Exploitation of the nicotinic anti-inflammatory pathway for the treatment of epithelial inflammatory diseases

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Abstract
Discoveries in the first few years of the 21st century have led to an understanding of important interactions between the nervous system and the inflammatory response at the molecular level, most notably the acetylcholine (ACh)-triggered, \( \alpha 7 \)-nicotinic acetylcholine receptor (\( \alpha 7 \)nAChR)-dependent nicotinic anti-inflammatory pathway. Studies using the \( \alpha 7 \)nAChR agonist, nicotine, for the treatment of mucosal inflammation have been undertaken but the efficacy of nicotine as a treatment for inflammatory bowel diseases remains debatable. Further understanding of the nicotinic anti-inflammatory pathway and other endogenous anti-inflammatory mechanisms is required in order to develop refined and specific therapeutic strategies for the treatment of a number of inflammatory diseases and conditions, including periodontitis, psoriasis, sarcoidosis, and ulcerative colitis.

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Key words: \( \alpha 7 \)-nicotinic acetylcholine receptor; Inflammation; Mucosa; Nicotine; Nicotinic anti-inflammatory pathway; Tobacco smoking

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INTRODUCTION
Tobacco smoke appears to affect susceptibility to and the severity of various skin and mucosal diseases differently. For example, tobacco smoking is associated with an increased incidence and clinical severity of psoriasis\(^6\)-\(^8\) and Crohn’s disease\(^9\)-\(^1\) but is associated with a lower incidence of pouchitis\(^6\), celiac disease\(^1\) and ulcerative colitis\(^8\) as well as improved symptoms of ulcerative colitis\(^9\). While smokers are more susceptible to developing inflammatory periodontal diseases, smoking masks overt signs of gingival inflammation, which represents a clinical conundrum for dental professionals\(^6,10\). In order to explain these associations, and to harness any therapeutic potential of tobacco components, it will be necessary to better understand the cellular and molecular mechanisms by which tobacco smoke and smoke components influence epithelial inflammation in the skin and mucosa.

This review describes the nicotinic anti-inflammatory pathway and provides some insight into the possible exploitation of this pathway for the treatment of epithelial inflammation and other inflammatory conditions.

In addition to observations of associations between tobacco use and specific inflammatory diseases, the use of nicotine delivery systems, for reasons other than tobacco cessation therapy, has received attention. However, such studies have been essentially limited to inflammatory bowel and neurodegenerative diseases. Clinical trials using transdermal nicotine have shown that nicotine can improve symptoms in individuals with ulcerative colitis\(^11-14\). Additionally, several studies suggest that nicotine treatment may be useful in improving learning and attention, but importantly, probably not memory function in subjects with Alzheimer’s disease and other neurodegenerative diseases associated with a loss of neuronal nAChR protein or function\(^15-18\). However, an increasing understanding of the mechanisms by which nicotine interacts with the inflammatory system may soon open up further avenues for the therapeutic use of nicotine and other cholinergic agonists in the combat of several inflammatory disease processes.
LPS recruits, activates, and promotes degranulation events in the most numerous inflammatory leukocyte; i.e., the neutrophil. LPS and neutrophil degranulation products each recruit monocytes and macrophages to the locus of infection. While neutrophils are, in relative terms, short-lived and transcriptionally quiescent, activated monocytes/macrophages are longer-lived cells that produce large amounts of pro-inflammatory cytokines de novo, including TNF, IL-1, IL-6, IL-12/IL-23, p40, IL-18, and HMGB-1, when stimulated by inflammatory mediators. Such macrophage-derived mediators amplify and direct inflammation and link the innate and adaptive immune responses. In addition to the pro-inflammatory cytokine functions of HMGB-1, the continued production of this protein is a requisite for survival in monocytes, with apoptosis occurring when HMGB-1 translation is suppressed.[19-22]

The ability of the host’s immune system to initially recognize and respond to bacteria and other insults is largely mediated by the innate immune system via the expression of a family of type I transmembrane receptors; i.e., the Toll-like receptors (TLRs) [23-26] that signal the production of pro-inflammatory cytokines when stimulated by their cognizant ligands. For example, LPS activation of TLR4 on monocytes and macrophages triggers the biosynthesis of diverse mediators of inflammation, such as TNF and IL-1-β, and activates the production of costimulatory molecules (B7, CD40, MHCI) and the immunoregulatory cytokine IL-12 required for the adaptive immune response [27-29]. These inflammatory events are critical for clearing bacterial pathogens locally and surviving systemic infections. However, inflammation is a leading cause of morbidity and mortality in humans. Pro-inflammatory cytokines, such as TNF, have been found to be key mediators of chronic inflammatory diseases, including periodontitis [30]; rheumatoid arthritis [31]; and inflammatory bowel disease [32]. Additionally, the onset of sepsis has been associated with a predominant production of multiple pro-inflammatory cytokines, including IL-1, TNF, IFN-γ and IL-12 [33]. There is a subsequent set of cytokines, including HMGB1, that play a predominant role in mediating mortality in the latter phase of septic shock [33]. Therefore, there is great interest in learning how to control the production and activity of immune cell-derived inflammatory mediators [34,35] and the potential of their targeted suppression is enormous.

Cholinergic agonists act via either muscarinic (G-protein coupled) or nicotinic receptors. Nicotinic acetylcholine receptors are ligand-gated ion channels, but have additional functions unrelated to ion-channeling. Functional AChRs are pentameric, are composed of multiple combinations of a possible 16 monomer subtypes (α1-7; α9-10; β1-4; δ; ε; and γ), and exhibit divergent pharmacological behaviors [36,37]. Thus, identifying the exact type of nAChR involved in specific events can be difficult. It has been known for 25 years that phagocytic cells express nAChRs [38], yet this knowledge has not been significantly explored until recently. Neutrophils are known to express multiple nAChR subtypes. nAChR expression on monocytes and macrophages, in contrast, is much more restricted and may be limited solely to the α7 subtype in humans [39]. Certainly, of the α-bungarotoxin sensitive human nAChRs (α1, α7, and α9), monocytes and macrophages appear to express only α7 receptors that are functional [32,35,38,39]. nAChR expression on human macrophages is shown in Figure 1.

Of the known AChRs, α7 nAChR exhibits a number of unique features [40]. First of all, it can assemble and function as a homopentamer [41], the ion channel exhibits high permeability for calcium ions in preference to sodium [42], and it is widely expressed in the central and peripheral nervous system [43] as well as on leukocytes [42]. The last few years have seen a great expansion of our knowledge of how nicotine interacts with α7 nAChRs on monocytes and suppresses pro-inflammatory activities in these cells. The most extensively studied signaling mechanism involved in nicotine-induced inflammatory suppression in monocytes is the cholinergic, or nicotinic, anti-inflammatory pathway.

**THE NICOTINIC ANTI-INFLAMMATORY PATHWAY**

In order to limit self-damage, excessive inflammation is normally controlled by several endogenous anti-inflammatory mechanisms. One such mechanism is the nicotinic anti-inflammatory pathway. It has been known for some time that products of the central nervous system, such as adrenocorticotropic hormone, glucocorticoids, substance P, and melanocyte-stimulating hormone, are immunomodulatory [43,44,45]. In 2000, Borovikova et al first showed that synthesis of TNF by macrophages was under the control of the vagus nerve [46]. The vagus nerve is part of the parasympathetic system, is finely branched, and because it is composed of sensory (input) and motor (output) fibres can theoretically react to cytokines and suppress their production [31]. Furthermore, the vagus nerve is the longest of the cranial nerves and innervates most peripheral organs in humans. Recently, it has been shown that vagus nerve stimulation does not block TNF production in splenectomized animals dosed with LPS and the cholinergic pathway is functionally hard-wired to the spleen via the celiac nerve [46]. It has been dramatically shown that electrical stimulation of the vagus nerve prevents TNF production from macrophages and protects against death from LPS-induced shock [45]. The same authors have also shown that severance of the vagus nerve increases LPS-sensitivity in mice. Recently, it has been shown by using (1) α-bungarotoxin, an inhibitor of the α7 AChR, in wild type mice and (2) α7 AChR-deficient mice, that acetylcholine (ACh) or nicotine interaction with the α7 AChR is critical in the suppression of TNF release in response to LPS [19]. It has been dramatically shown that α7 AChR-deficient mice are not only hypersensitive to LPS and produce high amounts of TNF, but they also exhibit an exaggerated production of the pro-inflammatory cytokines IL-1 and IL-6 [19]. It is currently known that the nicotine-dependent suppression of TNF release from primary macrophages is abrogated by α7 nAChR-specific, but not α1- or α10-specific, anti-sense oligonucleotides surrounding the translation-initiation codon of the α7 nAChR gene [32]. It is important to note that suppression of TNF and other cytokine release from macrophages...
by the cholinergic anti-inflammatory system is extremely rapid, acting \textit{via} a post-transcriptional mechanism\cite{32,45,49}, further enhancing the attractiveness of this pathway as a therapeutic target for mucosal inflammatory conditions, endotoxemia and sepsis, for example. The cholinergic anti-inflammatory pathway is presented in Figure 2.

A critical intracellular pathway involved in the production of pro-inflammatory cytokines in innate immune cells is the NF-κB pathway\cite{19,36}. Nicotine prevents or inhibits the degradation of the inhibitory IκB protein that masks the nuclear localization signal of NF-κB and thus prevents NF-κB translocation and activation in monocytes/macrophages, in a dose-dependent manner\cite{32,45,69}, as may also be the case for other cell types, such as TNF-stimulated endothelial cells\cite{84}. Therefore, the nicotinic anti-inflammatory pathway may not be limited to cells of monocyte lineage. As a further example, nicotine has been shown to inhibit LPS-induced TNF production by microglia cells\cite{65}. Laan et al have shown that nicotine dampens the inflammatory response to LPS in bronchial epithelial cells and suggested that the down-regulation of the LPS-induced transcription factor, AP-1, may be of importance in regulating this phenomenon\cite{56}. Thus, we are beginning to understand that, while tobacco smoke exerts a plethora of negative effects on the immune and inflammatory system, tobacco appears to have the potential to protect against highly specific pathological conditions; e.g. inflammatory bowel diseases\cite{57}, perhaps neurodegenerative diseases\cite{58} and overt periodontal inflammation\cite{59}. Obviously, the negative effects of smoking are likely to significantly outweigh any “positive” health impacts and given the enormous epidemiological and mechanistic data linking tobacco use and disease this must remain the primary public health message. Nevertheless, identification of mechanisms by which tobacco smoke components suppress certain aspects of inflammation may lead to the identification of novel therapeutic targets and may drive the future development of non-tobacco-derived agonists and antagonists. In this regard, much recent attention has focused on the translation of the anti-inflammatory agent, CNI-1493 (semapimod). CNI-1493 suppresses TNF production in multiple environments; e.g. retinopathy\cite{58}, ischemic heart failure and stroke\cite{21,36}, inflammatory bowel disease\cite{57}, Haemophilus influenza type b and LPS-induced sepsis\cite{48,68}. While its precise mode of action is unclear, it now seems that CNI-1493 activates vagus nerve electrical activity and acts \textit{via} the cholinergic anti-inflammatory pathway\cite{21}.

In macrophages, the nicotine-induced suppression of pro-inflammatory cytokine release may involve recruitment of the tyrosine kinase Jak2 to the α7 nACh receptor, the subsequent phosphorylation of the transcription factor STAT3, and the activation of STAT3 and SOCS3 signaling cascade\cite{61}, which is known to interact with the NF-κB system\cite{32,64} and to inhibit the expression of IL-1, IL-6, and TNF\cite{62}. There is an obvious need to further elucidate the signaling mechanisms activated on interaction of nicotine with the α7 nAChR.

**A NON-α7nAChR-DEPENDENT ANTI-INFLAMMATORY PATHWAY**

Matsunaga et al\cite{65} have shown that a non-α7nAChR-dependent, nicotine-induced anti-inflammatory pathway may also function in macrophages. Nicotine-treated,
Legionella pneumophila-infected murine alveolar macrophages exhibit enhanced intracellular bacterial replication and down-regulation of key pro-inflammatory cytokine release (IL-6, IL-12, and TNF) but not the anti-inflammatory cytokine IL-10. This inflammatory suppression was unaffected by a selective antagonist; i.e., α7-bungarotoxin. Thus, the suppression of macrophage cytokine production in the pulmonary environment may help to explain the increased susceptibility for respiratory infections in smokers.

THE NICOTINIC ANTI-INFLAMMATORY PATHWAY AND SPECIFIC DISEASES AND CONDITIONS

Periodontitis
Smoking is the primary environmental factor associated with increased susceptibility and severity of periodontitis in western populations, and more than 50% of periodontitis cases in the USA can be attributed to tobacco use[66]. However, as we have previously reported, smoking reduces overt, clinically apparent, periodontal inflammation (edema; gingival index; bleeding on probing)[10,67], which is not due to any acute vasoactive effect of tobacco smoke (unlike the obvious tobacco-induced vasodilatation and vasoconstriction known to occur in forehead skin and the thumb, respectively)[68], but rather is a chronic tobacco-induced angiogenic suppression[69] that is reversed within weeks of tobacco cessation[10]. A representative case of chronic periodontitis in a smoker is shown in Figure 3.

While conflicting data has been presented for TNF[70,71], several studies have shown reduced gingival crevicular fluid (GCF) levels of major pro-inflammatory mediators, such as IL-1[72-74] in smokers with periodontitis compared to non-smokers with periodontitis, whereas anti-inflammatory cytokines are increased in the GCF of smokers, including IL-10 and TGF-β[72,75]. This agrees well with recent and exciting data showing that nicotine activates the nicotinic anti-inflammatory pathway and suppresses pro-inflammatory cytokine production at the transcriptional and/or post-translational levels[21,9,45,56,61].
Further understanding of the interactions between tobacco components, the immune system and the development of periodontitis is needed.

**Psoriasis**

Like periodontitis, tobacco smoking is associated with an increased clinical severity of psoriasis, with up to 95% of subjects with a genetically-determined localized variant of psoriasis; i.e., palmoplantar pustulosis, being smokers. Smoking can influence nAChR expression in skin epidermis and, in palmoplantar pustulosis, skin epidermal α7-nAChR expression is abolished, whereas α7-nAChR staining of the endothelium is stronger, compared to controls. Such findings have led to the hypothesis that there is an abnormal inflammatory response to nicotine, or other tobacco smoke constituents, in subjects with palmoplantar pustulosis. These findings suggest that patients with palmoplantar pustulosis may not be able to activate the endogenous nicotinic anti-inflammatory pathway due to a lack of α7nAChR, and thus treatments that activate this pathway, or other anti-inflammatory pathways, may prove efficacious in such subjects.

**Sarcoidosis**

Sarcoidosis is a systemic granulomatous disease that can present in any organ but primarily involves the lungs and can lead to respiratory failure. In sarcoidosis, macrophages release multiple inflammatory mediators favoring an initial accumulation of Th1 cells and the generation of a polarized Th1-type environment (IL-12, TNF and IFN-γ), which has led to the targeting of specific cytokines as potential therapeutics with which to prevent the reduction in pulmonary function that accompanies granuloma formation. A higher frequency of sarcoidosis in non-smokers than in smokers has been reported by several authors. Thus, therapeutic activation of the nicotinic anti-inflammatory pathway represents a theoretical intervention with which to prevent progression of sarcoidosis.

**Ulcerative colitis**

While smokers may be at increased risk of Crohn’s disease, increasing evidence suggests that the risk for ulcerative colitis is significantly reduced in smokers, and that smoking itself may reduce disease symptoms. This has led to clinical trials that show potential therapeutic efficacy in nicotine treatment for this specific inflammatory bowel disease. Thus, the suppression of cytokine production and/or release by nicotine appears to contribute to a dampening of intestinal inflammation and an improved disease course. There is some evidence that ulcerative colitis may be a Th2-type inflammatory disease, and it is known that nicotine suppresses the production of the IL-12/IL-23 sub-unit p40, which is critical in promoting Th1 responses (our unpublished data, see Figure 4). This implies that refined targeting of the nicotinic anti-inflammatory pathway may allow the development of therapeutic interventions that are as successful in reducing the symptoms of ulcerative colitis as nicotine but that may not induce the side-effects, such as nausea, lightheadedness, headache, tremor, sleep disturbance, contact dermatitis, nausea and acute pancreatitis, that compromise the attractiveness of nicotine delivery as a treatment for ulcerative colitis.

**Crohn’s disease**

Tobacco smoking is associated with an increased incidence and clinical severity of Crohn’s disease. Considering that pro-inflammatory cytokines, particularly TNF, are considered to be key mediators of Crohn’s disease, it seems counter intuitive to suggest that activation of the nicotinic anti-inflammatory pathway may exacerbate this disease. It has, however, been hypothesized that tobacco-induced suppression of the normal inflammatory response of macrophages, by α7 and non-α7 nAChR-dependent mechanisms, impairs the macrophage response to intestinal bacteria, leaving smokers more prone to developing Crohn’s disease. Therefore, a better understanding of the nicotinic anti-inflammatory pathway may allow pharmaceutical manipulation of this pathway, the counteraction of nicotine-dependent inflammatory suppression, and recovery of the inflammatory response to a level sufficient for rescue macrophage effector function.

**Septic shock**

Severe sepsis, the organ dysfunction that occurs during...
systemic inflammation, is a major cause of mortality in developed nations, contributing to 9.3% of the total annual deaths in the United States. It is characterized by the rampant production of multiple pro-inflammatory cytokines, including TNF, IL-1β, HMGB-1, and IL-12 [21,86,87]. Therapies designed to combat individual pro-inflammatory mediators in order to prevent septic shock have not been as successful as hoped and it has thus been hypothesized that inhibition of several or all pro-inflammatory mediators may be required [21]. Alternatively, it is recognized that HMGB-1, which is a late mediator of sepsis, is critical in the disease process and can be inhibited by nicotine [21,22]. Therefore, there is now a great deal of interest in using nicotine as an inducer of the nicotinic anti-inflammatory pathway as a potential treatment for severe sepsis [21,22,31,32,39].

**FUTURE DIRECTIONS**

Cholinergic strategies have been suggested as potential treatments for multiple diseases. In this review we have largely limited ourselves to the discussion of the relevance...
of the nicotinic anti-inflammatory pathway to skin and mucosal pathologies.

It is essential to point out that the use of nicotine as an anti-inflammatory agent, while supported by the current evidence, nevertheless represents a “sledgehammer” strategy. It must be envisaged that non-nicotinic (and, indeed, non-tobacco-derived) cholinergic agonists will be developed and explored in order to avoid the psychoactive, vascular, and other actions of nicotine on multiple nAChR-initiated pathways and to avoid the side-effects and adverse reactions associated with nicotine delivery. As recently pointed out by Ulloa, better structural characterization of nAChRs will be crucial in designing such nicotinic agonists[23].

Furthermore, it must be expected that targets downstream of the nAChRs will be identified allowing therapeutic refinement and an avoidance of blanket targeting of nAChRs.

Finally, the nicotinic anti-inflammatory pathway is unlikely to exist as a single, self-contained entity, but rather it is anticipated that the nicotinic anti-inflammatory pathway interacts and converges with multiple other pathways, including the NF-κB pathway and others. For example, we have shown the convergence of the nicotinic anti-inflammatory and an endogenous GSK-3-dependent anti-inflammatory pathway[24] in monocytes (our unpublished data, see Figure 5). As our knowledge of these signaling interactions increases, we are likely to identify further attractive anti-inflammatory targets and refined selectivity. In conclusion, the manipulation of nAChR-initiated signaling pathways likely represents a potentially fruitful area for inflammation research in the coming years and the currently expanding literature suggests that the number of diseases in which the pathway is relevant, for example, pancreatitis[47] and various vascular pathologies will increase[50,89,90].

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