Outcomes of nicotinic modulation on markers of intestinal IgA antibody response

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Abstract. Acetylcholine (ACh), as a ligand of nicotinic acetylcholine receptors (nAChRs), plays a key role in the cholinergic anti-inflammatory pathway; however, its role in the immunoglobulin A (IgA) response remains unknown. Therefore, the present study aimed to investigate the role of ACh in the intestinal biomarkers involved in IgA synthesis and the polymeric immunoglobulin receptor (pIgR) involved in IgA transcytosis. Groups of mice were administered GTS-21 (an α7nAChR agonist) or mecamylamine (a non-selective nAChR antagonist) intraperitoneally for 7 days. Intestinal fluids were used for antibody concentration assessment by ELISA, cell suspensions from Peyer's patches and the lamina propria were obtained for flow cytometric analysis of plasma cells, and $CD4^+$ T-cells expressing intracellular transforming growth factor (TGF)-β and IgA-producing interleukin (IL)-4, -5, -6 and -10, and isolated epithelial cells to determine the levels of pIgR mRNA using reverse transcription-quantitative PCR. Regarding to the untreated control group, the concentration of IgA was reduced in the mecamylamine group and unaltered in the GTS-21 group while IgM levels exhibited no differences; the percentage of IgA^+ plasma cells from Peyer's patches and the lamina propria, and the percentage of TGF-β^+/$CD4^+$ T-cells from Peyer's patches were greater in the GTS-21-group. In both treatment groups, the percentages of IgM^+ plasma cells and IL-6^+/IL-10^+ $CD4^+$ T cells were greater in both compartments; pIgR mRNA expression levels decreased in epithelial cells. The percentage of IL-4 $CD4^+$ T-cells were greater in Peyer's patches and lower in the lamina propria in the mecamylamine group, and the percentage of IL-5 $CD4^+$ T-cells in the lamina propria were decreased in both treatment groups. These findings require further examination to address the impact of cholinergic modulation on IgA-transcytosis via pIgR. The present study may be an experimental reference for clinical trials that address the role of nicotinic system in intestinal dysfunctions as postoperative ileus.

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Introduction

The cholinergic system refers to the constituents for enzymatic synthesis, degradation, transport and receptors for acetylcholine (ACh), a neurotransmitter in neurons and non-neuronal cells (1). ACh is i) synthesized by choline acetyltransferase from choline and acetyl coenzyme A in the cytoplasm; ii) degraded by butyrylcholinesterase and acetylcholinesterase into choline and acetic acid; and iii) stored by the vesicular ACh transporter in neurons or organic cation transporters in non-neuronal cells (1,2). The actions of ACh are mediated via ligation with cholinergic receptors, including muscarinic ACh (mAChRs) and nicotinic ACh (nAChRs) receptors (2). mAChRs are G-protein coupled receptors, including inhibitory receptors (M2 and M4) that block adenylate cyclase activity, and excitatory receptors (M1, M3 and M5) that trigger the mobilization of intracellular calcium ions (3). nAChRs are ligand-gated channels of homo- or hetero-pentamers of several subunits, namely α1-α10, β1-β4, γ and δ (2). The interaction of ACh with nAChR evokes the change in subunit conformation, resulting in the opening of a non-selective cation channel to form a central pore that enables the influx of cations (1).

The cholinergic system drives the cholinergic anti-inflammatory pathway (CAIP) in the intestine through α7nAChRs

expressed on immune cells, specifically macrophages. ACh is released by vagal efferent terminal fibers (4). Unlike CAIP, which has been extensively documented in several animal models, the role of ACh and AChRs in the modulation of anti-inflammatory immunoglobulin A (IgA) remains unknown.

IgA, along with the polymeric immunoglobulin receptor (pIgR), are regarded as markers of intestinal homeostasis (5,6). Experimental assays using perfused porcine colonic explants and rat intestinal loops indicated that luminal IgA secretion is increased by mAChR agonists (carbachol, pilocarpine and bethanechol) or decreased by mAChR antagonists (atropine) (7,8). During vagotomy, neuronal inputs of the cholinergic system include downmodulation, as found in secretions of the human jejunum (9,10) and in the small intestines of mice (11,12). Vagotomy effects on IgA plasma cells also include downmodulation, as described in the lamina propria of mice (11), and increased modulation documented in the lamina propria of the rat jejunum (13).

To the best of our knowledge, the specific role of ACh in the anti-inflammatory IgA response remains unknown. Thus, the present study aimed to evaluate the outcomes of nAChR modulation in the IgA response. The present study may act as a novel theoretical basis for the development of strategies that contribute to the maintenance of IgA responses in diseases, such as inflammatory bowel diseases, in which the cholinergic system may be involved (1).

Materials and methods

Animals. BALB/c mice (age, 8 weeks; male; weight, 25-30 g) were obtained from Unidad de Production y Experimentation de Animales de Laboratorio, Universidad Autónoma Metropolitana, Unidad Xochimilco, and individually housed in acrylic cages on a 12-h light/dark cycle, with a room temperature of 20˚C and relative humidity of 55%. Water and a controlled diet, including >23% crude protein, >4.5% crude fat, <6% crude fiber, <2% moisture and <8% ash (Laboratory Rodent Diet 5001; LabDiet) were provided *ad libitum*. A 1-week period preceded any experimental intervention. All experiments were performed by a single handler and always took place prior to 10:00 a.m. The use of animals in the experimental protocols was approved (approval no. ESM-CICUAL-01/09-10-2018) by the Comité Interno para Uso y Cuidado de Animales de Laboratorio (CICUAL) of the Escuela Superior de Medicina (ESM), following the Mexican Federal regulations for animal care (NOM-062-ZOO-1999; Ministry of Agriculture, Mexico City, Mexico) and in accordance with the ARRIVE guidelines for reporting animal research (14).

Drugs. Drugs were purchased from MilliporeSigma. The vehicle for drug dissolution was sterile isotonic saline solution, and drugs were used in the following doses: GTS-21 dihydrochloride, a selective α7nAChR agonist (cat. no. 505277) at a dose of 4 mg/kg/day, and mecamylamine hydrochloride (MLA), a non-selective nAChR antagonist (cat. no. M9020) at a dose of 1 mg/kg/day (15,16). Drugs were administered once a day for 7 consecutive days via intraperitoneal injection. Isoflurane (Sofloran, Lab Pisa) was used for animal sacrifice.

Experimental model and sampling. A total of 10 mice were included in each group, and intraperitoneally injected daily with GTS-21 or MLA for 7 consecutive days. A group of mice without drug treatment was included as the control. At 24 h after the final dose, each mouse was euthanized using 5% isoflurane for 3 min, mice were subsequently exsanguinated using cardiac puncture. The whole small intestine was resected from the pyloric to the cecal junction and washed with a phosphate-buffered saline solution (PBS; pH 7.4) containing a protease inhibitor cocktail (cOmplete™; cat. no. 11836153001; Roche Diagnostics GmbH). Intestinal fluids were individually centrifuged at 10,000 x g for 20 min at 4˚C, and the supernatants were aliquoted and stored at -70˚C until further ELISA assays. Peyer's patches, the lamina propria and epithelial cells were collected and processed as previously described (17,18). Single cell suspension from Peyer's patches and the lamina propria were used for flow cytometric analysis, and epithelial cells were used for reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

ELISA. Sandwich ELISA was used to quantify the antibody concentrations using 96-microwell polystyrene plates (cat. no. 8590; Corning, Inc.). A total of 200 μl PBS containing 0.05% Tween-20 (PBST) was used for washing both before and after the incubation of plates at 4˚C or at 37˚C. A total of 100 μl PBST was used for washing of reactants and samples. Total protein was quantified using Bradford assay with commercial dye reagent (cat. no. 500-0006; Bio-Rad Laboratories, Inc.). The 96-microwell polystyrene plates were coated with 0.1 μg/ml goat anti-mouse IgA (cat. no. 626700; Invitrogen; Thermo Fisher Scientific, Inc.) or with 2.0 μg/ml goat anti-mouse IgM (cat. no. 115-001-020; Jackson ImmunoResearch Laboratories, Inc.) in carbonate-bicarbonate buffer (pH 9.6). Following overnight incubation at 4˚C and washing as previously described, non-specific binding sites were blocked with 5%/volume blotto (cat. no. 37530; Thermo Fisher Scientific, Inc.) in carbonate-bicarbonate buffer (pH 9.6). Following incubation for 2 h at 37˚C and washing, the plates were treated with intestinal fluid samples, tested in triplicate and normalized at various concentrations of total protein. Samples were dissolved in 5%/volume blotto in PBST. Following overnight incubation at 4˚C and washing, the horseradish (HRP) conjugates (both from Invitrogen; Thermo Fisher Scientific, Inc.) dissolved in 5% blotto/PBST, 1:5,000 goat anti-mouse IgA-HRP (cat. no. 626720) or 1:5,000 goat-anti mouse IgM-HRP (cat. no. PA184383) were added and incubated for 1 h at 37˚C. Subsequently, the plates were washed and the substrate solution [hydrogen peroxide and o-phenylenediamine in citrate phosphate buffer (pH 5.0)] was added, followed by incubation for 20 min in the dark at room temperature. The enzymatic reaction was terminated using 2.5 M sulfuric acid and the absorbance was measured at 492 nm using a microplate reader (EpochTM; BioTek Instruments, Inc.). Standard curves with purified α chain of mouse IgA (cat. no. M1421; MilliporeSigma) or mouse IgM (cat. no. CLCMGM00; Cedar Lane Labs) were prepared to quantify total IgA and IgM in μg per 100 mg of total protein.

Flow cytometry. A single suspension procedure was achieved according to a previously described protocol (17,18). Cell

suspensions including Peyer's patches and the lamina propria cells were adjusted to a final concentration of $1x10^6$/ml in PBS. Lymphocytes were stained at 4˚C for 25 min in PBS and 0.01% sodium azide. Fluorochrome-tagged antibodies for surface markers used [all from BD Biosciences, except the transforming growth factor (TGF)-β-APC from BioLegend, Inc.] were as follows: Anti-CD4 PERCP (1:20) (cat. no 553052), anti-CD8 FITC (1:10) (cat. no. 553030), anti-CD3 APC (1:20) (T-cells; cat. no 565643), anti-CD19 PE (1:20) (cat. no 557399), anti-CD138 APC (1:10) (cat. no 558626), anti-IgM PeCy7 (1:20) (cat. no. 552867) and anti-IgA FITC (1:20) (B-cells and plasma cells; cat. no. 559354). To detect intracellular cytokines, fixation and permeabilization were carried out using a BD Cytofix/Cytoperm kit (cat. no. 554714; BD Biosciences) and the following antibodies: IL-4 PE (1:20) (cat. no. 554389), IL-5 PE (1:20) (cat. no. 554395), IL-6 APC (1:20) (cat. no. 561367), IL-10 FITC (1:20) (cat. no. 554466) and TGF-β-APC (1:10) (cat. no. 1406). The fluorescent signal intensity was recorded and analyzed using a FACSCalibur flow cytometer (BD Biosciences). Events were collected from the lymphocyte gate on the forward scatter area/side scatter area dot plot. A total of 20,000 gated events were acquired from each sample using BD FACSDIVA software version 6.1 (BD Biosciences). Data were analyzed using Summit software version 4.3 (Dako; Agilent Technologies, Inc.).

RT-qPCR for pIgR. The mRNA expression of pIgR was analyzed in epithelial cells isolated following discontinuous 20% percoll gradient, as previously described (17,18). RT-qPCR was performed to assess the expression of pIgR. Briefly, total RNA was extracted from epithelial samples using TRIzol® reagent (50 mg/1 ml) according to the manufacturer's protocol (cat. no. 15596018; Invitrogen; Thermo Fisher Scientific, Inc.). The purity and concentration of RNA were determined spectrophotometrically using Nanodrop Lite (Thermo Fisher Scientific, Inc.). Samples with an A260/280 ratio of 1.8-2.0 were included. RNA samples were loaded onto 1.5% gels for electrophoresis and stained with ethidium bromide to visualize RNA integrity on a transilluminator (Fusion SL; Vilber Lourmat Deutschland GmbH). Reverse transcription using RNA as the template was performed using a cDNA synthesis kit Transcriptor First Strand cDNA for RT-qPCR (cat. no. 04896866001; Roche Diagnostics GmbH) as per the manufacturer's instructions. For cDNA amplification, TaqMan Master reaction mix (cat. no. 04535286001; Roche Diagnostics GmbH) was used as per the manufacturer's instructions. Amplification was conducted on a Techne Prime Pro48 Real-Time qPCR system (Thermo Fisher Scientific, Inc.) using the following conditions: Initial denaturation for 10 min at 95˚C, followed by 50 cycles of 10 sec at 94˚C, 20 sec at 90˚C and 5 sec at 72˚C. The nucleotide sequences of primers used for RT-qPCR were as follows: forward, 5'-CTGTGCCCGAAACTGGAT-3' and reverse, 5'-TCAGGT TGGCTTCTTGTATGAG-3', and these were designed using Probe Finder version 4.5 (http://www.universalprobelibrary.com). The samples were analyzed in quintuplets, and the relative gene expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method and normalized to the level of the 18S ribosomal RNA subunit (forward, 5'-GCCGCTAGAGGTGAAATTCTT-3' and reverse, 5'-CGTCTTCGAACCTCCGACT-3') (19).

Statistical analysis. A total of three independent experiments were performed (10 mice per group for each experiment), and data are presented as the mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. All analyses were carried out using GraphPad Prism version 8.0.2 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Antibody and plasma cell determination. In the present study, total IgA and antibody-secreting cells were evaluated as markers of intestinal homeostasis (5,6). Compared with the control group, the concentration of IgA was not significatly different following treatment with GTS-21, and decreased ($P<0.05$) following treatment with MLA (Fig. 1A). On the other hand, IgM concentration was not significatily different following treatment with either drug (Fig. 1B).

Plasma cell responses were analyzed in Peyer's patches as the main compartment of the gut-associated lymphoid tissue (GALT), and in the lamina propria as the effector site of the intestine (5). Compared with the control group, the percentage of IgA^+ plasma cells were significantly increased in Peyer's patches and the lamina propria following treament with GTS-21 ($P<0.001$) (Fig. 1C and E). Moreover, the percentage of IgM^+ plasma cells was significantly increased in these compartments, following treatment with either drug ($P<0.001$) (Fig. 1D and F).

Intracellular IL assessment in $CD4^+$ T-lymphocytes. IgA generation requires TGF-β, which determines the IgM^+ B to IgA^+ B cell class switch. It also requires IL-4, -5, -6 and -10, which reinforce the class switch, and favor the clonal expansion of IgA^+ B cells and their maturation in IgA^+ plasma cells (5,20). In the present study, flow cytometry was performed to determine the number of $CD4^+$T lymphocytes. The flow cytometry gating strategy is illustrated in Fig. 2A and a representative dot-plot from Peyer's patches is presented in Fig. 2B. Compared with the control group, intracellular TGF-β in $CD4^+$T lymphocytes was significantly increased ($P<0.001$) in Peyer's patches following treatment with GTS-21 (Fig. 2C), and significantly decreased ($P<0.001$) in the lamina propria following treatment with MLA or GTS-21 (Fig. 2D). Compared with the control, IL-6 and -10 $CD4^+$ T-cell responses were significantly increased ($P<0.001$) in Peyer's patches (Fig. 3A) and the lamina propria (Fig. 3B) following treatment with MLA or GTS-21. Compared with the control, the IL-4 $CD4^+$ T-cell response was increased ($P<0.001$) in Peyer's patches (Fig. 3A) and decreased ($P<0.001$) in the lamina propria following treatment with MLA (Fig. 3B). On the other hand, the IL-5 $CD4^+$ T-cell response was not significatly changed in Peyer's patches (Fig. 3A), and decreased ($P<0.001$) in the lamina propria following treatment with MLA or GTS-21 (Fig. 3B).

mRNA expression of pIgR. pIgR involved in IgA-transcytosis acts as a marker of homeostasis (6,21), and was therefore analyzed in the present study. The results indicated that compared with the control group, the mRNA expression of pIgR was significantly decreased in both drug-treated groups ($P<0.001$) (Fig. 4).

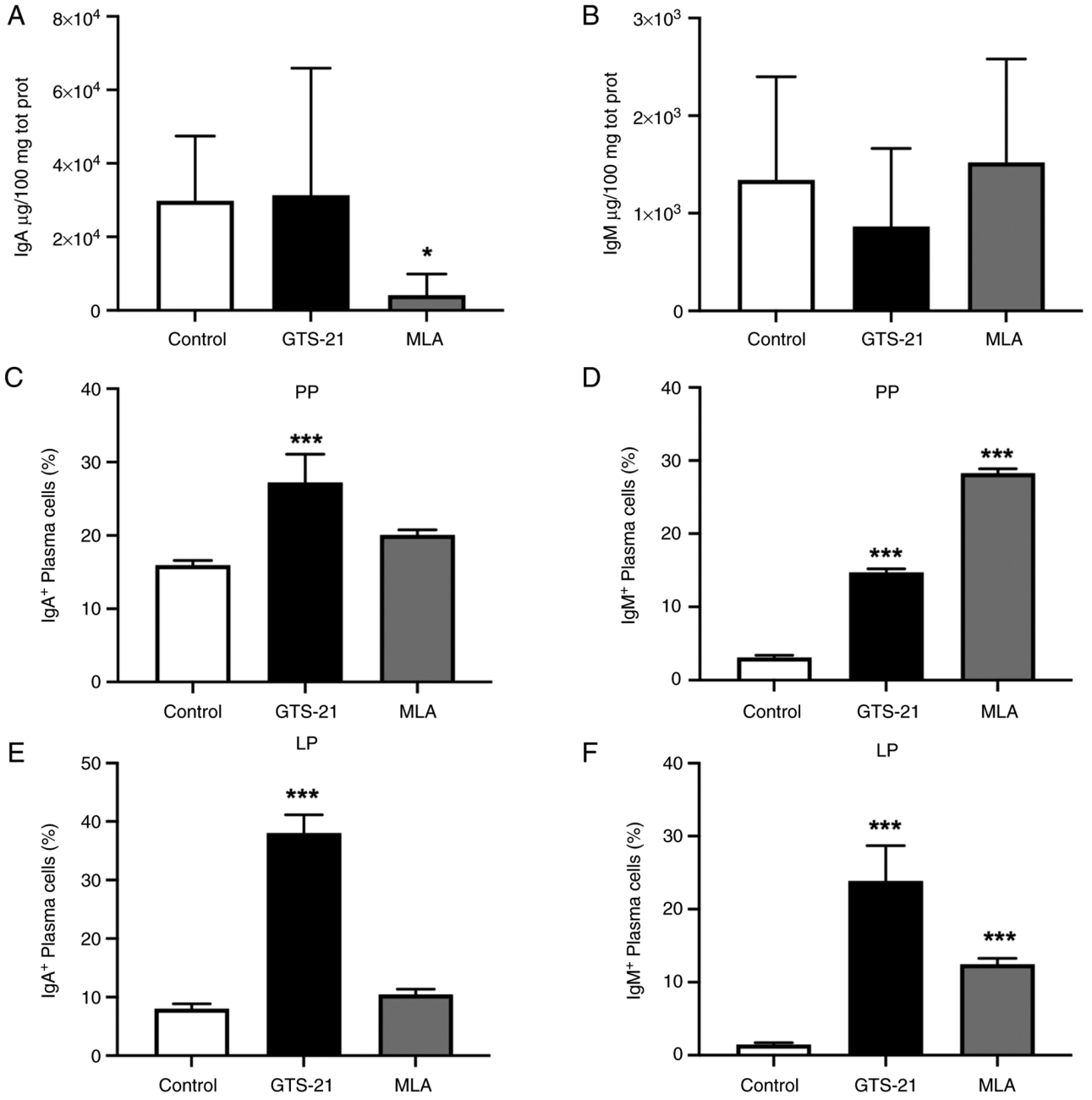

Figure 1. Antibody concentration (μg/100 mg total protein) of (A) total IgA and (B) total IgM in the intestinal secretions of the control, GTS-21 (α7AChR agonist) and MLA (non-selective nAChR antagonist) treatment groups. Percentage of (C) IgA$^+$ and (D) IgM$^+$ plasma cells in PP, and (E) IgA$^+$ and (F) IgM$^+$ plasma cells in the LP from the same groups. Results from one representative experiment (from three replicates) using 10 mice per group are expressed as the mean ± standard deviation. *P<0.05 and ***P<0.001 vs. the control group. MLA, mecamylamine; Ig, immunoglobulin; PP, Peyer's patches; LP, lamina propria.

Discussion

The impact of the cholinergic system on the IgA response has previously been addressed through analyzing mAChR modulation in terms of IgA secretion (7,8,22,23). Moreover, the role of the cholinergic system on IgA secretion and/or generation has been addressed in a limited number of vagotomy settings in humans (9,10), mice (11,12) and rats (13). To the best of our knowledge, the present study was the first to analyze the outcome of nAChR modulation on the IgA response and IgA-associated parameters in Peyer's patches and the lamina propria. The IgA generation via T-cell dependent mechanisms occurs at Peyer's patches as the main compartment of the GALT, whereas T-independent generation occurs in the lamina propria, a prominent intestinal effector site (5,20).

The present study demonstrated that IgA concentration was decreased following treatment with MLA (a non-selective nAChR antagonist). The down-modulatory effect on IgA secretion via nAChRs has also been reported through blocking mAChRs using atropine, a non-selective mAChR antagonist, as documented in perfused porcine colonic explants and intestinal loops of rats (7,8). In murine models of vagotomy, a decreased IgA concentration supports the neuronal inputs of the cholinergic system for IgA generation (11,12).

In the present study, GTS-21, a partial α7nAChR agonist, did not significantly alter IgA concentration, suggesting

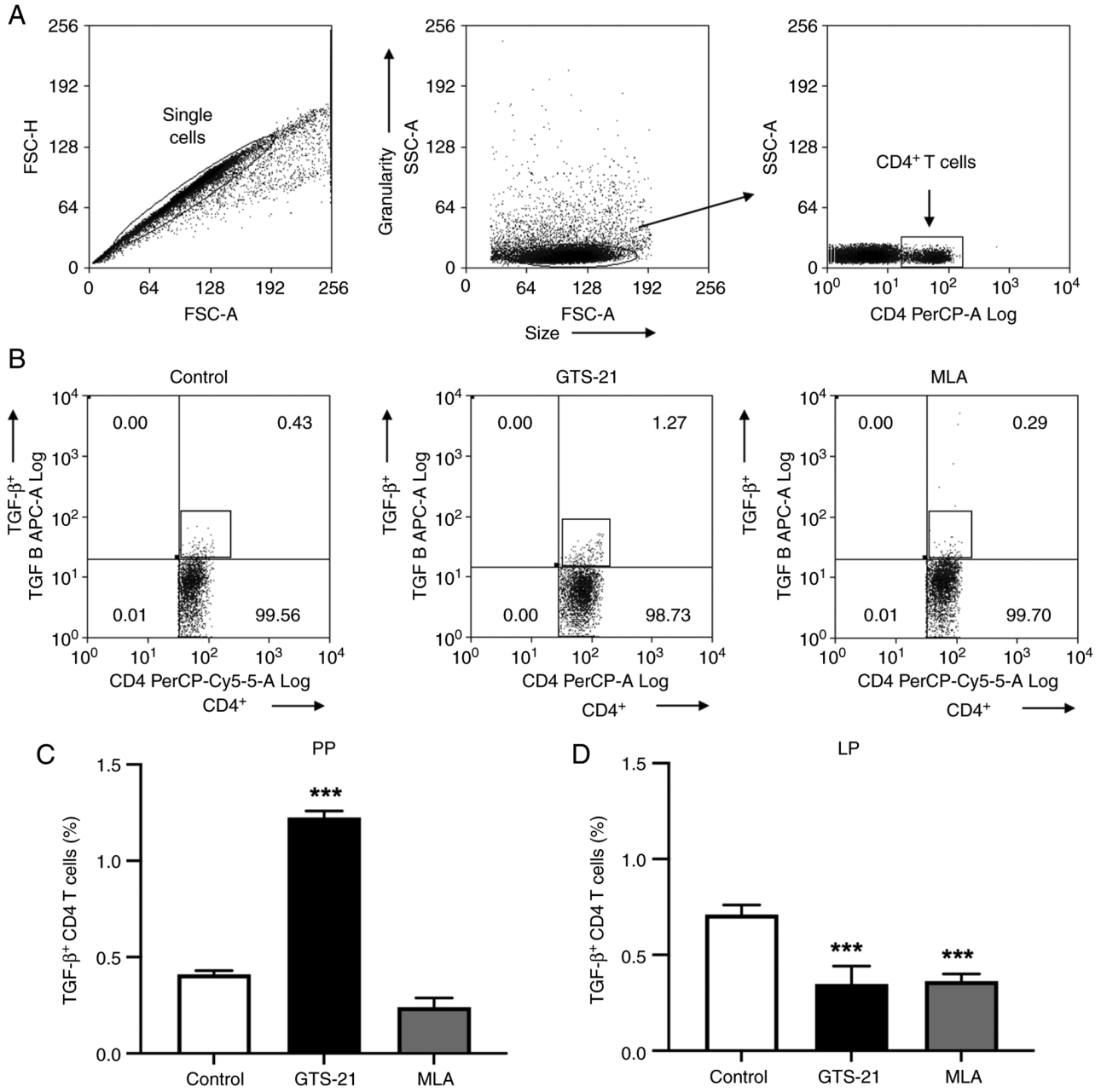

Figure 2. Representative cytometric analysis using TGF-β+ cells as a model. (A) Percentage of TGF-β+/CD4+ T-cells in the control, GTS-21 and MLA groups. Single cells were gated using FSC-A and FSC-H. Cells were gated using FSC-A and SSC-A based on size and granularity to determinate lymphocyte region. CD4+ T-cells were selected for further analysis. (B) The same strategy used for TGF- β+ was applied for the CD4+ T-cell expression of all intracellular cytokines analyzed. Percentage of TGF-β+/CD4+ T-cells expressed in (C) Peyer's patches and (D) the lamina propria. Results from one representative experiment (from three replicates) using 10 mice per group are expressed as the mean ± standard deviation. ***P<0.001 vs. the control group. TGF-β+, transforming growth factor β; MLA, mecamylamine; FSC-A, forward scatter area; FSC-H, forward scatter height; SSC-A, side scatter area.

that other α-nAChR subunits may be required to provide inputs for eliciting IgA secretion. The complete role of the cholinergic system is yet to be fully established; however, it appears to control IgA output through a complex interplay with enteric peptides, such as somatostatin, as described in isolated perfused porcine ileum (22) and cholecystokinin, as found in the intestinal mucosa of rats sensitized to ovalbumin (23). Data analysis evidenced no significant changes in IgM concentration were observed following treatment with MLA or GTS-21. These findings are comparable with those of a previous study, in which the unaltered IgM response in intestinal secretions was observed in the whole small intestine of mice that underwent posterior vagotomy (12). Although nAChR signals did not affect IgM secretion, the results of a previous study using regionalized analysis demonstrated that IgM concentration was triggered in the proximal and distal segments of the small intestine of mice that underwent anterior vagotomy (11). Thus, neuronal cholinergic inputs may differentially drive the IgM response in each region of the small intestine.

The analysis of antibody-secreting cells in both compartments indicated that the percentage of IgA+ plasma cells was elicited following treatment with GTS-21, whereas the percentage of IgM+ plasma cells was triggered following

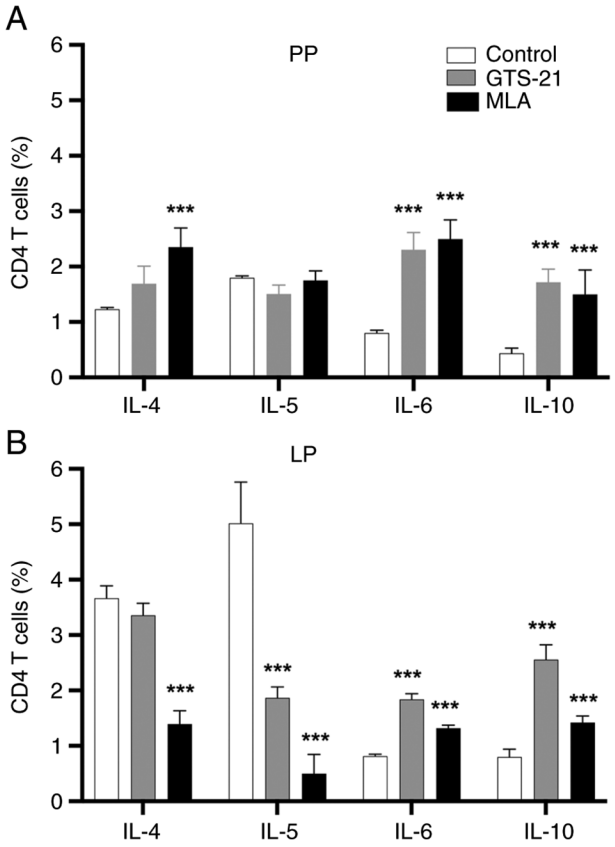

Figure 3. Percentage of CD4$^+$ T-cells expressing IL-4, -5, -6 and -10 in (A) PP and (B) the LP. Results from one representative experiment (from three replicates) using 10 mice per group are expressed as the mean ± standard deviation. ***P<0.001 vs. the control group. IL, interleukin; PP, Peyer's patches; LP, lamina propria.

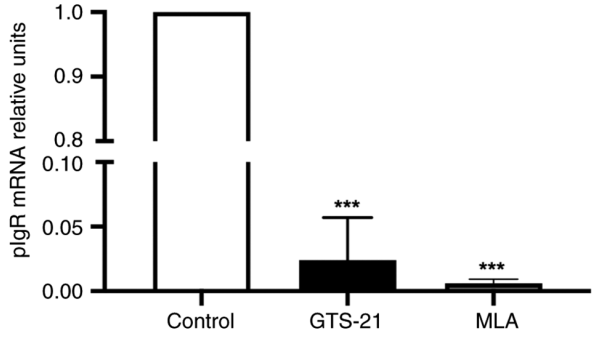

Figure 4. mRNA expression of pIgR in epithelial cells from the small intestine of the control, GTS-21 and MLA treatment groups. Results from one representative experiment (from two replicates) using 10 mice per group are expressed as the mean ± standard deviation. ***P<0.001 vs. the control group. pIgR, polymeric immunoglobulin receptor; MLA, mecamylamine.

treatment with MLA or GTS-21. The results of a previous study demonstrated that the increase in the number of IgA$^+$ plasma cells in the jejunal lamina propria was ascribed to an impaired intestinal motility in rats that underwent both proximal gastric vagotomy and pyloroplasty (13). Moreover, cholinergic modulation on antibody-secreting cells encompassed both up- or downmodulating effects, according to the compartment (Peyer's patches or the lamina propria) and the region of the small intestine (proximal or distal), as documented in vagotomized mice (11,12). Thus, cholinergic inputs may differentially drive the plasma cell response in each region of the small intestine.

In the present experimental setting, flow cytometric analysis of intracellular ILs demonstrated that the percentage of CD4$^+$ T-cells expressing TGF-β was increased in Peyer's patches following treatment with GTS-21, or decreased in the lamina propria following treatment with either drug. The results of a previous study demonstrated that the percentage of TGF-β$^+$/CD4$^+$ T-cells was significantly reduced in the proximal and distal small intestine of mice that underwent anterior vagotomy (11). The present study, for the first time to the best of our knowledge, demonstrated that nAChRs provide signals at Peyer's patches to modulate the cells expressing TGF-β, a critical factor that determines the class switch of IgM$^+$ B-cells to IgA$^+$ B-cells (5,20). In contrast to CD4$^+$ T-cell responses expressing TGF-β, the impact of GTS-21 or MLA on CD4$^+$ T-cells expressing IL-4, -5, -6 and -10 involved in IgA-generation was not observed (Fig. 3A and B). The mechanisms underlying these findings are unknown; however, they may mirror the complex interplay of non-neuronal and neuronal nAChR signals that drive divergent outcomes in inductive or effector sites in each region of the small intestine. The latter premise is based on findings derived from regionalized analysis in mice that underwent anterior vagotomy, in which the percentage of CD4$^+$ T-cells expressing IgA-producing ILs was modulated divergently in each region of the small intestine (11).

In the current assay, pIgR mRNA expression levels were suppressed following treatment with MLA or GTS-21. The effects of the cholinergic system on pIgR expression via nAChR regulation has not yet been fully elucidated. However, the results of a previous study demonstrated that carbachol, a non-selective mAChR agonist, increased both anion transport and pIgR-mediated IgA transcytosis. Notably, these events are independent of each other (7). To the best of our knowledge, the regulation of IgA transport by assessing pIgR expression with nAChR agonist is unknown. Thus, the role of Ca^{2+} intracellular pathway signaling after nAChR activation on IgA transport by determining pIgR expression needs further addressing in future assays.

The results of the present study demonstrated that the inhibition of nAChRs following treatment with MLA reduces both IgA secretion and pIgR mRNA expression, which suggests that mechanisms of IgA-transport are dependent on pIgR-mediated transcytosis. These data suggested that IL4$^+$/CD4$^+$ T cells downmodulation may result in a blunted response of secreted IL-4, an important cytokine involved in *PIGR* gene modulation for *de novo* synthesis of pIgR protein (24). Thus, the effect of MLA may be associated with the reduction of IL-4$^+$/CD4$^+$ T cells in the lamina propria, resulting in the decrease in pIgR mRNA expression.

Moreover, GTS-21 treatment did not alter IgA concentration; however, it did decrease pIgR mRNA expression. GTS-21 is a partial α7 nAChR agonist, which suggests that other nAChR subunits may play a role in the regulation of IgA transport via pIgR. As has been described in *in vitro* assays on different cell types including monocytes, macrophages and endothelial cells, α7 nAChR mediated-activation inhibits the nuclear translocation of NF-κB (25). It has been revealed

that NF-κB activation via classical and alternative pathways is involved in *PIGR* gene transcription (24). These findings may provide a potential mechanism for supporting the role of α7 nAChR on downmodulation of *PIGR* gene transcription by impeding NF-κB translocation.

There are a number of limitations to the present study. Namely, immunohistochemical analysis of pIgR to gain insight into the effects of nAChR on IgA-transcytosis was not included. Therefore, experimental settings focused to assess the protein expression of pIgR, via western blotting and/or immunohistochemical analysis, as well as secretory component and IgA concentration in intestinal fluids, by means of immunoenzymatic assays, may address presumable mechanisms of the participation of nicotinic subunits other than α7 and their role on cholinergic modulation on IgA transcytosis via pIgR.

In conclusion, the selective activation of α7nAChR elicited IgA^+ plasma cells in both compartments, and the percentage of $CD4^+$ T-cells expressing TGF-β in Peyer's patches. Notably, the non-selective inhibition of nAChRs decreased IgA levels and mRNA pIgR expression, which may highlight their involvement in pIgR-mediated IgA transcytosis. The aforementioned aspects require further examination. This basic study may be an experimental reference for clinical trials that address the role of the nicotinic system in intestinal dysfunctions as postoperative ileus (26).

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RCR proposed the original idea and experimental design. JECL, MEDS and JPY were involved in the conceptualization of the study. JECL, IMAM, AARA and MGL were involved in the study methodology. JECL, IMAM and MGL were involved in the formal analysis. JECL and MEDS were involved in data curation. MEDS was involved in the writing and preparation of the original draft. JECL and JPY were involved in the writing, reviewing and editing of the manuscript. JPY supervised the study and was involved in project administration. All authors confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Experiments using animals were approved (approval no. ESM-CICUAL-01/09-10-2018) by the Institutional Review Board of Instituto Politécnico Nacional (Ciudad de México, México).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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