The vagus nerve as a modulator of intestinal inflammation

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Effects of smoking on nicotinic AChR expression and susceptibility to cholinergic immunomodulation in human monocytes

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ABSTRACT

Smoking and nicotine positively affect the disease course in ulcerative colitis. As previous data suggest that nicotinic receptor $\text{CHRNA7}$ activation reduces inflammatory responses in monocytes, we evaluated whether smoking could affect monocyte $\text{CHRNA7}$ expression. Repeated nicotine exposure up-regulated $\text{CHRNA7}$ expression on THP-I monocytes and in conjunction, in a pilot study, $\text{CHRNA7}$ was only detectable in isolated blood monocytes of smokers. The duplicated $\text{CHRNA7}$ variant gene $\text{CHRFAM7A}$ was ubiquitously expressed on human monocytes and was not affected by nicotine exposure, while $\text{CHRNB2}$ was not detectable. In a volunteer study (n=11), we next aimed to demonstrate that smoking rendered blood monocytes more susceptible to cholinergic immuno-modulation, either by application of nicotine or by administration of fat nutrition. Pro-inflammatory cytokine secretion was inhibited by nicotinic receptor activation in THP-I monocytes, but this response was not consistently seen in blood monocytes from smoking individuals.
INTRODUCTION

It is increasingly recognized that the vagus nerve has immuno-modulating properties via activation of cholinergic receptors on non-neuronal cells. Cholinergic signaling attenuates pro-inflammatory cytokine release and improves clinical outcome in experimental animal models of inflammatory disease, such as sepsis, pancreatitis, post-operative ileus and rheumatoid arthritis. It has been proposed that electric stimulation of the efferent vagus nerve leads to the release of acetylcholine, which could bind to nicotinic acetylcholine receptors (nAChRs) that are broadly expressed on different types of immune cells, resulting in attenuation of pro-inflammatory cytokine release in monocytes and macrophages. However, more recent results indicate that the vagus nerve and cholinergic agonists inhibit systemic inflammation by activating the noradrenergic splenic nerve. Earlier studies have indicated that the anti-inflammatory effect of ACh is mediated through the alpha7 nicotinic acetylcholine receptor (CHRNA7) expressed on the surface of primary human monocytes. Besides the CHRNA7, a partially duplicated hybrid form of this gene was identified (CHRFAM7A) in which exon 5 to 10 of the alpha7 genes have been duplicated in a “tail to head” organization and combined with four novel exons (A to D) to comprise a new gene. The CHRFAM7A gene transcript is expressed in human monocytes has been identified in brain and human monocyte cell lines. This CHRFAM7A mRNA is transcribed to 45 kDa protein which lacks a nicotine binding domain, and hence it is unclear whether it is part of a functional nicotinic receptor. However, recent work indicates that its expression is transcriptionally regulated by LPS responsive genes and may contribute to the cholinergic regulation of the immune response. To date, the physiological and immunological implications of this receptor remain unknown.

Importantly, as most of the above work considering cholinergic inhibition of inflammation has been done in vitro and in experimental mouse models, the relevance of cholinergic immunomodulation to human disease remains sketchy. In one prominent human inflammatory disease, ulcerative colitis, cholinergic immunomodulation may play a significant role and provide therapeutic advantages for the treatment of the disorder. It is widely acknowledged that nicotine and smoking have a protective effect in the development of ulcerative colitis and reduces its severity. As a consequence of this clinical observation, nicotine patches and nicotine enemas have been successfully tested in patients with ulcerative colitis, but showed no advantage as compared to standard therapy. However, the protective mechanism and its clinical implications remain unknown.

We reasoned that the protective effect of smoking in UC patients could be due to preferential activation of the ‘cholinergic anti-inflammatory pathway’ in smokers via enhanced exposure to nicotine in tobacco smoke. Human immune cells display a variation in nAChR expression due to genetic or environmental factors. One of
these environmental factors resulting in altered nAChR expression could be cigarette smoking. In brain regions, it is already demonstrated that long-term consumption of tobacco elicits nAChR upregulation\textsuperscript{24-26}. Hence, in this study, we hypothesized that regular exposure to nicotine in tobacco smoke, affects \textit{CHRNA7} and \textit{CHRFAM7A} expression on human monocytes. Aims of the study were to determine whether priming to nicotine, either by cigarette smoking or by \textit{in vitro} nicotine exposure, altered nAChR expression levels on human monocytes. Moreover, we determined whether repeated nicotine exposure rendered human monocytes more susceptible to cholinergic immunomodulation.

We show here that pre-exposure with nicotine as well as tobacco smoking specifically elevates nAChR \textit{CHRNA7} expression on human monocytes. However, this nicotine priming or cigarette smoking does not consistently affect LPS-induced TNF production in human monocytes or whole blood samples. In addition, we demonstrate that human peripheral blood monocytes show a large intra-individual variation in response to nAChR activation or high fat nutrition, which has been put forward as a modulator of the cholinergic anti-inflammatory pathway\textsuperscript{27}.

**MATERIALS AND METHODS**

**Cell culture and cell treatment.** The human acute monocytic leukemia cell line THP-I was cultured in RPMI 1640 (Sigma Aldrich), supplemented with 1% fetal bovine serum (FBS), 100units/ml penicillin, 100µg/ml streptomycin and 2mM L-glutamine. Cells were maintained at a recommended density between 2×10\textsuperscript{5}/ml and 1×10\textsuperscript{6}/ml at 37 °C in 5% CO\textsubscript{2}. Before experiments, cells were seeded at a density of 1×10\textsuperscript{6}/ml in 12-Well plates. Cells were incubated with different doses of nicotine (0, 1µM, 10µM) at the time-points indicated, followed by vehicle or 10ng/ml LPS challenge for two hrs. Medium was collected and TNF levels were measured using sandwich ELISA (R&amp;D Systems, Minneapolis, Michigan).

**RNA isolation and Polymeric Chain Reaction.** For RNA isolation, 1×10\textsuperscript{6} THP-I or 2×10\textsuperscript{6} whole blood isolated monocytes were centrifuged (8’ 1500rpm), washed in ice-cold PBS and total RNA was extracted using the RNasy minikit (Qiagen, Venlo, the Netherlands), according to the provided protocol. RNA concentration was determined using a nanodrop ND-1000 spectrophotometer (Thermo scientific). Subsequently, samples were treated with DNase, and reverse transcribed. Amplification from 100 ng of RNA was carried out using Reddymix (Thermoscientific) in a thermocycler with 30 cycles of denaturation at 94° for 30 seconds, annealing at 60° for 30 s, and elongation for 30 s at 72°C. The bands were visualised on an ethidium bromide-stained 2.5% agarose gel. For lightcycler analysis, The complementary DNA (cDNA; 100ng) was subjected to Light Cycler polymerase
nAChR expression and cholinergic immune-modulation in human monocytes

chain reaction (Roche, Woerden, the Netherlands) for 35 cycles at 60°C. Following primers were used: **CHRNA7**, forward: 5’-CCCAAGTGGACCAGAGTCTCAT-3’ reverse: 5’-GCCACACACTACCCCAGAGT-3’. **CHRFAM7A**, forward: 5’-GGAGGTGAGGGGAAGATGTC -3’, reverse: 5’-CAGGTCCCTGCTGACTCAGGT -3’. **CHRNB2** forward: 5’-TGGAAGATTATCGCCTCAC -3’, reverse: 5v- AGACCACCGCATTGGAATAG -3’. **B2M** was used as a reference gene.

**Volunteers olive oil study.** 11 healthy volunteers (6 male, 5 female; aged 25-35) were recruited. All volunteers refrained from caffeine/alcohol-containing beverages and food for at least 12 hrs before blood collection. At 8.30 a.m., 60ml of venous blood was drawn from the cubital vein into sodium heparine tubes (Vacutainer system, BD biosciences, Plymouth, UK). Fifteen minutes afterwards, 50ml (44g) of olive oil (extra virgin) was orally administered to the volunteers in a single dose. Thirty minutes after intake, another 60ml of venous blood was drawn. Characteristics of the 11 healthy subjects considering gender, age and smoking habits are represented in table 1.

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**Table 1: characteristics healthy subjects**

Characteristics of the eleven healthy volunteers that participated in the study considering gender, age and smoking. Four of the eleven healthy volunteers (subject 1-4) were regular cigarette smokers.

**Whole blood assay.** Whole-blood was diluted 1:1 in RPMI 1640 medium supplemented with antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, (Sigma-Aldrich, Mannheim, Germany)) and L-glutamine. The blood was incubated in 96 wells plates (total volume 200μL/Well) and pretreated with nicotine (0-1000nM) or vehicle. Thirty minutes after nicotine pretreatment, whole blood samples were stimulated with 10ng/ml LPS for 4h. TNF levels were determined using sandwich ELISA.

**Monocyte isolation.** For the isolation of monocytes of the eleven healthy volunteers, 50ml of anti-coagulated blood was diluted with buffered saline (PBS). Aliquots (25ml) of this suspension were layered over an equal volume of Ficoll-Hypaque (GE.
Healthcare, Little Chalfont, U.K.) and gradients were subjected to centrifugation (800xg for 20min at RT). PBMC were washed three times in sterile PBS, after which they were counted. CD14+ cells were positively separated by high-gradient magnetic sorting using the MIDI-magnetic cell sorting (MACS) and Clini-MACS techniques according to the protocol provided by the manufacturer (Miltenyi Biotec, Bergisch Gladbach, Germany). In short, PBMC were resuspended in MACS buffer (0.5% BSA, 2mM EDTA in PBS) at a density of 12.5 × 10^7 cells/mL. CD14 MicroBeads (20 μL/10^7 cells) were added and the samples were kept at 4 °C for 15 min. Subsequently, cells were washed in 10 mL buffer by centrifugation (8 min, 300 × g, 4 °C), resuspended in buffer (500μL/10^8 cells) and transferred to LS columns placed in the magnetic field of the MACS separator. CD14+ positive cells were selected by adhesion to the magnetized column, supernatant containing unselected cells was discarded, the labeled and positively enriched cells were eluted after removal of the columns from the magnetic device.

**Statistical analysis.** Statistical analysis was performed in Prism (Graphpad) version 4. The Friedman 2-way analysis of variance was used to explore multiple dependent value assays. If the Friedman analysis was significant, individual values compared with the saline were tested with a Mann-Whitney U test. P values < .05 were considered statistically significant, and results were expressed as mean ± SEM.

**RESULTS**

**Nicotine significantly reduces TNF production in human monocytes**

To determine whether nicotine reduced TNF production in human monocytes, we pre-incubated the human monocyte THP-I cell-line with 1 or 10 μM of nicotine, prior to 3hrs of LPS challenge (10ng/ml). In line with previous data^{28}, nicotine caused a concentration dependent inhibition of the LPS-induced TNF production in THP-1 cells. At the highest concentration of 10µM nicotine, TNF production was significantly reduced from 100,0 ± 5.4% down to 68,3 ± 3,4% of vehicle (Fig. 1).

**Nicotine priming induces CHRNA7 expression in human monocytes**

As is shown in figure 2A, THP-I cells express both CHRNA7 and CHRFAM7A although the expression if the CHRFAM7A is 7 fold higher than the CHRNA7 expression. CHRNA7 expression levels are low, but not undetectable, which is in contrast with earlier studies^{29} that reported a total absence of CHRNA7 expression in THP-1 cells.

To relate the CHRNA7 transcriptional expression pattern with the anti-inflammatory effect of nicotine exposure in smokers, we next hypothesized that
chronic exposure to nicotine would induce \textit{CHRNA7} expression on human immune cells. To test this hypothesis, we primed human THP-I monocytes with vehicle or nicotine (10μM) for 20 minutes. After 24 hrs, cells were re-exposed to nicotine or vehicle for another 3 hrs, subsequently cells were challenged with 10ng/ml LPS for 2 hrs. Expression levels of \textit{CHRNA7} and \textit{CHRFAM7A} were determined using PCR and bands were visualized on an agarose gel. As is shown in figure 2B, nicotine priming at day 1, followed by 3hrs of nicotine re-exposure at day 2, induced \textit{CHRNA7} expression in THP-I cells. In figure 2C, where RNA expression levels were quantified using LightCycler analysis, we showed that short (20 min.) pre-incubation with nicotine, or single 3hrs exposure, did not alter \textit{CHRNA7} expression levels in monocytes. However, when cells were primed with nicotine for 20 minutes and re-exposed to nicotine for 3 hrs 24hrs later, expression levels of \textit{CHRNA7} were significantly enhanced (Fig 2C). As the \textit{CHRNA7} has been associated with inflammatory responses, we wondered whether expression of \textit{CHRNA7} might be modified by pro-inflammatory stimuli. Therefore, we primed THP-I cells with nicotine (20 min.) or vehicle, re-exposed them with nicotine for 3hrs and subsequently stimulated them with 10ng/ml LPS for two hours. In LPS stimulated THP-I cells, \textit{CHRNA7} expression was significantly down-regulated as compared to non LPS challenged cells. When cells were primed and re-exposed to nicotine before endotoxin challenge, this down-regulation was not significant anymore, indicating that repeated nicotine exposure also induces \textit{CHRNA7} expression in LPS-treated cells.

Besides \textit{CHRNA7}, we also determined effects of nicotine exposure on expression levels of the \textit{CHRFAM7A}. Nicotine priming or re-exposure to nicotine did not alter \textit{CHRFAM7A} expression levels, as is shown in Fig 2B and Fig 2D. In line with a recent
Chapter 7

Figure 2: 

**Figure 2: CHRNA7 and CHRFAM7A expression in THP-1 cells after nicotine exposure.** (A). mRNA of THP-1 cells was isolated and expression levels of CHRNA7 and CHRFAM7A were determined using lightcycler experiments. B2M was used as a reference gene. (B). THP-1 cells were primed with vehicle or 10µM nicotine at day 1 for 20' and washed afterwards. 24 Hrs later, at day 2, cells were treated with vehicle or nicotine for 3hrs, washed and challenged with 0 or 10ng/ml LPS for 2hrs. A scheme of the experiment set-up is represented in figure 2B, right panel. RNA was isolated and CHRN7A and CHRFAM7A expression levels were determined using RT-PCR. PCR products were visualized on agarose gel electrophoresis. (C and D) CHRN7A (C) and CHRFAM7A (D) mRNA levels were expressed as percentage of B2M expression. Data are representative of three independent experiments in duplicate, Asterisks indicate significant (p<0.05) differences from vehicle treated and unstimulated cells using Mann-Whitney U test.
study by Benfante et al, the levels of the *CHRFAM7A* transcript were significantly down-regulated in the LPS-treated cells to about 60% of the untreated cells.\(^3\)

**Repeated nicotine exposure does not reduce LPS-induced TNF levels in human THP-I cells**

Next, we determined the effect of nicotine priming, followed by nicotine re-exposure on cytokine production in human THP-I cells. Again, cells were primed with nicotine for 20 min., re-exposed to nicotine 24hrs later for 3hrs, followed by 2hrs LPS challenge (10ng/ml). In figure 3, it is shown that nicotine exposure for 3hrs on day 2 reduced LPS-induced TNF production from 100±2.3% to 68±7.9%. Nicotine priming followed by nicotine re-exposure did attenuate LPS-induced TNF production in THP-I cells, but this reduction did not reach significance. These data suggest that enhanced *CHRNA7* expression induced by nicotine priming and re-exposure, does not result in a significant reduction in LPS-induced TNF production.

**CHRNA7 is present on monocytes of cigarette smokers, but not on monocytes of non-smoking individuals**

Since nicotine priming enhanced *CHRNA7* expression in human THP-I cells, we questioned whether *CHRNA7* expression levels are elevated in human monocytes of regular cigarette smokers as compared to non-smokers. To address this, we collected whole blood samples from 11 healthy volunteers. The characteristics considering gender, age and smoking habits are represented in table 1. We isolated monocytes from these whole blood samples and quantified the RNA levels of *CHRNA7*.

![Figure 3](image.png)

*Figure 3: Nicotine priming does not affect LPS-induced TNF production in THP-I cells. Human THP-I monocytes were pre-incubated for 20 minutes with 10 uM nicotine or vehicle at day 1. At day 2 (24 hrs later), cells were re-exposed with 10uM nicotine for 3hrs, followed by 2hrs of saline, or 10ng/ml LPS stimulation. Medium was harvested for ELISA to measure TNF production. Data are mean ± S.E.M. of two independent experiments done in duplicate. *, \(P < 0.05\)*
Chapter 7

CHRFAM7A and CHRN2. mRNA expression levels of all receptors were very low and hardly detectable in monocytes. Interestingly, CHRNA7 expression was only observed in monocytes of the cigarette smoking individuals (Fig. 4; subj.1-4), while the other 7 subjects lacked expression of this receptor. On the other hand, CHRFAM7A was detected on monocytes of ten individuals, whereas CHRN2 was expressed in monocytes of two volunteers (Fig. 4; subj. 3 and 11). These data indicate that cigarette smoking enhances expression of CHRNA7 on circulating monocytes.

Nicotine effects on whole blood TNF release vary per individual

To determine the effect of nicotine on LPS-induced TNF production in whole blood samples of the healthy volunteers, we pre-incubated whole blood samples for 30 min with different concentrations of nicotine (0, 100 or 1000 nM), followed by 4 hrs LPS stimulation (10 ng/ml). Nicotine significantly reduced TNF production in whole blood samples of four healthy subjects, varying from ~56% to ~97% reduction in TNF release at a concentration of 1000 nM nicotine (Fig. 5, subjects 1, 5 and 10). However, in whole blood samples of seven healthy subjects, LPS-induced TNF levels were unaffected by nicotine, whereas one person showed a significant increase in TNF release (210±31.0% increase; Fig. 5, subject 2). Remarkably, in whole blood samples of the four smokers (subject 1-4), nicotine reduced LPS-induced TNF production in only one person, was ineffective in two others, while it enhanced TNF production in another. These data indicate that, despite the finding that cigarette smoking induced expression of

Figure 4. nAChR expression on monocytes of 11 healthy volunteers. nAChR expression levels on circulating monocytes of eleven healthy volunteers. Monocytes were isolated from whole blood samples from 11 healthy volunteers. Subject 1-4 are regular cigarette smokers, while the other 7 are non-smokers. The presence of CHRNA7 (black bars), CHRFAM7A (grey bars) and CHRN2 (white bars) mRNA was determined using QPCR, presented as percentage B2M expression.
nAChR expression and cholinergic immune-modulation in human monocytes

On monocytes, it did not render whole blood samples more susceptible to nicotinic reduction of TNF.

**CHRNA7** on monocytes, it did not render whole blood samples more susceptible to nicotinic reduction of TNF.

**TNF levels after olive oil intake**

As high fat enteral nutrition has been put forward as a modulator of the cholinergic anti-inflammatory pathway, we tested the effect of oral ingestion of long chain fatty acids in form of olive oil on whole blood LPS-induced TNF production. A schematic

![Figure 5. Nicotinic effects on LPS-induced TNF production in whole blood samples of healthy volunteers.](image)

**Figure 5. Nicotinic effects on LPS-induced TNF production in whole blood samples of healthy volunteers.** Whole blood samples of healthy volunteers were incubated with nicotine (0-1000nM) for 30 min, followed by 4hrs LPS stimulation (10ng/ml). TNF levels were measured using ELISA, and represented as % of vehicle treatment. Experiment was performed in quadruple, Asterisks indicate significant differences (*p<0.05).

**Figure 6. Design olive oil study.** Figure 6 represents a study design of the olive oil study. Eleven healthy volunteers participated in the study, they refrained from food and caffeine/alcohol containing beverages for 12 hrs. At t=0, 60ml of venous whole blood was taken, followed by 50ml oral ingestion of extra virgin olive oil at t=15'. Another 60ml of whole blood was drawn at t=30'. Whole blood was diluted 1:1 with RPMI medium, cells were plated and cells were pre-incubated with 10µM nicotine at t=1hr, followed by 10ng/ml LPS stimulation at t=1,5hr. After 4 hrs, medium was harvested and TNF levels were measured using ELISA.
Chapter 7

Overview of the study design is represented in figure 6. Eleven healthy volunteers refrained from food and caffeine/alcohol-containing beverages for at least 12 hrs before whole blood collection. Whole blood samples were challenged with 10ng/ml LPS for 4hrs before and after oral olive oil administration.

In three healthy subjects, LPS-induced TNF production was significantly diminished after olive oil intake, with a maximum inhibition of 50.4±7.7% (Fig.7; subjects 1,5 and 6). On the other hand, in three volunteers, oral administration of 44(g) olive oil enhanced endotoxin-challenged TNF release up to 249±46% (Fig. 7; subjects 4, 10 and 11), whereas olive oil administration had no effect in whole blood stimulation in the other five subjects. Again, no difference was observed between olive oil effects on whole blood samples of smoking subjects as compared to non-smokers.

DISCUSSION

The finding that nicotine inhibits activation of immune cells, together with the observation that vagus nerve signaling or specific CHRNA7 agonists attenuate disease in several inflammatory animal models 31-34, implies that therapeutic agents modifying cholinergic signaling might be beneficial in humans. However, since vagus nerve stimulation in humans is a rather invasive procedure, data on human studies are limited.
Cigarette smoking is an important environmental factor in ulcerative colitis (UC). Ulcerative colitis patients with a history of smoking usually acquire their disease after they have stopped smoking \(^{35-37}\) and patients who smoke intermittently often experience an improvement in their colitis symptoms during the periods when they smoke \(^{38-40}\). The reason behind the protective effect of smoking in UC remains obscure. The effects of smoking and nicotine are numerous and since the pathogenesis of inflammatory bowel disease is only partially understood, any discussion on the possible mechanisms can only be speculative. Potentially, nicotine in cigarette smoke can restrain the immune response in UC via activation of nicotinic acetylcholine receptors (nAChRs) on immune cells, which may explain its protective effect. Given the purported role of 41 in mediating the cholinergic anti-inflammatory pathway, we hypothesized that chronic nicotine exposure induced 41 expression on human monocytes.

In this study, we demonstrated that repeated nicotine exposure up-regulated 41 expression on THP-I monocytes. In conjunction, in a volunteer study, 41 was only detectable on monocytes of smoking individuals, while it was lacking on monocytes of non-smokers. These data suggest that enhanced 41 monocyte expression by repeated nicotine exposure could be important in the protective effect of smoking in UC. However, enhanced 41 expression did not render human monocytes more susceptible to nicotinic immune-modulation. Seemingly, the 41 is not the only mediator effectuating the cholinergic anti-inflammatory effect and another nAChR might be involved. For example, although it remains unclear whether the 42 is appropriately translated and processed to a functional receptor, the finding that 42 is down-regulated in monocytes by LPS challenge suggests that it could play a role in inflammation. Benfante et al speculated that receptors of mixed subunits of 42 and 42 are formed that can modulate the signaling potential of immune cells to respond to ACh released from the vagus nerve during infection.

In human subjects, we failed to demonstrate a direct relation between 41 expression and TNF release. In this respect, it should be noted that alternative mechanisms may play a role vagal immuno-modulation in vivo. Recent data indicate for instance that the spleen may play a role in effectuating the anti-inflammatory effects of vagus nerve activity, as electrical vagus nerve stimulation fails to attenuate serum TNF levels in splenectomized mice treated with endotoxin. The authors propose that ACh released by the vagus nerve does not reach the spleen directly, but acts on 41 at the level of the ganglia of the celiac-superior mesenteric plexus to modulate splenic nerve function. They suggest that attenuation of TNF production by spleen macrophages induced by vagus nerve stimulation is mediated by norepinephrine released from splenic nerve endings. These data put the role of the 41 in a new perspective; although in vitro ACh modulates macrophage function via 41, the in vivo effects of vagus nerve stimulation may rely on 41 on neurons, rather
than on monocytes or macrophages. This implies that, although nAChR expression on circulating monocytes in humans is rather low, cholinergic activation could still display *in vivo* immuno-modulating functions via neuronal nAChRs.

We tested the effect of nicotine pretreatment on TNF production by endotoxin-stimulated whole blood samples of 11 healthy volunteers. We choose to work with whole blood samples since this was the closest to the *in vivo* situation, and cytokines produced from whole-blood were found to be strongly correlated with monocytic cytokines. Nicotine dose-dependently reduced TNF production in 4 of 11 human whole blood samples, whereas in one sample, TNF release was enhanced. Other studies report that in other cell types, such as peripheral blood derived human macrophages and mononuclear cells, nicotine reduced TNF production in all samples. On the other hand, Kox et al. published that nicotine attenuated TNF release equally on PBMCs, monocytes and human whole blood samples, but only at a high dosage of 1 mM. These differences may be due to differential nAChR expression, since it is demonstrated that nAChR expression on human leukocytes may vary due to genetic or environmental factors. Indeed, we observed that *CHRNA7*, *CHRFA7A* and *CHRNB2* expression varied between individuals. However, expression of all nAChRs tested was extremely low and hard to detect. Moreover, we found no correlation between nAChR expression levels and nicotine effects.

Activating the so called ‘cholinergic anti-inflammatory pathway’ via electrical vagus nerve stimulation, or the use of applied cholinergic agonists targeting distinct nAChR subtypes, could be a potential therapeutic asset in the treatment of inflammatory disorders in human. However, more physiological applied high-fat enteral nutrition can activate this ‘cholinergic anti-inflammatory pathway’ as well. High-fat enteral nutrition, sensed in the gastrointestinal tract, activates the parasympathetic nervous system, and leads to inhibition of the inflammatory response by way of efferent vagal fibers. In addition, enteral nutrition with long chain fatty acids in form of olive oil suppresses LPS-induced TNF release of macrophages in the gut wall in mice. Moreover, extra virgin olive oil enriched diet (10%) showed protective effects in experimental mouse models of DSS-colitis and endotoxic shock. In our study, olive oil administration reduced LPS-induced TNF production in whole blood samples of four healthy subjects, while it enhanced TNF production in three other volunteers. However, although it is presumed that olive oil stimulates the vagus nerve, we did not have a proper read out for vagus nerve activity in our study. There is evidence that exposure to fat for only three minutes is sufficient to elicit a pancreatic polypeptide response. Secretion of pancreatic polypeptide, a polypeptide secreted by pancreatic peptide cells in the endocrine pancreas, is regulated by cholinergic stimulation. In the future, measurement of pancreatic polypeptide serum levels would be indicative of vagus nerve activity. On the other hand, it is questionable whether acetylcholine released from vagus nerve termini upon olive oil administration, actually reaches immune cells in the blood. However, olive oil
administration did alter TNF production in whole blood samples of seven volunteers, indicating that olive oil can modulate the immune response in whole blood. If this is indeed vagus nerve mediated, and what causes the individual variation, needs to be further established.

In conclusion, we demonstrated that chronic exposure to nicotine enhances CHRNA7 expression on circulating monocytes in humans, in vitro and in vivo. However, this CHRNA7 upregulation does not render human monocytes more susceptible to cholinergic immune-modulation. Results obtained in a wide range of in vitro and in vivo models of inflammation imply that therapeutic agents targeting the cholinergic anti-inflammatory pathway can be an important asset in the treatment of immune disorders in human. Our data indicate that there might be an individual variation in response to future therapeutic agents modifying the cholinergic anti-inflammatory pathway in humans.


42. Benfante R, Antonini RA, De PM, Gotti C, Clementi F, Locati M, Fornasari D. Expression of the alpha7 nAChR subunit duplicate form (CHRFA7α7A) is down-regulated in the monocytic cell line THP-1 on treatment with LPS. J Neuroimmunol 2011;230:74-84.


