T-cell-mediated immunity by neuro-immunomodulators. A general mechanism is proposed in which nerve terminals might release neurotransmitters (molecule X) in the T-cell-rich areas of lymphoid tissues (a,b). Neurotransmitter X might trigger stimulatory or inhibitory receptors expressed on the (a) T-cell or (b) DC surface, thereby modulating T-cell activation and antigen presentation, respectively. During synaptic interaction, DCs begin to release neuro-immunomodulators (molecule Z) by a transporter-mediated mechanism or by vesicular exocytosis (c). Neurotransmitter Z might in turn activate stimulatory or inhibitory receptors expressed on the T-cell surface (d), modulating T-cell activation or T-cell fate decisions. Alternatively, molecule Z might act in an autocrine way, stimulating positive or negative signals in DCs, depending on the pattern of specific receptors expressed for molecule Z on these cells (e). Similar to DCs, once T cells are activated, they begin to release neurotransmitters (molecule Y) (f). Neurotransmitter Y might modulate (g) T-cell activation as well as (h) DC function positively or negatively.

coupled to the Gs/cAMP/protein kinase (PK)A-pathway in the nervous system, whereas it can also be coupled to the Gq/PLC- β /Ca²⁺/PKC-pathway in B cells [27,28]. A third example is the NK-1 receptor for substance P; although it is coupled to PLC/Ca²⁺/PKC in the CNS [29], its stimulation in macrophages or DCs can trigger activation of NF- κ B in a calcium-independent fashion [30]. It should be also noted that one of the key mediators of neurotransmitter effects, the voltage-gated calcium channels (Cav), behaves differently in neurons and in T cells. Depolarization appears to have a minor role in Cav-channel opening in T cells thereby indicating novel regulation and gating mechanisms [31].

With regard to the global modulation of leukocytes by the stimulation of dedicated neurotransmitter receptors, there are general trends defined by the signaling-pathways coupled to these receptors. Receptors coupled to increasing cAMP promote the inhibition or suppression of the immune response and, in some instances, it might be involved in polarization towards Th2 responses. Thus, stimulation of mGluR5 [20,25], vasoactive intestinal peptide receptor (VIP1) [32], calcitonin gene-related peptide receptor (CGRP1) [5,32] and dopamine receptors 1/5 (D1/5) [33] inhibits T-cell activation. T-cell signaling through the neuropeptide Y receptor 1 (Y1) induces polarization towards Th2 responses [5,34]. Activation of β_2 -AR inhibits the development of Th1 responses and promotes the production of suppressor cytokines, including interleukin (IL)-10 and transforming growth factor (TGF)- β [35]. By contrast, signaling through neurotransmitter receptors coupled to the inhibition of adenylate cyclase (somatostatin sst2/3 receptors, D2/D3 receptors or 5-HT₇ receptors) in T and B lymphocytes often has an immune-stimulatory effect [9,16,19,36]. However, neurotransmitter receptors coupled to the PLC/IP₃⁺ diacyl glycerol (DAG)/PKC⁺ Ca²⁺ pathway and mitogen-activated protein kinase (MAPK) activation induce positive modulation in the function of the target cell. For example, the stimulation of neurokinin (NK)-1 provides a co-stimulatory signal that enhances Th1 activation, augments NK-cell cytotoxic activity and enhances neutrophil and eosinophil function (including cytokine release, degranulation and chemotaxis) [37].

Unlike many cells that are responsive to neurotransmitters, leukocytes can exhibit complex life-cycles that can include maturation, activation and, in some cases, a return to quiescence. Concomitant with life-cycle progression, leukocytes can also modulate their capacity for the synthesis and release of neurotransmitters or their sensitivity to neurotransmitter signal transduction through differential expression of cognate receptors. The latter is exemplified by T-cell expression of glutamate receptors. Resting T cells express mGluR5 and the ionotropic receptor iGluR3, which induce increases in cAMP and intracellular Ca²⁺/Na⁺/K⁺, respectively. Thus, mGluR5 stimulation inhibits T-cell activation, whereas stimulation of iGluR3 is involved in adhesion and chemotactic migration [1]. Following activation, T cells express the inducible mGluR1 and release the serine protease granzyme B, which inactivates iGluR3 [35]. Under such conditions, stimulation of mGluR1 overcomes the effect of mGluR5 signaling by an ERK1/2-dependent mechanism and leads to Th1 polarization and the production of

proinflammatory cytokines [1,25]. In this way, glutamate inhibits activation and leads to a pattern of chemotactic migration in resting, patrolling T cells, whereas it provides a co-stimulatory signal in T cells primed for activation (Figure 3). Activation of dopamine D_{2/3} receptors inhibits cAMP production and enhances the intracellular Ca²⁺ concentration ([Ca²⁺]_i), thereby stimulating chemotaxis, adhesion and the production of tumor necrosis factor (TNF)- α and IL-10. Conversely, signaling via D_{1/5} receptors increases cAMP and impairs the proliferation and function of cytotoxic CD8⁺ T cells [6,9,33]. Of note, distinct receptors have different affinities for dopamine and the exact circumstances in which dopamine receptor subtypes are expressed on the T-cell surface are not yet well established. Similarly, there is also evidence for maturation-dependent responsiveness to ACh. In peripheral tissues, the release of ACh from the efferent vagal nerve can terminate macrophage activation and proinflammatory activity [38,39]. Signaling through α 7 nicotinic (n) ACh receptors (ligand-gated ion channels) leads to Janus kinase 2 (JAK2)/Signal transducer and activator of transcription 3 (STAT3)-dependent inhibition of NF- κ B and concomitant suppression of proinflammatory molecules, including TNF- α , IL-6, High-mobility group box 1 (HMGB1) and macrophage inflammatory protein (MIP)-2 [39,40]. Conversely, monocytes are relatively refractory to anti-inflammatory signaling from the cholinergic pathway [38,39]. Signaling through T-cell-expressed muscarinic (m) and nACh receptors leads to increased [Ca²⁺]_i and the induction of gene expression that is required for activation, IL-2 synthesis and proliferation [41]. Typically, activation occurs in the paracortex of lymph nodes or the periarteriolar lymphoid sheath of the spleen, wherein T cells are clustered densely. Because ACh signaling is terminated rapidly through degradation by cholinesterases that are abundant in plasma and tissues, this intimate environment and juxtaposition of T cells would promote both autocrine and paracrine signaling.

Role of neurotransmitters in immune-related diseases

Disruption of the complex interactions between the nervous and immune systems, including the altered release of neurotransmitters, receptor expression or signal transduction, contributes to the pathogenesis of inflammatory and immune-mediated diseases. Cholinergic signaling provides anti-inflammatory feedback to leukocytes expressing cholinergic receptors, however, dysregulation of ACh signaling is associated with both hypo- and hyper-immune dysfunction. For example, myasthenia gravis is a prototypical autoimmune disease characterized by antibody-mediated blockade of nACh receptors, decreased receptor expression and the induction of ACh receptor-specific CD4⁺ effector T cells [42]. Similarly, it is thought that deregulation of autonomic anti-inflammatory signaling might be a contributory pathogenic factor in diabetes, rheumatoid arthritis, systemic lupus erythematosus and Crohn's disease [43]. Insufficient cholinergic signaling might lead to uncontrolled, chronic inflammation. Consistent with this, α 7nACh-receptor-deficient mice show increased sensitivity to systemic inflammation and endotoxemia [44,45]. Conversely, chronic cholinergic stimulation inhibits

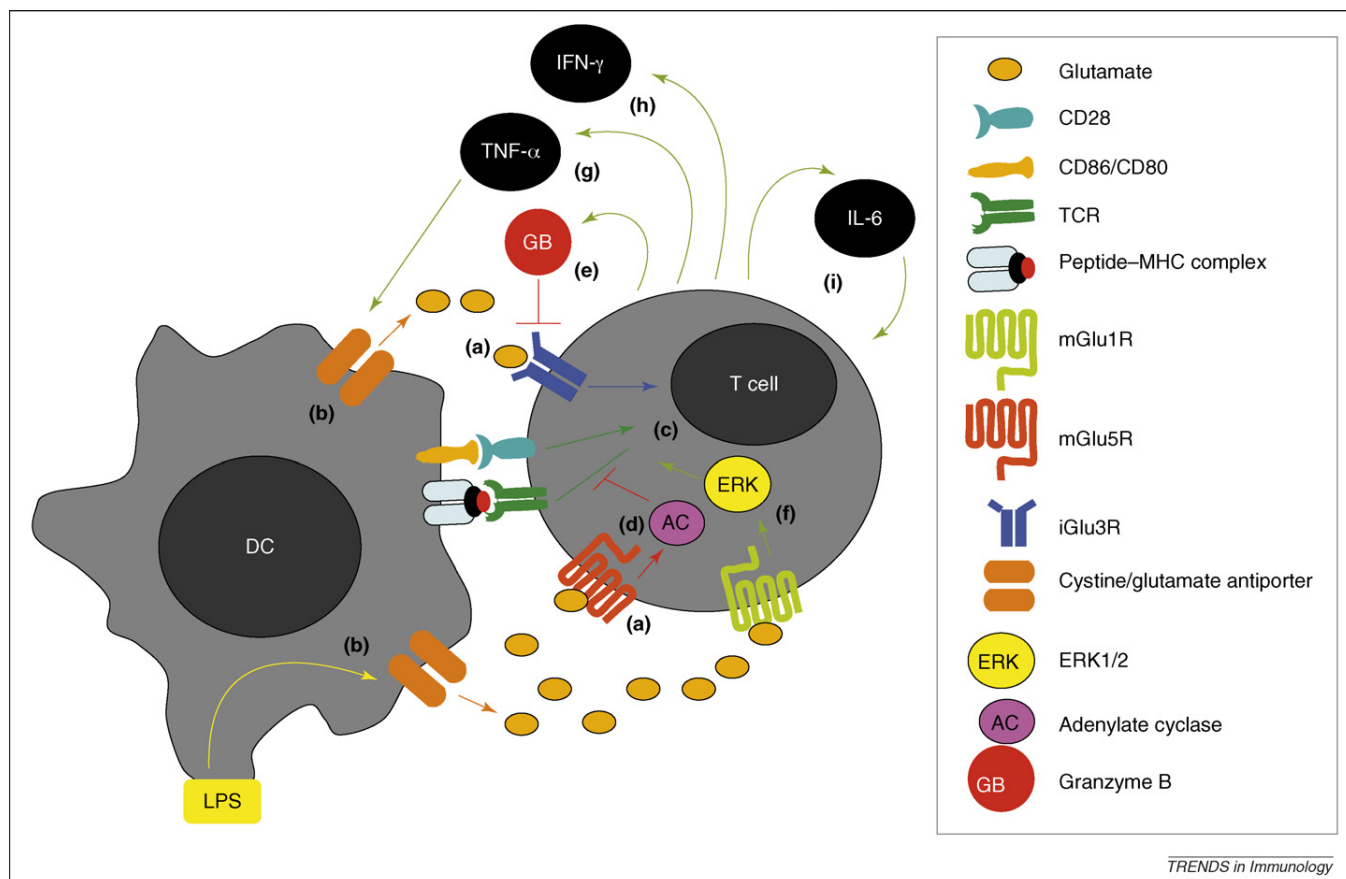


Figure 3. The role of glutamate in T-cell-mediated immunity. Resting T cells express ionotropic glutamate receptor 3 (iGlu3R) and metabotropic glutamate receptor 5 (mGlu5R), which are coupled to the entry of cations inside cells or to stimulation of adenylate cyclase, respectively (a). By stimulating iGlu3R, plasma glutamate promotes a given pattern of adhesion and migration towards certain chemokines and, by stimulating mGlu5R, impairs T-cell activation. When DCs are stimulated to mature by pathogen-associated molecules, such as lipopolysaccharide (LPS), they begin to release high amounts of glutamate through the cystine/glutamate antiporter (b). These mature glutamate-releasing DCs migrate to lymph nodes where they encounter patrolling naive T cells. When mature DCs present cognate antigen to these T cells, stimulatory signals triggered through the TCR and co-stimulation together promote T-cell activation (c). When DCs present non-cognate antigen to T cells, stimulation through the TCR is not strong enough and stimulation of adenylate cyclase-coupled mGlu5R avoids aberrant T-cell activation (d). However, when a cognate antigen promotes a signal strong enough to elicit activation, T cells begin to release granzyme B, which, in turn, degrades iGlu3R (e). Activated T cells also begin to express the ERK1/2-coupled mGlu1R and its subsequent stimulation bypasses the mGlu5R-mediated inhibitory signal and promotes a co-stimulatory effect on T-cell activation (f) by potentiating T-cell proliferation and enhancing the secretion of TNF- α , IFN- γ and IL-6. Enhanced TNF- α induces increased glutamate release by DCs (g), IFN- γ promotes a Th1-polarized response (h) and IL-6 triggers additional co-stimulation (i). Arrowheads represent stimulation or up-regulation, whereas the flat heads represent inhibition or down-regulation.

immune responsiveness, which is consistent with the immune-suppression that is associated with nicotine intake by smokers [14]. These observations have led to the exploration of $\alpha 7$ nACh receptor agonists for therapy of uncontrolled inflammation, particularly the systemic inflammation that accompanies severe sepsis and ulcerative colitis [45].

Altered dopaminergic signaling is also implicated in the pathogenesis of autoimmune disease. Expression of D₅, the inhibitory dopaminergic receptor, by T cells is down-regulated in patients with multiple sclerosis and might contribute to the T-cell hyper-responsiveness that characterizes this disease [46]. Moreover, plasma dopamine concentrations are elevated in humans under stressful conditions, which results in the well established suppression of T-cell function by a D₁-receptor-mediated mechanism [33].

Glutamate has an important role in modulating T-cell fate decisions, in large part owing to differential GluR expression and the associated signal transduction of glutamate and GluR [1]. However, the role of glutamate in

immune-mediated disease is not yet well established. Nonetheless, there is consistent and substantial evidence that elevated extracellular glutamate in the CNS is a key mediator of neurological damage, whereas elevated plasma glutamate is a feature shared by a number of neuroimmune-related diseases [1]. In the CNS, glutamate probably stimulates mGluR1 expressed by activated autoreactive T cells that enter the CNS following neuronal damage, trauma or infection. Signaling through T-cell-expressed mGluR1 promotes secretion of the main Th1 cytokine interferon (IFN)- γ [20], which induces augmented uptake of glutamate by microglia, thus preventing the glutamate-induced damage and decreasing pathology [47,48]. In fact, depletion of T regulatory cells was beneficial in a mouse strain with a spontaneous T-cell-dependent ability to withstand the consequences of glutamate-induced neuronal damage [49,50]. This was explained by T regulatory cell-induced down-regulation of Th1-cell-mediated functions [50,51]. It has been proposed that glutamate might have dual but opposing roles in autoimmune neurological disorders, such as multiple sclerosis: first, mediating neuronal damage and, second,

inducing IFN- γ secretion by infiltrating T cells, thereby reducing pathology [1]. With regard to elevated plasma glutamate levels, it is conceivable that hyper-stimulation of inhibitory mGluR5 expressed by T cells would inhibit their effector function in immune-mediated pathologies, such as amyotrophic lateral sclerosis, epilepsy, headache, AIDS and HIV-associated dementia and some malignancies [1].

Gut enterochromaffin cells are a primary source of 5-HT synthesis in normal subjects; however, 5-HT is reduced markedly in patients with inflammatory bowel disease (IBD) [52]. Accumulating evidence indicates that anti-inflammatory signaling by the hypothalamus (glucocorticoids) and the autonomic nervous system (ACh) is uncoupled in IBD [39,40]. This scenario is consistent with the persistence of IFN- γ -producing Th1 cells [53] and the elevated expression of the tryptophan-degrading enzyme indoleamine 2, 3-dioxygenase within the inflamed bowel [54]. Under these conditions, it is likely that local 5-HT synthesis is impaired owing to the relative scarcity of tryptophan. Because 5-HT provides a necessary autocrine signal for T-cell activation [16], we propose that these conditions might prevent the *de novo* induction and/or persistence of natural T regulatory cells that are associated with the prevention and cure of experimental IBD [55,56]. Careful analysis of 5-HT signal transduction in T-cell subpopulations is required to test this hypothesis.

Classical allergic disorders, such as eczema and asthma, display high local concentrations of 5-HT during the acute disease phase [57,58]. DCs have significant roles both in the pathogenesis of allergic disorders [59,60] and the temporal and spatial control of 5-HT availability [19]. It is conceivable that allergen exposure triggers the release of 5-HT from immature skin DCs. Signaling by 5-HT₇ receptors drives the maturation of IL-8- and IL-10-producing DCs [61,62] and can potentiate allergic responses by promoting the activation of Th2 cells. Thus, regulation of microenvironmental 5-HT, as well as potentially other biogenic amines, might be important for the maintenance of resting DCs in peripheral tissues. Indeed, evidence supporting this hypothesis is provided from studying patients with Langerhans cell (LC) histiocytosis, a disorder characterized by the uncontrolled accumulation of LCs within peripheral tissues. In these patients, LCs do not express the type-2 vesicular monoamine transporter necessary for sequestration of biogenic amines from the cytosol to intracellular vesicles [63]. Thus, the capacity to sequester and, ultimately, secrete biogenic amines might contribute to the homeostatic control of DCs.

Future perspectives

The knowledge that leukocytes can release neurotransmitters that elicit autocrine or paracrine effects within the immune system opens a new perspective in understanding the fine tuning of immune responses. To gain further insight into this field, it will be necessary to investigate whether other compounds can be released by leukocytes, as shown recently for serotonin and glutamate. Identifying the mechanism of release (vesicular versus non-vesicular), the identity of transporters and the triggers that regulate release will be

an important challenge for future investigations. Comparison between neuronal and immune synapses will give rise to some new and interesting paradigms. For instance, in nerve-driven immune responses (Figure 1), it appears that signaling is achieved by volume transmission (i.e. diffusion from the neuron to leukocyte cell surface receptors). Conversely, in leukocyte-driven immunity, it is possible that wiring transmission can occur by release of transmitter into the immune synapse and in close proximity to the receptor (Figure 1). Thus, lymphocyte function could be modified by neurotransmitters produced by peripheral nerves and secreted within lymphoid tissues (volume transmission) or theoretically released during DC-lymphocyte synaptic interactions (wiring transmission). The latter pathway would enable the rapid and concentrated delivery of neurotransmitters through a spatially targeted mechanism. Alternatively, neurotransmitters released by leukocytes might signal peripheral nerves by volume transmission, a pathway not considered previously.

To date, research has focused on understanding the neurotransmitter composition of cells in lymphoid organs but not the composition in terms of neurotransmitter level in lymph or in the extracellular fluid of lymphoid organs. This is probably owing to technical difficulties that could be overcome by using techniques more common in neuroscience. Also, it would be worth investigating the presence of 'post-immunosynaptic' receptors and 'pre-immunosynaptic' transport systems to determine the exact resemblance between synapse-based neural and immune communication systems. It would also be relevant to explore the occurrence of leukocyte subpopulations that express a specific neurotransmitter receptor subtype or a specific transporter system as it occurs in neurons, which can be, for example, dopaminergic or glutamatergic.

Because changes in the expression of receptor subtypes occur in neural development and function, something similar can be envisaged for immune development and function. Also, a comparison of neurotransmitter receptor-mediated signaling by leukocytes or neurons is also required to compile detailed similarities and differences in signal transduction occurring at the neurological versus the immunological synapse. A concerted effort will be necessary to comprehend the putative role of neurotransmitters in immunological memory, in T-cell differentiation and in other important aspects, such as in regulating intercellular exchanges of membrane patches (trocytosis) and, in general, their impact in immune plasticity.

T cells are important effector cells in natural antiviral and anticancer immunity. As described here, there is accumulating evidence supporting the view that the final outcome of antigen receptor-mediated immune processes is at least partially determined by physiologically abundant small signaling molecules in the extracellular environment of lymphocytes. This includes not only neurotransmitters but compounds known as neuromodulators. Extracellular purines (ATP and adenosine) and their receptors have been studied as an example of participation in immune-driven immunity [64–66]; the role of other neuromodulators in regulating T-cell receptor-mediated T-cell responses should be equally investigated.

It is now evident that neurotransmitter receptors form heteromers, which provide an enormous degree of plasticity and complexity in signal transduction [67]. This is because receptor heteromers act as processors that modulate cell signaling. Although some leukocytes express multiple neurotransmitter receptor subtypes, it has not yet been determined if heteromerization occurs in cells of the immune system. Stimulation of one receptor in the heteromer leads to the allosteric modification of the adjacent receptor and thereby might change its functional characteristics. If this phenomenon also occurs in leukocytes, detailed analysis of receptor signal transduction pathways will be necessary to fully elucidate the role of neurotransmitters in the induction and regulation of immune responses. Such a finding will also reveal a new layer of complexity in leukocyte signaling.

Acknowledgements

Supported by Grants from the Spanish Ministerio de Ciencia y Tecnología (SAF2006-05481 to RF), from Fondecyt (3070018 to RP), from Milenio (P04/030-F to RP) and from NIH (to GPA and PJO) and by the Premiers' Research Excellence Award (PREA to PJO).

References

- Pacheco, R. *et al.* (2007) Role of glutamate on T-cell mediated immunity. *J. Neuroimmunol.* 185, 9–19
- Silverman, M.N. *et al.* (2005) Immune modulation of the hypothalamic–pituitary–adrenal (HPA) axis during viral infection. *Viral Immunol.* 18, 41–78
- Peeva, E. *et al.* (2004) Prolactin as a modulator of B cell function: implications for SLE. *Biomed. Pharmacother.* 58, 310–319
- Wrona, D. (2006) Neural-immune interactions: an integrative view of the bidirectional relationship between the brain and immune systems. *J. Neuroimmunol.* 172, 38–58
- Levite, M. (2000) Nerve-driven immunity. The direct effects of neurotransmitters on T-cell function. *Ann N. Y. Acad. Sci.* 917, 307–321
- Besser, M.J. *et al.* (2005) Dopamine by itself activates either D2, D3 or D1/D5 dopaminergic receptors in normal human T-cells and triggers the selective secretion of either IL-10, TNF α or both. *J. Neuroimmunol.* 169, 161–171
- Torres, K.C. *et al.* (2005) Norepinephrine, dopamine and dexamethasone modulate discrete leukocyte subpopulations and cytokine profiles from human PBMC. *J. Neuroimmunol.* 166, 144–157
- Takahashi, H. *et al.* (2005) Contribution of the sympathetic nervous system to the burn-associated impairment of CCL3 production. *Cytokine* 29, 208–214
- Watanabe, Y. *et al.* (2006) Dopamine selectively induces migration and homing of naïve CD8⁺ T cells via dopamine receptor D₃. *J. Immunol.* 176, 848–856
- Cutolo, M. *et al.* (2002) Androgens and estrogens modulate the immune and inflammatory response in rheumatoid arthritis. *Ann. N. Y. Acad. Sci.* 966, 131–142
- Marshall, G.D., Jr and Agarwal, S.K. (2000) Stress, immune regulation, and immunity: applications for asthma. *Allergy Asthma Proc.* 21, 241–246
- Fujii, T. *et al.* (1995) Expression of choline acetyltransferase mRNA and protein in T-lymphocytes. *Proc Japan Acad* 71B, 231–235
- Rinner, I., Kawasima, K. and Schauenstein, K. (1998) Rat lymphocytes produce and secrete acetylcholine independent of differentiation and activation. *J. Neuroimmunol.* 81, 31–37
- Kawasima, K. and Fujii, T. (2003) The lymphocytic cholinergic system and its biological function. *Life Sci.* 72, 2101–2109
- Gordon, J. and Barnes, N.M. (2003) Lymphocytes transport serotonin and dopamine: agony or ecstasy? *Trends Immunol.* 24, 438–443
- Leon-Ponte, M. *et al.* (2007) Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. *Blood* 109, 3139–3146
- Laberge, S. *et al.* (1996) Secretion of IL-16 (lymphocyte chemoattractant factor) from serotonin-stimulated CD8⁺ T cells *in vitro*. *J. Immunol.* 156, 310–315
- Ameisen, J.C. *et al.* (1989) A new interpretation of the involvement of serotonin in delayed-type hypersensitivity. serotonin-2 receptor antagonists inhibit contact sensitivity by an effect on T cells. *J. Immunol.* 142, 3171–3179
- O'Connell, P.J. *et al.* (2006) A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. *Blood* 107, 1010–1017
- Pacheco, R. *et al.* (2006) Glutamate released by dendritic cells as a novel modulator of T cell activation. *J. Immunol.* 177, 6695–6704
- Bergquist, J. *et al.* (1998) Catecholaminergic suppression of immunocompetent cells. *Immunol. Today* 19, 562–567
- Rajda, C. *et al.* (2006) The norepinephrine level is decreased in the lymphocytes of long-term interferon- β -treated multiple sclerosis patients. *Mult. Scler.* 12, 265–270
- Cosentino, M. *et al.* (2007) Human CD4⁺CD25⁺ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine-paracrine inhibitory functional loop. *Blood* 109, 632–642
- Dustin, M.L. and Colman, D.R. (2002) Neural and immunological synaptic relations. *Science* 298, 785–789
- Pacheco, R. *et al.* (2004) Group I metabotropic glutamate receptors mediate a dual role of glutamate in T cell activation. *J. Biol. Chem.* 279, 33352–33358
- Pin, J.P. and Duvoisin, R. (1995) The metabotropic glutamate receptors: structure and functions. *Neuropharmacology* 34, 1–26
- 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
- Kohm, A.P. *et al.* (2002) B cell receptor- and β 2-adrenergic receptor-induced regulation of B7-2 (CD86) expression in B cells. *J. Immunol.* 168, 6314–6322
- O'Connor, T.M. *et al.* (2004) The role of substance P in inflammatory disease. *J. Cell Physiol.* 201, 167–180
- Marriott, I. *et al.* (2000) Substance P activates NF- κ B independent of elevations in intracellular calcium in murine macrophages and dendritic cells. *J. Neuroimmunol.* 102, 163–171
- Badou, A. *et al.* (2006) Critical role for the β regulatory subunits of Cav channels in T lymphocyte function. *Proc. Natl. Acad. Sci. USA* 103, 15529–15534
- Krantic, S. (2000) Peptides as regulators of the immune system: emphasis on somatostatin. *Peptides* 21, 1941–1964
- Saha, B. *et al.* (2001) Physiological concentrations of dopamine inhibit the proliferation and cytotoxicity of human CD4⁺ and CD8⁺ T cells *in vitro*: a receptor-mediated mechanism. *Neuroimmunomodulation* 9, 23–33
- Bedoui, S. *et al.* (2002) NPY modulates epinephrine-induced leukocytosis via Y-1 and Y-5 receptor activation *in vivo*: sympathetic co-transmission during leukocyte mobilization. *J. Neuroimmunol.* 132, 25–33
- Ganor, Y. *et al.* (2007) TCR activation eliminates glutamate receptor GluR3 from the cell surface of normal human T cells, via an autocrine/paracrine granzyme B-mediated proteolytic cleavage. *J. Immunol.* 178, 683–692
- Krantic, S. *et al.* (2004) Novel modalities of somatostatin actions. *Eur. J. Endocrinol.* 151, 643–655
- Weinstock, J.V. (2004) The role of substance P, hemokinin and their receptor in governing mucosal inflammation and granulomatous responses. *Front. Biosci.* 9, 1936–1943
- Borovikova, L.V. *et al.* (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405, 458–462
- Czura, C.J. and Tracey, K.J. (2005) Autonomic neural regulation of immunity. *J. Intern. Med.* 257, 156–166
- Sternberg, E.M. (2006) Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat. Rev. Immunol.* 6, 318–328
- Fujii, T. (2007) Roles played by lymphocyte function-associated antigen-1 in the regulation of lymphocytic cholinergic activity. *Life Sci.* 80, 2320–2324
- Levinson, A.I. *et al.* (2004) The intrathymic pathogenesis of myasthenia gravis. *Clin. Dev. Immunol.* 11, 215–220
- Jara, L.J. *et al.* (2006) Immune–neuroendocrine interactions and autoimmune diseases. *Clin. Dev. Immunol.* 13, 109–123
- Wang, H. *et al.* (2003) Nicotinic acetylcholine receptor α 7 subunit is an essential regulator of inflammation. *Nature* 421, 384–388

- 45 Ulloa, L. (2005) The vagus nerve and the nicotinic anti-inflammatory pathway. *Nat. Rev. Drug Discov.* 4, 673–684
- 46 Giorelli, M. *et al.* (2005) Dopamine fails to regulate activation of peripheral blood lymphocytes from multiple sclerosis patients: effects of IFN- β . *J. Interferon. Cytokine Res.* 25, 395–406
- 47 Shaked, I. *et al.* (2005) Protective autoimmunity: interferon-g enables microglia to remove glutamate without evoking inflammatory mediators. *J. Neurochem.* 92, 997–1009
- 48 Shaked, I. *et al.* (2004) Early activation of microglia as antigen-presenting cells correlates with T cell-mediated protection and repair of the injured central nervous system. *J. Neuroimmunol.* 146, 84–93
- 49 Kipnis, J. *et al.* (2002) Neuroprotective autoimmunity: naturally occurring CD4⁺CD25⁺ regulatory T cells suppress the ability to withstand injury to the central nervous system. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15620–15625
- 50 Kipnis, J. *et al.* (2004) Dual effect of CD4⁺CD25⁺ regulatory T cells in neurodegeneration: a dialogue with microglia. *Proc. Natl. Acad. Sci. U. S. A.* 101, 14663–14669
- 51 Kipnis, J. *et al.* (2002b). Myelin specific Th1 cells are necessary for post-traumatic protective autoimmunity. *J. Neuroimmunol.* 130, 78–85
- 52 Coates, M.D. *et al.* (2004) Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* 12, 1657–1664
- 53 Parronchi, P. *et al.* (1997) Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am. J. Pathol.* 150, 823–832
- 54 Wolf, A.M. *et al.* (2004) Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease. *Clin. Immunol.* 113, 47–55
- 55 Liu, H. *et al.* (2003) CD4⁺CD25⁺ regulatory T cells cure murine colitis: the role of IL-10, TGF- β , and CTLA4. *J. Immunol* 171, 5012–5017
- 56 Coombes, J.L. and Maloy, K.J. (2007) Control of intestinal homeostasis by regulatory T cells and dendritic cells. *Semin Immunol.* 19, 116–126
- 57 Huang, J. *et al.* (2004) Immunohistochemical study of serotonin in lesions of chronic eczema. *Int. J. Dermatol.* 43, 723–726
- 58 Lechin, F. *et al.* (1996) Increased levels of free serotonin in plasma of symptomatic asthmatic patients. *Ann. Allergy Asthma Immunol.* 77, 245–253
- 59 Miraglia del Giudice, M. *et al.* (2006) Immune dysregulation in atopic dermatitis. *Allergy Asthma Proc.* 27, 451–455
- 60 Lambrecht, B.N. and van Rijt, L.S. (2006) Infections and asthma pathogenesis: a critical role for dendritic cells? *Novartis Found Symp.* 279, 187–200 discussion 200–205, review 216–219
- 61 Idzko, M. *et al.* (2004) The serotonergic receptors of human dendritic cells: identification and coupling to cytokine release. *J. Immunol.* 172, 6011–6019
- 62 Katoh, N. *et al.* (2006) Effect of serotonin on the differentiation of human monocytes into dendritic cells. *Clin. Exp. Immunol.* 146, 354–361
- 63 Anlauf, M. *et al.* (2004) The vesicular monoamine transporter 2 (VMAT2) is expressed by normal and tumor cutaneous mast cells and Langerhans cells of the skin but is absent from Langerhans cell histiocytosis. *J. Histochem. Cytochem.* 52, 779–788
- 64 Apasov, S. *et al.* (1996) Role of extracellular ATP and P1 and P2 classes of purinergic receptors in T-cell development and cytotoxic T lymphocyte effector functions. *Immunol. Rev.* 146, 5–19
- 65 Sitkovsky, M.V. *et al.* (2004) Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. *Annu. Rev. Immunol.* 22, 657–682
- 66 Sitkovsky, M. and Lukashev, D. (2005) Regulation of immune cells by local-tissue oxygen tension: HIF1 α and adenosine receptors. *Nat. Rev. Immunol.* 5, 712–721
- 67 Agnati, L.F. *et al.* (2003) Mechanisms and therapeutic implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. *Pharmacol. Rev.* 55, 509–550

Elsevier.com – linking scientists to new research and thinking

Designed for scientists' information needs, Elsevier.com is powered by the latest technology with customer-focused navigation and an intuitive architecture for an improved user experience and greater productivity.

The easy-to-use navigational tools and structure connect scientists with vital information – all from one entry point. Users can perform rapid and precise searches with our advanced search functionality, using the FAST technology of Scirus.com, the free science search engine. Users can define their searches by any number of criteria to pinpoint information and resources. Search by a specific author or editor, book publication date, subject area – life sciences, health sciences, physical sciences and social sciences – or by product type. Elsevier's portfolio includes more than 1800 Elsevier journals, 2200 new books every year and a range of innovative electronic products. In addition, tailored content for authors, editors and librarians provides timely news and updates on new products and services.

Elsevier is proud to be a partner with the scientific and medical community. Find out more about our mission and values at Elsevier.com. Discover how we support the scientific, technical and medical communities worldwide through partnerships with libraries and other publishers, and grant awards from The Elsevier Foundation.

As a world-leading publisher of scientific, technical and health information, Elsevier is dedicated to linking researchers and professionals to the best thinking in their fields. We offer the widest and deepest coverage in a range of media types to enhance cross-pollination of information, breakthroughs in research and discovery, and the sharing and preservation of knowledge.

Elsevier. Building insights. Breaking boundaries.
www.elsevier.com