Nicotine and serotonin in immune regulation and inflammatory processes: a perspective

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Abstract: Nicotine and serotonin modulate the innate and adaptive immune responses and the inflammatory states. Several nicotinic cholinergic and serotonergic receptor subtypes have been characterized in B and T lymphocytes, monocytes, macrophages, and dendritic cells. The use of knockout mice has allowed a better characterization of nicotinic receptors and their role in anti-inflammatory processes in these cells. Cytokines play a crucial role in controlling inflammatory reactions. Nicotine and serotonin have been reported to regulate cytokine release. Cholinergic mechanisms also play an important role in inflammation through endogenous acetylcholine. Nicotine mimics this effect by activating the cholinergic anti-inflammatory pathways. New concepts of reciprocal interactions between nicotine and serotonin are emerging. The role of nicotine as an anti-inflammatory agent has been established, whereas that of serotonin remains more controversial. *J. Leukoc. Biol.* 81: 599–606; 2007.

Key Words: *immune cells · inflammation · cytokine · neuromodulator · 5-HT receptors · nicotinic cholinergic receptors*

INTRODUCTION

Neurotransmitters such as acetylcholine (ACh) and serotonin [5-hydroxytryptamine (5-HT)] play crucial roles in brain homeostasis and functioning. Several studies confirm the existence of in vivo reciprocal interactions between cholinergic and 5-HT axon terminals in specific brain regions such as hippocampus and cortex, which occur through the activation of muscarinic ACh receptors (mAChRs), nicotinic cholinergic AChRs (nAChRs), and 5-HT receptors. These interactions underlie the effects of nicotine on anxiety states observed in smokers [1] and exposed animal models [2, 3].

The mAChRs, nAChRs, and 5-HT receptors have been identified recently in various blood cell populations as well as in peripheral organs and tissues with reported modulatory functions. Among cholinergic receptors, only the nAChR subtypes are activated directly by nicotine, which is their exogenous ligand present in tobacco smoke. Activation of 5-HT receptors by 5-HT and that of nAChRs by endogenous ACh or exogenous nicotine result in modulation of a broad range of immunological functions including innate and adaptive immune responses.

As compared with the CNS, the existence of reciprocal interactions between extraneuronal 5-HT and ACh in peripheral systems has not yet been clearly demonstrated. With regard to 5-HT and nicotine, such interactions are likely, at least in smokers, as 5-HT is released in the vicinity of blood cells [4] and might interfere with nicotine's effects in these cells.

All of the recent published observations reinforce the view that activated blood cells—lymphocytes, in particular—may be exposed to endogenous ACh and 5-HT, as well as to nicotine in smokers. The consequences of tobacco smoke and nicotine on some aspects of immune responses such as proliferation and calcium signaling in lymphocytes have been reviewed [5]. New concepts for immune regulation and control of inflammatory processes have also emerged. By focusing mainly on nicotine and 5-HT, we present recent data about their occurrence and roles in blood cells and on cytokine release. The possible mechanisms of reciprocal actions between nicotine and 5-HT are also discussed.

AVAILABILITY OF NICOTINE AND 5-HT FOR BLOOD CELLS

Nicotine

During cigarette smoking and transdermal nicotine treatment, nicotine reaches the brain rapidly (less than 20 s) and triggers addiction in smokers. It also accumulates in the blood, where its concentration can increase to several hundred nanomolar levels [6, 7]. Nicotine mimics some of the ACh effects that are mediated by nAChRs. The origin and the function of ACh in blood have been reviewed extensively [8]. One of the important aspects is that blood lymphocytes possess all the required enzymatic components to constitute an independent, extraneuronal cholinergic system involved in the regulation of immune functions. Recent observations suggest that ACh is released by the vagus nerve and diffuses through the parenchyma of organs

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of the reticuloendothelial system, in proximity of tissue macrophages [9, 10]. The existence of a functional relationship between vagus nerve, released ACh, and the reticuloendothelial system was confirmed by lesioning approaches [11]. In smokers, nicotine may activate the same targets as those activated by endogenous ACh and control the blood cell activities directly.

5-HT

5-HT is synthesized by enterochromaffin cells (EC) of the gastrointestinal (GI) tract, released under stimulation and taken up by circulating platelets. At inflammatory sites, activation of platelets by factors such as platelet-activating factor, complements anaphylatoxin C5a and IgE-containing immune complexes leads to their aggregation and a rapid release of micromolar concentrations of 5-HT in proximity of blood cells [12]. The C5a also triggers mast cells to release 5-HT, and this aspect is particularly important, as it is believed to favor an immune resistance to primary tumors and metastases [13]. The autonomic innervations of lymphoid tissues represent a local source of 5-HT: 5-HT colocalizes with noradrenaline in nerve terminals, and it can be released under nerve stimulation [12]. Accordingly, activation of parasympathetic neurons has been shown to enhance plasmatic concentrations of free 5-HT [14]. Finally, 5-HT concentrations can also be regulated by its transport into monocytes, macrophages, dendritic cells (DC), and lymphocytes through activation of 5-HT uptake systems {5-HT transporter (5-HTT)} [12, 15].

PERIPHERAL, RECIPROCAL INTERACTIONS BETWEEN NICOTINE AND 5-HT

Nicotine is shown to partly control the 5-HT transport and content within platelets by distinct cellular mechanisms. One example of such mechanisms is the activation of nAChRs in EC, which leads to an increase in 5-HT concentrations in human platelets [16]. Nicotine also stimulates 5-HT release from human blood platelets [17]. Finally, decreased densities of platelet vesicular monoamine transporters have been reported in habitual smokers [18], which is likely due to nicotine's effects. These observations provide clear evidence with regard to nicotine-5-HT interactions including nicotine's interference with 5-HT effects on immune cells by controlling its availability.

Activation of mAChRs and nAChRs has also been reported to increase 5-HT release from guinea-pig EC [19, 20]. Therefore, reciprocal interactions among nicotine, ACh, and 5-HT may also occur at the GI level and contribute to the molecular mechanisms involved in peripheral inflammatory diseases. It has been reported that smokers have a lower incidence of GI disorders such as inflammatory bowel disease as compared with nonsmokers [21]. It is interesting that nicotine appears to be beneficial for clinical treatment of ulcerative colitis [22]. 5-HT plays an important role in the regulation of GI functions. 5-HT concentrations have been shown to decrease in colonic mucosa of individuals suffering from ulcerative colitis and irritable bowel syndrome [23]. In addition to its anti-inflammatory properties, nicotine may therefore prevent the development of such GI disorders by increasing the local concentrations of 5-HT.

The GI receives vagus afferent nerves. As a consequence, 5-HT released from EC in the intestinal mucosa is expected to activate 5-HT receptors expressed on the adjacent vagus afferent nerves [20]. As mentioned above, 5-HT release from EC is controlled by cholinergic mechanisms. Therefore, reciprocal interactions between nicotine/ACh and 5-HT might occur at vagus nerve and GI levels. The consequences of vagus nerve stimulation on EC activity and 5-HT release in control and experimental models of ulcerative colitis have not been investigated yet.

The maintenance of 5-HT availability also depends on the levels of the amino acid tryptophan (Trp), which is the precursor for its synthesis. Under various pathological conditions, Trp may become less available. For example, severe trauma, sepsis, adult respiratory distress syndrome, and autoimmune diseases can lead to production of IFN-γ [24], which will lead to the activation of indoleamine 2, 3-dioxygenase (IDO), which will in turn lead to Trp degradation with concomitant accumulation of kynurenine metabolites [25, 26]. Among the kynurenine metabolites, kynurenic acid is an antagonist drug at central and peripheral nAChRs [27]. Therefore, depletion of Trp through IDO activation may simultaneously decrease 5-HT content and antagonize cellular functions, which are mediated by nAChRs. In accordance with such hypothesis, recent observations indicate that in vivo concentrations of kynurenic acid are sufficient to lower the activity of nicotinic monomeric α7 receptors in mice brain [27]. This new concept might represent another important mechanism of reciprocal interactions between 5-HT and nicotine in blood cells, which may in turn control the inflammatory response.

NICOTINE AND 5-HT TARGETS IN BLOOD CELLS

The presence of mAChRs and nAChRs in blood cells has been evidenced and confirmed in genetically engineered knockout (KO) mice. For the purpose of this review, we will only focus on nAChRs, which are ligand-gated ion channels, mainly expressed in the brain and at the neuromuscular junction. Their pharmacological, physiological, and kinetic properties have been studied extensively and reviewed in our laboratory [28–31]. nAChRs have a pentameric structure organized around an axis, which delineates the pore of the channel with at least two ligand-binding sites (**Fig. 1A**). Seventeen different nAChR subunits (α1–α10, β1–β4, γ, δ, and ε) encoding nAChR subunits have been identified and cloned in mammals. Depending on their site of expression, subunits assemble with diverse stoichiometries to form homo- and heteropentameric receptor subtypes, which are found in blood cells (Fig. 1B). The nAChRs are expressed in macrophages [33], T lymphocytes [34], B lymphocyte-derived cell lines [35], and mouse B lymphocytes [36, 37].

In blood cells, nicotine might exert its effects independently without the activation of nAChRs, by diffusing into the cells and direct modulation of intracellular components and signaling pathways [38, 39].

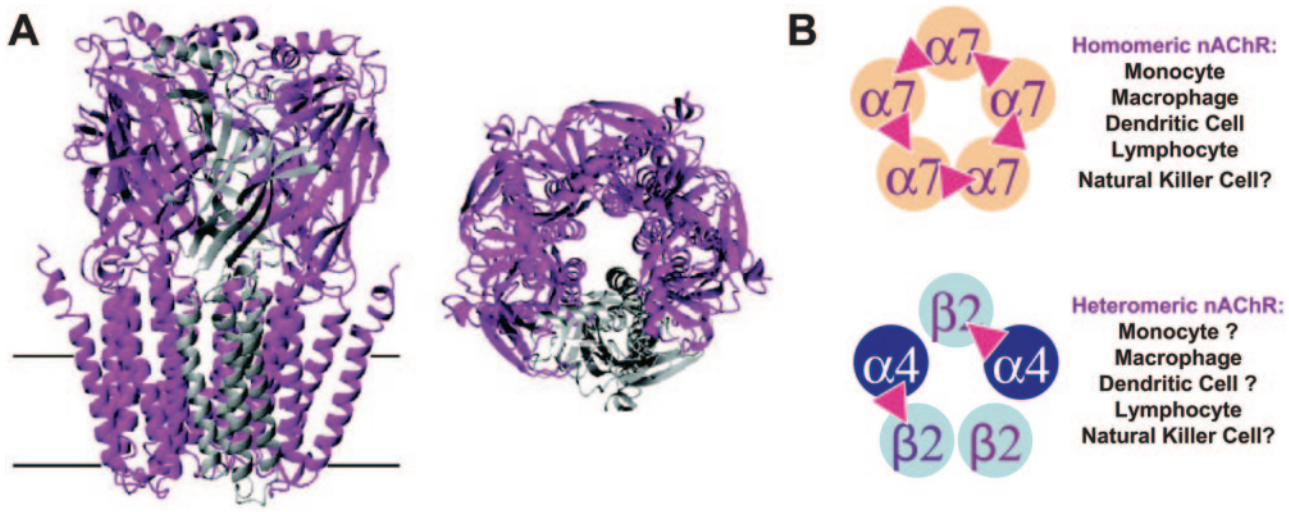

Fig. 1. Subclasses of nAChRs are formed by the assembly of five subunits. (A) Three-dimensional, computerized model of the pentameric α7 nAChR (published in refs. [30, 32]). (B) Pentameric organization of homomeric and heteromeric pentamers with respective locations in blood cell subpopulations. Pharmacological studies have identified two main classes of nicotinic receptors. One class consists of low-affinity binding sites labeled by [^{125}I]α-bungarotoxin formed by α7 homomers. The other consists of a high-affinity nAChR composed mainly of different classes of heteromeric receptors, which are labeled with [^{125}I]epibatidine.

Long-term exposure to nicotine up-regulates nAChRs in many neuronal cells [40–42]. This effect may be due to the fact that nAChRs undergo rapid desensitization and subsequent inactivation, which follow prolonged exposure to nicotine or nicotine agonists. We recently showed that nAChR up-regulation was not a result of alterations in transcriptional processes but resulted from the facilitating effect of nicotine on nAChR maturation [39, 43]. Up-regulation of nAChRs occurs in leukocytes of smokers and in mice exposed to nicotine [44]. Nicotine also up-regulates α4 and α7 subunits in B lymphocyte-derived cell lines [35]. In smokers, changes in the number and/or functionality of neuronal nAChRs under nicotine exposure may underlie, at least in part, the development of addiction. However, the functional consequences of up-regulation are not well understood, and the presence of multiple receptor subtypes may complicate the interpretation of such mechanisms in neural and peripheral cells. Moreover, nicotine concentrations in the blood of smokers may increase or decrease transiently depending on the frequency of nicotine intake. Consequently, the nAChRs may also transiently resensitize after their initial desensitization and inactivation. Therefore, nicotine response may differ depending on the state of nAChRs. This aspect should be considered when interpreting data from experiments with continuous chronic nicotine exposure, which are significantly different from the conditions generally observed in smokers.

5-HT receptors comprise at least 15 subtypes and display overlapping pharmacological properties, amino acid sequences, and second messenger-coupling pathways [45, 46]. These receptors are classified into seven groups, 5-HT$_{1–7}$. Each group is divided in subgroups, which contain receptors that are homologous but encoded by discrete genes. With the exception of the 5-HT$_3$ receptor, which is a cation channel, all the other 5-HT receptors are G protein-coupled receptors that may activate or inhibit a large number of signaling pathways [47].

KO mouse models for 5-HT receptors have been produced for diverse 5-HT receptor subtypes and should represent valuable tools for studying the role of selective 5-HT receptors in immune cell functions. However, by contrast to nAChR receptors, the KO mouse model has not yet been used to study the precise role of selective 5-HT receptor subtypes in immune cells. The distribution of 5-HT receptors in blood cells is presented in **Table 1**. So far, nine 5-HT receptor subtypes, namely 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1E}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_{3A}$, 5-HT$_4$, and 5-HT$_7$, have been identified on these cells.

TABLE 1. Current Knowledge of the Distribution of 5-HT Receptor Subtypes and 5-HTT in Blood Cells

	Cell type				
	B cell	T cell	Monocyte/macrophage	Dendritic cell	Refs.
5-HT$_1$	+ (1A)	+ (1A)	+ (1A)	+ (1B, 1E)	[12, 48, 49]
5-HT$_2$	+ (2A)	+ (2A, 2C)	+ (2A, monocyte)	+ (2A, 2B)	[12, 48, 50, 51]
5-HT$_3$	+	+ (3A)	+ (3A)	?	[12, 50, 52]
5-HT$_4$	?	?	+	+	[48, 50, 51]
5-HT$_7$	+	?	?	+	[12, 48, 50, 51]
5-HTT	+	+	+	+	[12, 15]

The diverse molecular properties of cholinergic and serotonergic receptors together with their distinct signaling pathways may account for the numerous regulatory roles that ACh and nicotine together and 5-HT may play on the activities of blood cells. These include regulation of proliferation, phagocytosis, apoptosis, and cytokine production. We focus this review on the effects of nicotine and 5-HT on cytokine production, which has not been fully reviewed yet, and their impacts in inflammatory processes, in particular, those that are observable in smokers.

MODULATION OF CYTOKINE RELEASE

The equilibrium between the secretion levels of pro- and anti-inflammatory cytokines and their sequential release may be one of the key determinant factors that accounts for the severity of inflammatory responses. Any alteration in this equilibrium may convert a beneficial inflammatory response into a severe pathological, inflammatory outcome. For example, overproduction of proinflammatory cytokines such as TNF and IL-1 leads to severe sepsis and lethal multiple organ failure. Consequently, a great attention is currently devoted to cellular mechanisms, which control cytokine levels under normal conditions. Among these, the roles of ACh and 5-HT as neuroactive substances as well as nicotine, which may use pathways similar to ACh, have been studied extensively.

The concept of a "cholinergic anti-inflammatory pathway", by which brain modulates the systemic inflammatory response to endotoxin (LPS), was introduced recently [53]. Vagus nerve stimulation attenuates TNF release from macrophages within the reticuloendothelial system. This effect is abolished in α7 KO mice and is believed to be the result of a post-transcriptional inhibition of cytokine synthesis [33]. Activation of the vagus nerve cholinergic pathway blocks the recruitment of leukocytes during inflammation [54]. Anti-inflammatory effects of cholinergic activities have also been evidenced experimentally by using acetylcholinesterase inhibitors, which prevent ACh degradation with resulting inhibition of TNF and IL-1β production [55]. In LPS-stimulated macrophages, ACh inhibits the release of IL-1β, IL-6, and IL-18 but does not modify IL-10 production [56]. Recent studies have shown that central mAChRs activate the cholinergic anti-inflammatory pathway and inhibit systemic TNF in endotoxemic rats [57].

Nicotine has also been reported to modulate cytokine secretion. It inhibits TNF release from macrophages through the activation of α7 receptors, even more strongly than ACh itself [56]. These findings raise two questions: Are other nAChR subtypes, distinct from the α7 subtype, involved in the regulation of cytokine release by macrophages? Does regulation of cytokine release by nicotine take place in other cells such as monocytes, DC, lymphocytes, and NK cells?

The involvement of nAChRs, distinct from the α7 subtype, is strongly suggested by the study of Matsunaga et al. [58]. These authors propose the participation of α4 and β2 subunits in down-regulation by nicotine of IL-6, IL-12, and TNF but not that of IL-10 production from murine alveolar macrophages. Any variations in proinflammatory cytokine secretion may alter the recruitment and activation of circulating leukocytes such as neutrophils, which are involved in bacterial killing. Nicotine has been reported to lower endocytosis and phagocytosis activities in human DC and decrease the levels of IL-12, which is a key cytokine for the recruitment of T cells in response to LPS stimulation [59]. Finally, nicotine may exert indirect effects on DC activities by regulating their local cytokine environment, thereby modifying their function as the initiators of a primary specific immune response [60]. It is interesting that down-regulation of key proinflammatory cytokines is believed to contribute to the mechanisms that are involved in increased susceptibility of smokers to respiratory infections [61]. The impairment of DC functions by nicotine may also be related to the increased occurrence of infections in smokers.

Down-regulation of TNF release and cytokine mRNA expression (i.e., TNF, IL-10, IFN-γ) by nicotine has been observed experimentally using a murine alveolar macrophage cell line [62]. Nicotine was also found to inhibit the production of IL-2, TNF, and IFN-γ in response to anti-CD3 stimulation in human PBMC [63] and that of IL-6, TNF, and IFN-γ in LPS-induced murine splenocyte [64]. The modulatory effects of nicotine, which were demonstrated for TNF, can be extended to other key cytokines such as IL-1 and IFN-γ in the inflammatory cascades and may involve different cell populations.

5-HT effects on cytokine release in blood cells are more complex than those observed with nicotine. In particular, 5-HT displays opposite effects on the production of cytokines, which present similar cellular functions. This is true for TNF and IL-1β, which are, respectively, decreased and increased by 5-HT in human DC [48], human PBMC [50], and human monocytes [65]. Other cytokines, such as IL-1α, IL-6, IL-10, and IL-1 receptor antagonist, are not affected by 5-HT in human PBMC [50]. By contrast, 5-HT has been reported to facilitate the release of IFN-γ in human NK cells and the chemokine IL-16 in peripheral blood leukocytes and in CD-8+ T cells [51]. Therefore, based on current knowledge, 5-HT appears to strongly control the development of inflammatory processes by regulating different patterns of cytokine secretion.

Distinct 5-HT receptors mediating 5-HT effects on cytokine release have been identified in human blood cell populations. Activation of 5-HT_{2A} receptors and that of 5-HT_4 and 5-HT_7 receptors inhibit TNF production in PBMC [50] and monocytes [65], respectively. In DC, activation of 5-HT_4 and 5-HT_7 receptors increases the production of IL-1β and IL-8 and reduces that of IL-12 and TNF [48]. In monocytes, 5-HT_3 receptors have been shown to increase the production of IL-1β and IL-6 [65]. Overall, nicotine and 5-HT act as potent inhibitors of TNF release but display opposite effects on IL-1β and IFN-γ productions. The increase in IL-1β secretion by 5-HT may partly counteract the inhibitory effects of TNF with possible consequences on neutrophil activation and protective responses against infection. IFN-γ and IL-12 play key roles in T cell differentiation and modulation of Th1/Th2 responses [66, 67]. With regard to IFN-γ production, the opposite effects of nicotine and 5-HT strongly suggest the involvement of these mediators in regulation of T cell differentiation and Th1 and Th2 cell responses in different manners. IFN-γ production stimulates Th1 cell responses. Therefore, the inhibitory effect of nicotine on IFN-γ production favors a positive action toward

Th2 cell responses. However, such action has not been established clearly, as ulcerative colitis (a Th2 disease) is less prevalent in smokers than Crohn's disease (a Th1 disease) [68, 69]. In addition, chronic exposure to nicotine leads to the inhibition of the T-dependent antibody response [70]. Nicotine has also been shown to decrease IL-12 production in DC [58]. The effect of 5-HT on this cytokine has not been investigated yet; however, this would be of great interest in understanding its role(s) in maintaining a Th1:Th2 ratio and response. The major effects of nicotine and 5-HT on cytokine release are presented schematically in **Figure 2**. Nicotine and 5-HT are potent inhibitors of TNF release but display opposite effects on IL-1β and IFN-γ production. Nicotine also inhibits the release of other cytokines such as IL-12 and IL-18, which may interfere with NK cell and T cell activities. These data suggest that nicotine and 5-HT regulate T cell differentiation and Th1 and Th2 responses in different manners. However, the precise mechanisms involved have not been investigated.

PRO- AND/OR ANTI-INFLAMMATORY?

TNF is the prototype cytokine, released in the early phases of inflammatory processes, which initiates signaling cascades and controls innate responses. One of the common features of nicotine, ACh, and 5-HT is their capacity to inhibit TNF production from monocytes and monocyte-derived cells and their activity as anti-inflammatory agents. Indeed, nicotine has been shown to display anti-inflammatory properties with suppressive effects on various cell types, which participate in inflammatory processes. These effects include the inhibition of proinflammatory cytokine release, as discussed above, phagocytosis and proliferation [35, 36, 71], alteration in Th cell:T suppressor cell ratios [72], decrease in number and the activity of NK cells, and serum IgG levels [37]. Nicotine has been shown to reduce mortality and improve survival in experimental models of sepsis such as polymicrobial peritonitis through activation of the anti-inflammatory pathway [73]. However, the mechanisms by which nicotine controls inflammation are not all clearly defined and probably occur through complex interactions with inflammatory pathways. Inflammation is associated with dysregulated apoptosis, and there are controversial reports about nicotine's effect and its pro- and antiapoptotic actions [74, 75]. Nicotine's involvement in apoptosis has been highlighted particularly based on its control through elimination of damaged cells and restoration of homeostasis at inflammatory loci.

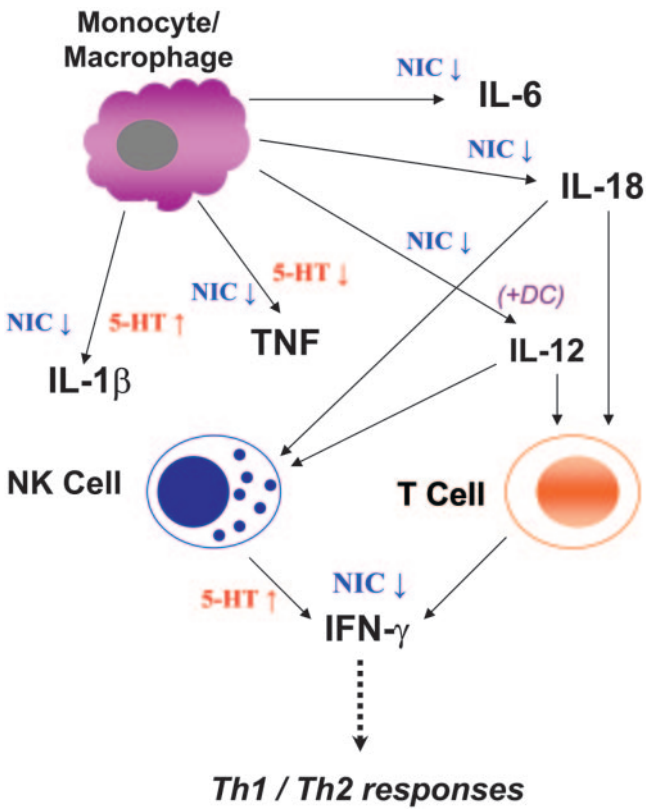

Fig. 2. Proposed schematic representation of the effects of nicotine and 5-HT on cytokine release in different blood cell populations. Nicotine and 5-HT are potent inhibitors of TNF release but display opposite effects on IL-1β and IFN-γ production. The opposite effects of nicotine and 5-HT on IFN-γ production suggest that these mediators regulate T cell differentiation and Th1 and Th2 responses in different manners. 5-HT, Serotonin; NIC, nicotine; symbols of effects: ↓, inhibitory; ↑, stimulatory.

Despite its reported anti-inflammatory properties, nicotine is associated with the development of specific diseases such as respiratory tract infections, chronic airway disease, asthma, allergies, and lung cancers. This is most likely the result of nicotine's inhibitory effect on TNF, IL-1β, and IFN-γ production. Defects in the production of these cytokines are thought to increase smokers' susceptibility to infections.

With regard to 5-HT, previous studies have documented its involvement in diverse inflammatory responses despite its controversial effects on cytokine release. A clear inverse relationship between 5-HT levels and inflammation has been reported in depressed patients [76]. However, paradoxical effects of 5-HT in inflammation with biphasic responses have been reported at the central and peripheral systems [77] and in blood cells [78]. The presence of multiple subtypes of 5-HT receptors, which mediate the inhibitory and stimulatory effects, may be responsible for its complex activities in inflammation. 5-HT effects on cytokine production are dependent on its concentrations in the vicinity of immune cells. For example, at 1 μM, 5-HT inhibits TNF production without displaying any increasing effect on IL-1β production [50]. Therefore, nicotine, by controlling 5-HT availability in smokers, may modify its concentrations and consequently, its effects on cytokine production. In nonsmokers, 5-HT levels may also be controlled by the activation of mAChR by endogenous ACh as discussed above.

The possibility that the anti-inflammatory properties of nicotine in sepsis would be counteracted through IDO activation and Trp degradation cannot be excluded. The consequent accumulation of kynerunic acid would antagonize α7 nAChR-mediated effects and nicotine's anti-inflammatory properties. The fact that nicotine treatment may be beneficial in experimental models of sepsis does not comfort this hypothesis. Additional studies are therefore needed to evaluate the importance of the IDO activation pathway in diverse inflammatory diseases through its inhibition of α7 nAChR-mediated effects.

Finally, a few recent studies have reported the effects of nicotine and 5-HT on intracellular signaling cascades, which are activated in inflammatory processes. Nicotinic stimulation prevents the activation of the NK-κB pathway and inhibits high mobility group box 1 protein secretion, a late mediator of sepsis, from human macrophages [73]. The anti-inflammatory properties of nicotine are confirmed by its stimulatory action on the anti-inflammatory cascade STAT3-suppressor of cytokine signaling (SOCS) 3 in macrophages [79]. In addition, stimulation of the cholinergic anti-inflammatory pathway has been proposed to ameliorate enteric inflammation during postoperative ileus via α7 nAChR-Jak2 interactions [79].

5-HT has also been reported to activate the intracellular signaling pathways and stimulate NF-κB transcription in splenocytes [80]. This is believed to be related to stimulation by 5-HT of lymphocyte proliferation. It has been shown that the 5-HT_3 receptor antagonist, tropisetron, inhibits PMA plus ionomycin-induced NF-κB activation in Jurkat cells, whereas no effect was observed on TNF-mediated NF-κB activation [81]. Opposing effects on NF-κB activation by 5-HT or by nicotine may be a result of the involvement of different cell types involved.

CONCLUSION

Increasing data clearly confirm the role of nicotine and 5-HT in regulation of immune response and inflammatory cascades through the control of cytokine levels. More research should provide further evidence and better understanding of molecular and cellular pathways, which characterize nicotine and 5-HT interactions and their reciprocal effects in inflammatory processes. It should also help in understanding the links between nicotine intake by smokers and serotonin function in relation to psychological disorders such as suicidal behavior and depression. New insights into the mechanisms underlying these interactions may constitute the basis for novel therapies.

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REFERENCES

1. Pomerleau, O. F., Turk, D. C., Fertig, J. B. (1984) The effects of cigarette smoking on pain and anxiety. *Addict. Behav.* **9,** 265–271.
2. Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001) The dorsal raphe nucleus is a crucial structure mediating nicotine's anxiolytic effects and the development of tolerance and withdrawal responses. *Psychopharmacology (Berl.)* **155,** 78–85.
3. File, S. E., Cheeta, S., Kenny, P. J. (2000) Neurobiological mechanisms by which nicotine mediates different types of anxiety. *Eur. J. Pharmacol.* **393,** 231–236.
4. Verbeuren, T. J., Jordaens, F. H., Herman, A. G. (1983) Accumulation and release of (3H)-5-hydroxytryptamine in saphenous veins and cerebral arteries of the dog. *J. Pharmacol. Exp. Ther.* **226,** 579–588.
5. Sopori, M. L., Kozak, W., Savage, S. M., Geng, Y., Soszynski, D., Kluger, M. J., Perryman, E. K., Snow, G. E. (1998) Effects of nicotine on the immune system: possible regulation of immune responses by central and peripheral mechanisms. *Psychoneuroendocrinology* **23,** 189–204.
6. Benowitz, N. L. (1988) Drug therapy. Pharmacologic aspects of cigarette smoking and nicotine addition. *N. Engl. J. Med.* **319,** 1318–1330.
7. Fagerstrom, K. O., Hughes, J. R. (2002) Nicotine concentrations with concurrent use of cigarettes and nicotine replacement: a review. *Nicotine Tob. Res.* **4** (Suppl. 2), S73–S79.
8. Kawashima, K., Fujii, T. (2000) Extraneuronal cholinergic system in lymphocytes. *Pharmacol. Ther.* **86,** 29–48.
9. Czura, C. J., Tracey, K. J. (2005) Autonomic neural regulation of immunity. *J. Intern. Med.* **257,** 156–166.
10. Pavlov, V. A., Tracey, K. J. (2005) The cholinergic anti-inflammatory pathway. *Brain Behav. Immun.* **19,** 493–499.
11. Huston, J. M., Ochani, M., Rosas-Ballina, M., Liao, H., Ochani, K., Pavlov, V. A., Gallowitsch-Puerta, M., Ashok, M., Czura, C. J., Foxwell, B., Tracey, K. J., Ulloa, L. (2006) Splenectomy inactivates the cholinergic anti-inflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J. Exp. Med.* **203,** 1623–1628.
12. Mossner, R., Lesch, K. P. (1998) Role of serotonin in the immune system and in neuroimmune interactions. *Brain Behav. Immun.* **12,** 249–271.
13. Askenase, P. W., Szczepanik, M., Itakura, A., Kiener, C., Campos, R. A. (2004) Extravascular T-cell recruitment requires initiation begun by Vα14+ NKT cells and B-1 B cells. *Trends Immunol.* **25,** 441–449.
14. Lechin, F. (2000) Central and plasma 5-HT, vagal tone and airways. *Trends Pharmacol. Sci.* **21,** 425.
15. O'Connell, P. J., Wang, X., Leon-Ponte, M., Griffiths, C., Pingle, S. C., Ahern, G. P. (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.
16. Racke, K., Schworer, H., Simson, G. (1992) Effects of cigarette smoking or ingestion of nicotine on platelet 5-hydroxytryptamine (5-HT) levels in smokers and non-smokers. *Clin. Investig.* **70,** 201–204.
17. Rausch, J. L., Fefferman, M., Ladisich-Rogers, D. G., Menard, M. (1989) Effect of nicotine on human blood platelet serotonin uptake and efflux. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **13,** 907–916.
18. Schwartz, K., Weizman, A., Rehavi, M. (2005) Decreased platelet vesicular monoamine transporter density in habitual smokers. *Eur. Neuropsychopharmacol.* **15,** 235–238.
19. Schworer, H., Racke, K., Kilbinger, H. (1987) Cholinergic modulation of the release of 5-hydroxytryptamine from the guinea-pig ileum. *Naunyn Schmiedebergs Arch. Pharmacol.* **336,** 127–132.
20. Minami, M., Endo, T., Hirafuji, M., Hamaue, N., Liu, Y., Hiroshige, T., Nemmoto, M., Saito, H., Yoshioka, M. (2003) Pharmacological aspects of anticancer drug-induced emesis with emphasis on serotonin release and vagal nerve activity. *Pharmacol. Ther.* **99,** 149–165.
21. Calkins, B. M. (1989) A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig. Dis. Sci.* **34,** 1841–1854.
22. Pullan, R. D. (1994) Transdermal nicotine for active ulcerative colitis. *N. Engl. J. Med.* **330,** 811–815.
23. Coates, M. D., Mahoney, C. R., Linden, D. R., Sampson, J. E., Chen, J., Blaszyk, H., Crowell, M. D., Sharkey, K. A., Gershon, M. D., Mawe, G. M., Moses, P. L. (2004) Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* **126,** 1657–1664.
24. Wirleitner, B., Neurauter, G., Schrocksnadel, K., Frick, B., Fuchs, D. (2003) Interferon-γ-induced conversion of tryptophan: immunologic and neuropsychiatric aspects. *Curr. Med. Chem.* **10,** 1581–1591.
25. Pellegrin, K., Neurauter, G., Wirleitner, B., Fleming, A. W., Peterson, V. M., Fuchs, D. (2005) Enhanced enzymatic degradation of tryptophan by indoleamine 2,3-dioxygenase contributes to the tryptophan-deficient state seen after major trauma. *Shock* **23,** 209–215.
26. Grohmann, U., Fallarino, F., Puccetti, P. (2003) Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol.* **24,** 242–248.
27. Alkondon, M., Pereira, E. F., Yu, P., Arruda, E. Z., Almeida, L. E., Guidetti, P., Fawcett, W. P., Sapko, M. T., Randall, W. R., Schwarcz, R., Tagle, D. A., Albuquerque, E. X. (2004) Targeted deletion of the kynurenine aminotransferase II gene reveals a critical role of endogenous kynurenic acid in the regulation of synaptic transmission via α7 nicotinic receptors in the hippocampus. *J. Neurosci.* **24,** 4635–4648.
28. Changeux, J. P., Bertrand, D., Corringer, P-J., Dehaene, S., Edelstein, S., Lena, C., Le Novère, N., Marubio, L., Picciotto, M., Zoli, M. (1998) Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. *Brain Res. Brain Res. Rev.* **26,** 198–216.
29. Champtiaux, N., Changeux, J. P. (2004) Knockout and knockin mice to investigate the role of nicotinic receptors in the central nervous system. *Prog. Brain Res.* **145,** 235–251.
30. Changeux, J. P., Edelstein, S. J. (2005) Allosteric mechanisms of signal transduction. *Science* **308,** 1424–1428.

31. (2006) *Nicotinic Acetylcholine Receptors* (J. P. Changeux, S. J. Edelstein, eds.), Paris, France, Odile Jacob.
32. Taly, A., Delarue, M., Grutter, T., Nilges, M., Le Novère, N., Corringer, P-J., Changeux, J-P. (2005) Normal mode analysis suggests a quaternary twist model for the nicotinic receptor gating mechanism. *Biophys. J.* **88,** 3954–3965.
33. Wang, H., Yu, M., Ochani, M., Amelia, C. A., Tanovic, M., Susurla, S., Li, J. H., Wang, H., Yang, H., Ulloa, L., Al-Abed, Y., Czura, C. J., Tracey, K. J. (2003) Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature* **421,** 384–388.
34. Middlebrook, A. J., Martina, C., Chang, Y., Lukas, R. J., DeLuca, D. (2002) Effects of nicotine exposure on T cell development in fetal thymus organ culture: arrest of T cell maturation. *J. Immunol.* **169,** 2915–2924.
35. Skok, M. V., Kalashnik, E. N., Koval, L. N., Tsetlin, V. I., Utkin, Y. N., Changeux, J-P., Grailhe, R. (2003) Functional nicotinic acetylcholine receptors are expressed in B lymphocyte-derived cell lines. *Mol. Pharmacol.* **64,** 885–889.
36. Skok, M., Grailhe, R., Changeux, J-P. (2005) Nicotinic receptors regulate B lymphocyte activation and immune response. *Eur. J. Pharmacol.* **517,** 246–251.
37. Skok, M., Grailhe, R., Agenes, F., Changeux, J-P. (2006) The role of nicotinic acetylcholine receptors in lymphocyte development. *J. Neuroimmunol.* **171,** 86–98.
38. Hawkins, B. T., Brown, R. C., Davis, T. P. (2002) Smoking and ischemic stroke: a role for nicotine? *Trends Pharmacol. Sci.* **23,** 78–82.
39. Sallette, J., Pons, S., Devillers-Thiery, A., Soudan, M., Prado de Cavalho, L., Changeux, J-P., Corringer, J-P. (2005) Nicotine upregulates its own receptors through enhanced intracellular maturation. *Neuron* **46,** 595–607.
40. Wonnacott, S. (1990) The paradox of nicotinic acetylcholine receptor upregulation by nicotine. *Trends Pharmacol. Sci.* **11,** 216–219.
41. Buisson, B., Bertrand, D. (2002) Nicotine addiction: the possible role of functional upregulation. *Trends Pharmacol. Sci.* **23,** 130–136.
42. Gentry, C. L., Lukas, R. J. (2002) Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. *Curr. Drug Targets CNS Neurol. Discord.* **1,** 359–385.
43. Corringer, P-J., Sallette, J., Changeux, J-P. (2006) Nicotine enhances intracellular nicotinic receptor maturation: a novel mechanism of neural plasticity? *J. Physiol. (Paris)* **99,** 162–171.
44. Cormier, A., Paas, Y., Zini, R., Tillement, J-P., Lagrue, G., Changeux, J-P., Grailhe, R. (2004) Long-term exposure to nicotine modulates the level and activity of acetylcholine receptors in white blood cells of smokers and model mice. *Mol. Pharmacol.* **66,** 1712–1718.
45. Hoyer, D., Hannon, J. P., Martin, G. R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.* **71,** 533–554.
46. (2006) *The Serotonin Receptors, from Molecular Pharmacology to Human Therapeutics* (B. L. Roth, eds.), Totowa, NJ, Humana.
47. Adayev, T., Ranasinghe, B., Banerjee, P. (2005) Transmembrane signaling in the brain by serotonin, a key regulator of physiology and emotion. *Biosci. Rep.* **25,** 363–385.
48. Idzko, M., Panther, E., Stratz, C., Muller, T., Bayer, H., Zissel, G., Durk, T., Sorichter, S., DiVirgilio, F., Geissler, M., Fiebich, B., Herouy, Y., Elsner, P., Norgauer, J., Ferrari, D. (2004) The serotoninergic receptors of human dendritic cells: identification and coupling to cytokine release. *J. Immunol.* **172,** 6011–6019.
49. Abdouh, M., Albert, P. R., Dobetsky, E., Filep, J. G., Kouassi, E. (2004) 5-HT_{1A}-mediated promotion of mitogen-activated T and B cell survival and proliferation is associated with increased translocation of NF-κB to the nucleus. *Brain Behav. Immun.* **18,** 24–34.
50. Cloëz-Tayarani, I., Petit-Bertron, A. F., Venters, H. D., Cavaillon, J. M. (2003) Differential effect of serotonin on cytokine production in lipopolysaccharide-stimulated human peripheral blood mononuclear cells: involvement of 5-hydroxytryptamine2A receptors. *Int. Immunol.* **15,** 233–240.
51. Foon, K. A., Wahl, S. M., Oppenheim, J. J., Rosenstreich, D. L. (1976) Serotonin-induced production of a monocyte chemotactic factor by human peripheral blood leukocytes. *J. Immunol.* **117,** 1545–1552.
52. Fiebich, B. L., Akunda, R. S., Lieb, K., Candelario-Jalil, E., Gmeimer, D., Haus, U., Muller, W., Stratz, T., Munoz, E. (2004) Expression of 5-HT3A receptors in cells of the immune system. *Scand. J. Rheumatol. Suppl.* **119,** 9–11.
53. Pavlov, V. A., Wang, H., Czura, C. J., Friedman, S. G., Tracey, K. J. (2003) The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol. Med.* **9,** 125–134.
54. Saeed, R. W., Varma, S., Peng-Nemeroff, T., Sherry, B., Balakhaneh, D., Huston, J., Tracey, K. J., Al-abed, Y., Metz, C. N. (2005) Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. *J. Exp. Med.* **201,** 1113–1123.
55. Nizri, E., Hamra-Amitay, Y., Sicsic, C., Lavon, I., Brenner, T. (2006) Anti-inflammatory properties of cholinergic up-regulation: a new role for acetylcholinesterase inhibitors. *Neuropharmacology* **50,** 540–547.
56. Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., Wang, H., Abumrad, N., Eaton, J. W., Tracey, K. J. (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* **405,** 458–462.
57. Pavlov, V. A., Ochani, M., Gallowitsch-Puerta, M., Ochani, K., Huston, J. M., Czura, C. J., Al-Abed, Y., Tracey, K. J. (2006) Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proc. Natl. Acad. Sci. USA* **103,** 5219–5223.
58. Matsunaga, K., Klein, T. W., Friedman, H., Yamamoto, Y. (2001) Involvement of nicotinic acetylcholine receptors in suppression of antimicrobial activity and cytokine responses of alveolar macrophages to *Legionella pneumophila* infection by nicotine. *J. Immunol.* **167,** 6518–6524.
59. Nouri-Shirazi, M., Guinet, E. (2003) Evidence for the immunosuppressive role of nicotine on human dendritic cell functions. *Immunology* **109,** 365–373.
60. Banchereau, J., Steinman, R. M. (1998) Dendritic cells and the control of immunity. *Nature* **392,** 245–252.
61. Janson, C., Chinn, S., Jarvis, D., Zock, J-P., Torén, K., Burney, P. (2001) Effect of passive smoking on respiratory symptoms, bronchial responsiveness, lung function, and total serum IgE in the European Community Respiratory Health Survey: a cross-sectional study. *Lancet* **358,** 2103–2109.
62. Blanchet, M. R., Israël-Assayag, E., Cormier, Y. (2004) Inhibitory effect of nicotine on experimental hypersensitivity pneumonitis in vivo and in vitro. *Am. J. Respir. Crit. Care Med.* **169,** 903–909.
63. Ouyang, Y., Virasch, N., Hao, P., Aubrey, M. T., Mukerjee, N., Bierer, B. E., Freed, B. M. (2000) Suppression of human IL-1β, IL-2, IFN-γ, and TNF-α production by cigarette smoke extracts. *J. Allergy Clin. Immunol.* **106,** 280–287.
64. Hakki, A., Hallquist, N., Friedman, H., Pross, S. (2000) Differential impact of nicotine on cellular proliferation and cytokine production by LPS-stimulated murine splenocytes. *Int. J. Immunopharmacol.* **22,** 403–410.
65. Dürk, T., Panther, E., Müller, T., Sorichter, S., Ferrari, D., Pizzirani, C., Di Virgilio, F., Myrtek, D., Norgauer, J., Idzko, M. (2005) 5-Hydroxytryptamine modulates cytokine and chemokine production in LPS-primed human monocytes via stimulation of different 5-HTR subtypes. *Int. Immunol.* **17,** 599–606.
66. Billiau, A., Heremans, H., Vermeire, K., Matthys, P. (1998) Immunomodulatory properties of interferon-γ. An update. *Ann. N. Y. Acad. Sci.* **856,** 22–32.
67. Packard, K. A., Khan, M. M. (2003) Effects of histamine on Th1/Th2 cytokine balance. *Int. Immunopharmacol.* **3,** 909–920.
68. Madretsma, S., Wolters, L. M., Van Dick, J. P., Tak, C. J., Wilson, J. H., Zijlstra, F. J. (1996) In vivo effect of nicotine on cytokine production by human non-adherent mononuclear cells. *Eur. J. Gastroenterol. Hepatol.* **8,** 1017–1020.
69. Vohra, P. (2000) Inflammatory bowel disease. *Indian J. Pediatr.* **67,** 747–756.
70. Geng, Y., Savage, S. M., Razani-Boroujerdi, S., Sopori, M. L. (1996) Effects of nicotine on the immune response. II Chronic nicotine treatment induces T cell anergy. *J. Immunol.* **156,** 2384–2390.
71. McAllister-Sistilli, C. G., Caggiula, A. R., Knof, S., Rose, C. R., Miller, A. L., Donny, E. C. (1998) The effects of nicotine on the immune system. *Psychoneuroendocrinology* **23,** 175–187.
72. Hallquist, N., Hakki, A., Wecker, L., Friedman, H., Pross, S. (2000) Differential effects of nicotine and aging on splenocyte proliferation and the production of Th1-versus Th2-type cytokines. *Proc. Soc. Exp. Biol. Med.* **224,** 141–146.
73. Wang, H., Liao, H., Ochani, M., Justiniani, M., Lin, X., Yang, L., Al-abed, Y., Wang, H., Metz, C., Miller, E. J., Tracey, K. J., Ulloa, L. (2004) Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat. Med.* **10,** 1216–1221.
74. Mai, H., May, W. S., Gao, F., Jin, Z., Deng, X. (2003) A functional role for nicotine in Bcl2 phosphorylation and suppression of apoptosis. *J. Biol. Chem.* **278,** 1886–1891.
75. Chen, Y. C., Shen, S-C., Lin, H-Y., Tsai, S-H., Lee, T. J. F. (2004) Nicotine enhancement of lipopolysaccharide/interferon-γ-induced cytotoxicity with elevating nictric oxide production. *Toxicol. Lett.* **153,** 191–200.
76. Raison, C. L., Capuron, L., Miller, A. H. (2006) Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.* **27,** 24–31.

77. Maleki, N., Nayebi, A. M., Garjani, A. (2005) Effects of central and peripheral depletion of serotonergic system on carrageenan-induced paw oedema. *Int. Immunopharmacol.* **5,** 1723–1730.
78. Schuff-Werner, P., Splettstoesser, W. (1999) Antioxidative properties of serotonin and the bactericidal function of polymorphonuclear phagocytes. *Adv. Exp. Med. Biol.* **467,** 321–325.
79. Metz, C. N., Tracey, K. J. (2005) It takes nerve to dampen inflammation. *Nat. Immunol.* **6,** 756–767.
80. Abdouh, M., Storring, J. M., Riad, M., Paquette, Y., Albert, P. R., Drobetsky, E., Kouassi, E. (2001) Transcriptional mechanisms for induction of 5-HT_{1A} receptor mRNA and protein in activated B and T lymphocytes. *J. Biol. Chem.* **276,** 4382–4388.
81. Vega L de, L., Munoz, E., Calzado, M. A., Lieb, K., Candelario-jalil, E., Gschaidmeir, H., Färber, L., Mueller, W., Stratz, T., Fiebich, B. L. (2005) The 5-HT3 receptor antagonist tropisetron inhibits T cell activation by targeting the calcineurin pathway. *Biochem. Pharmacol.* **70,** 369–380.