Muscarinic Acetylcholine Receptors Modulate Interleukin-6 Production and Immunoglobulin Class Switching in Daudi Cells

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INTRODUCTION

Immune cells, including T cells, B cells and macrophages, express such cholinergic system components as an acetylcholine (ACh)-synthesizing protein (choline acetyltransferase (ChAT)) and both muscarinic and nicotinic acetylcholine receptors (mAChRs and nAChRs, respectively). Moreover, in vivo and in vitro studies using cells and mice deficient in mAChR subtypes or nAChR subunits have provided evidence that signaling via both mAChRs and nAChRs impacts immune function. For example, after immunization, M1 and M5 mAChR double-deficient mice produce significantly less, while α7 nAChR-deficient mice produce significantly more, total and antigen-specific immunoglobulin G (IgG) and interleukin (IL)-6 than do their wild-type counterparts.6) IgM production, by contrast, is unaffected. This suggests that both mAChRs and nAChRs affect B cell function by regulating IL-6 production.

MATERIALS AND METHODS

Cell Culture Daudi cells (human Burkitt’s lymphoma, B cell leukemia) were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin at 37 °C under a humidified atmosphere with 5% CO2. Cells were cultured with 0.2% Pansorbin for 24 h to detect mAChR and nAChR gene expression and for 5 d (restimulation with Pansorbin plus an AChR agonist with or without a corresponding antagonist after 3 d) to induce IgG production.

Real-Time PCR Total RNAs were extracted from Daudi cells using Sepasol RNA II Super (Nacalai Tesque, Kyoto, Japan), and cDNAs were prepared by reverse transcription using a Prime Script RT reagent Kit (TaKaRa Bio, Shiga, Japan) in a S1000 Thermal Cycler (Bio-rad, Hercules, CA, U.S.A.). Real-time PCR analysis was conducted using CYBR premix EX taq, FAM-labeled probes, and predesigned primers (TaKaRa Bio) with a Thermal Cycler Dice Real Time System (TaKaRa Bio). The catalog numbers of predesigned primers were as follows: M1 mAChR, HA229529; M2 mAChR, HA243585; M3 mAChR, HA229529; M4 mAChR, HA221666; M5 mAChR, HA274938; α4 nAChR, HA188413; α7 nAChR, HA164722; β2 nAChR, HA100887; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), HA067812.

Enzyme-Linked Immunosorbent Assay (ELISA) Levels of IL-6 in culture supernatants were quantified using a sandwich ELISA. The capture antibody for IL-6 (20 µg/mL, MP5-20F3, BD Biosciences, Franklin Lakes, NJ, U.S.A.) was coated onto 96-well plates. Then after blocking with 0.5% bovine serum albumin, plates were incubated with serial dilutions of culture supernatants followed by biotinylated anti-human IL-6 antibody and streptavidin-peroxidase. The enzyme activity was measured using the ELISA kit (Promega, Madison, WI, U.S.A.).

Note

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Fig. 1. Pansorbin Induces mAChR and nAChR Gene Expression in Daudi Cells

Daudi cells exposed to Pansorbin (0.2%, 24 h) were subjected to real-time PCR using primers specific for M1-M5 mAChRs (A) and the α4, α7 and β2 nAChR subunits (B). GAPDH were used as an internal control to normalize the variability in expression levels. Bars depict means ± S.E.M. (n = 3). *p < 0.05, **p < 0.01.

Fig. 2. mAChR Activation Enhances Immunoglobulin Class Switching from IgM to IgG in Daudi Cells

A. Percentages of Daudi cells with surface expression of IgM and IgG in the absence or presence of nicotine and Oxo-M. Cells were exposed to nicotine (500 µM) or Oxo-M (300 µM) for 5d and then subjected to flow cytometry using FITC-conjugated anti-IgG and APC-conjugated anti-IgM. Bars represent means ± S.E.M. for at least three samples. B. Representative flow cytometry data showing surface expression of IgM (right) and IgG (left) in Daudi cells. Vehicle (filled gray), Pansorbin (black line), Pansorbin + nicotine (light gray line), Pansorbin + Oxo-M (gray line). C–E. Percentages of cells with surface expression of IgM and IgG in the presence Pansorbin plus an mAChR or nAChR agonist (Oxo-M, nicotine) with or without a corresponding antagonist (scopolamine, mecamylamine, DHβE, 4-DAMP, AFDX-383 (all 10 µM)). Bars depict means ± S.E.M. (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001.
vine serum albumin (BSA) in PBS containing 0.5% Tween 20, diluted samples and recombinant protein standards were added to the plates and incubated for 1 h at room temperature. They were then incubated for an additional 1 h with biotin-conjugated detection Abs (1 µg/mL MP5-32C11, BD Biosciences) at 37 °C and reacted with streptavidin-conjugated horseradish peroxidase, followed by α-phenylenediamine. The reaction was terminated by addition of 0.5 M H₂SO₄. The absorbance at 490 nm was then measured, and a graph was created using data from three samples.

Flow Cytometry To detect IgG and IgM, Daudi cells were stained using fluorescein isothiocyanate (FITC)-conjugated anti-IgG Ab (RM4.5, Thermo Fisher Scientific, Waltham, MA, U.S.A.) and APC-conjugated anti-IgM Ab (PC61.5, Thermo Fisher Scientific) in Hanks’ balanced salt solution supplemented with 0.1% BSA and 0.1% NaN₃ and subjected to flow cytometry (Cytoselect, Beckman Coulter, Brea, CA, U.S.A.). A gate was set on the lymphocytes using appropriate forward scatter and side scatter parameters. Isotype-matched FITC and APC-conjugated mouse IgG1 Abs were used as controls. The acquired data were analyzed using CytExpert (Beckman Coulter).

Statistical Analysis Data are presented as means ± standard error of the mean (S.E.M.). All experiments were repeated three times. Statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, CA, U.S.A.). Differences between two groups were evaluated using Student’s t-test, and between three or more groups using one- or two-way ANOVA with post hoc Dunnett’s or Tukey’s test, respectively. Values of p < 0.05 were considered significant.

RESULTS AND DISCUSSION

B Cell Activation Alters mAChR and nAChR Gene Expression in Daudi Cells Several types of mAChRs and nAChRs are reportedly expressed in B cells.²⁷ We investigated whether the expression levels of these receptors were changed by immune stimulation in Daudi human leukemic B cells. Pansorbin cells, which are heat-killed, formalin-fixed Staphylococcus aureus B cells. Pansorbin cells, which are heat-killed, formalin-fixed Staphylococcus aureus, which are heat-killed, formalin-fixed Staphylococcus aureus, were used to trigger B cell activation through binding to toll-like receptor 2 (TLR2).²⁸ Stimulation with Pansorbin for 24 h significantly increased gene expression of the M₁–M₄ mAChR subtypes and the α₄ nAChR subunit (Fig. 1).

mAChR Activation Enhances Pansorbin-Induced Immunoglobulin Class Switching from IgM to IgG We used flow cytometry to investigate whether mAChRs and/or nAChRs are involved in immunoglobulin class switching in Daudi cells. Incubating Daudi cells for 5 d with nicotine or Oxo-M, a mAChR agonist, did not affect IgM content or induce IgG production (Fig. 2A). By contrast, Pansorbin increased IgG production and slightly decreased IgM content (Figs. 2B, C). Treatment with Pansorbin plus Oxo-M, but not nicotine, further enhanced IgG production (Figs. 2B, C). Scopolamine, a non-selective mAChR antagonist, eliminated the Oxo-M-induced increase in IgG production (Fig. 2D). By contrast, mecamylamine, a nAChR antagonist, and dihydro-β-erythroidine (DHβE), a α4β2 nAChR antagonist, did not affect IgG production (Fig. 2E). In addition, 4-diphenylacetoxy-N-methyl-piperidine methiodide (4-DAMP), a Gq/11-coupled M₁, M₃, M₄ mAChR antagonist, but not ADFX-384, a Gi/o-coupled M₂, M₄ mAChR antagonist, suppressed Oxo-M-induced increases in IgG production in Daudi cells. These results indicate that Gq/11-coupled mAChR activation enhances immunoglobulin class switching.

mAChR Activation Enhances Pansorbin-Induced IL-6 Release The cytokine IL-6 is secreted by several immune cell types, including B cells, and promotes differentiation of B cells into plasma cells.⁶ Treatment with Oxo-M promoted Pansorbin-induced IL-6 production in Daudi cells, and this effect was inhibited by pretreatment with scopolamine (Fig. 3).

Our findings summarized above indicate that some mAChR subtypes and nAChR subunits are upregulated upon B cell activation, and that activation of mAChRs, but not nAChRs, promotes immunoglobulin class switching to IgG as well as production and release IL-6. These findings are consistent with earlier observations from M₁/M₅ mAChR-deficient mice.⁶ IL-6 is required for B cell differentiation, and its production following TLR2 activation is regulated by several kinases and transcription factors, including extracellular signal-regulated kinase (ERK) and nuclear factor-kappaB (NFκB).¹⁰ M₂, M₃, and M₄ mAChRs are coupled to Gq/11 proteins, which activate phospholipase C (PLC)-β to signal through calcium and protein kinase C (PKC) signaling pathways. The fact that PKC can activate ERK signaling (¹¹) may provide a clue to the pathway via which Gq/11-coupled M₂, M₃, M₄ mAChR activation enhances IL-6 production, which in turn may promote differentiation into plasma cells and immunoglobulin class switching. M₁–M₄ mAChR gene expression was upregulated in response to Pansorbin. Determining which type of Gq/11-coupled mAChR mediates the response will be the aim of a future study.

Although Pansorbin increased expression of α4 subunit mRNA, nAChR activation does not appear to be involved in IgG production in Daudi cells. This finding differs from earlier observations in a7 nAChR-deficient mice.⁷ Our recent study indicates that a7 nAChRs expressed in antigen-presenting cells such as macrophages and dendritic cells interfere with their antigen presentation, thereby inhibiting T cell differentiation, including differentiation into T helper 2 (Th2) cells.⁷ Those findings together with the results of our present study suggest that a7 nAChRs expressed in B cells do not affect B cell differentiation or immunoglobulin class switching. By contrast, a7 nAChRs expressed in antigen presenting cells appear to suppress both T cell differentiation into Th2 cells and B cell differentiation into plasma cells as well as IgG secretion.
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Conflict of Interest  The authors declare no conflict of interest.

REFERENCES


