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Enhanced serum antigen-specific IgG_1 and proinflammatory cytokine production in nicotinic acetylcholine receptor α7 subunit gene knockout mice

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Abstract

Human and murine immune cells such as mononuclear leukocytes consisting of mainly T and B cells, bone marrow derived dendritic cells (DCs) and macrophages all express various nicotinic acetylcholine (ACh) receptor (nAChR) subunits. Activated T cells and DCs have the ability to synthesize ACh by choline acetyltransferase, suggesting the role of non-neuronal cholinergic system expressed in immune cells in the regulation of immune cell function. Stimulation of human leukemic T and B cell lines with nicotine causes a transient Ca^{2+}-signaling that is antagonized by α-bungarotoxin, suggesting the involvement of α7 subunit. Furthermore, α7 nAChRs have been shown to negatively regulate synthesis and release of tumor necrosis factor (TNF)-α in macrophages. These findings suggest that immune cell function is regulated by its own non-neuronal cholinergic system, at least in part, via α7 nAChR-mediated pathways. In the present study, we tested the role of α7 nAChRs in the regulation of immune function by measuring total serum and antigen-specific IgG_1 and IgM, and production of TNF-α, gamma interferon (IFN-γ) and interleukin (IL)-6 in activated spleen cells of nAChR α7 subunit gene knockout (α7 KO) and wild-type C57BL/6J mice immunized with ovalbumin (OVA). We found that serum levels of total and anti-OVA-specific IgG_1 were significantly elevated in α7 KO mice, though there were no significant differences in serum levels of total and anti-OVA-specific IgM between the two genotypes. Production of TNF-α, IFN-γ and IL-6 in spleen cells was significantly facilitated in α7 KO mice. Expression of AChE mRNA was not different between the two genotypes. These results suggest that α7 nAChRs are involved in the regulation of cytokine production, through which modulates TNF-α, IFN-γ and IL-6 productions, leading to modification of antibody production, but are not involved in expression of cholinergic components in immune cells.

Keywords: Acetylcholine; α7 subunit; IgG_1; IFN-γ; IL-6; TNF-α

1. Introduction

Human mononuclear leukocytes (MNLs) and leukemic T and B cell lines, and murine immune cells such as T and B cells, bone marrow derived dendritic cells (DCs) and macrophages express various nicotinic acetylcholine (ACh) receptor (nAChR) subunits as well as five distinct subtypes of muscarinic ACh receptors (M_1–M_5 mAChRs) (see reviews by Kawashima and Fujii, 2000, 2003a,b, 2004; Fujii and Kawashima, 2001; Kawashima et al., 2007). Furthermore, the findings that immunologically activated T cells and DCs have the ability to synthesize ACh by choline acetyltransferase (ChAT) suggest the role of non-neuronal cholinergic system expressed in immune cells in the regulation of immune function. In fact, we have shown recently that levels of total serum IgG_1 and antigen-specific IgG_1, and interleukin (IL)-6 production are all diminished in M_1/M_5 mAChR gene knockout (M_1/M_5 KO) mice 1 wk after immunization with ovalbumin (OVA), though there is no change in serum levels of total serum IgM and antigen-specific IgM. These results suggested the involvement of M_1 and/or M_5 mAChRs in the regulation of immune

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function, at least in part through altered production of the Th2 type cytokine IL-6, leading to modulation of antibody class switching from IgM to IgG_1 (Fujii et al., in press).

Stimulation of human leukemic T and B cell lines expressing nAChR α2, α5, α6, α7, α9, α10 and β2 and β4 subunits with nicotine or epibatidine elicited a transient Ca^{2+}-signaling that was antagonized effectively by α-bungarotoxin, suggesting that the α7 nAChRs are at least partly responsible for nicotine-induced Ca^{2+}-signaling in lymphocytes (Kimura et al., 2003; Kawashima and Fujii, 2004). While the expression level and pattern of nAChR subunits may vary depending upon the cells' stage of development and the stimulus given during development, nAChR α5 and α7 subunits appear to be always expressed in T cells from C57BL/6J mice (Kuo et al., 2002). Collectively, these findings suggest the role of α7 subunit in the regulation of immune cell function. Nicotine as well as exposure to cigarette smoke induces suppression of various immunological parameters such as T cell-dependent antibody and T cell mitogenic responses (see a review by Sopori, 2002). Skok et al. (2005) found reduced pre-immune level of serum IgG with little change in IgM in α4, α7 or β2 KO mice and enhanced serum IgG response to primary immunization with cytochrome c in either α4 KO or β2 KO mice, suggesting that the role of nAChRs in the regulation of antibody synthesis in B cells. However, the role of α7 nAChRs in the regulation of humoral response to antigen has not been explored yet.

In the present study, in order to investigate the role played by non-neuronal cholinergic system in the regulation of in vivo immune function, focusing on α7 nAChRs, we examined the production of total serum and antigen-specific IgM and IgG_1 in nAChR α7 subunit gene knockout (α7 KO) mice at 2 wk after immunization with ovalbumin (OVA). Furthermore, in order to investigate the role of α7 nAChRs in antigen-specific cytokine production, we determined levels of TNF-α, gamma interferon (IFN-γ) and IL-6 in OVA-activated spleen cell cultures.

2. Materials and methods

2.1. Animals

α7 KO (*Acra7*-deficient, α7−/−) C57BL/6J background mice were bred from the founders' strain generated by Orr-Urterger et al. (1997). Four male and 3 female 10 to 15-wk-old α7 KO mice and 3 male and 4 female age-matched wild-type C57BL/6J were used for study.

2.2. Immunization and bleeding

Mice were immunized by intraperitoneal injection of 100 μg of OVA (Sigma, St. Louis, MO, USA) mixed with 5×10^8 CFUs of *Bordetella pertussis* (LSL, Tokyo, Japan) and Alu-Gel-S (Serva, Heidelberg, Germany). Two wk after the immunization, blood samples were obtained by cardiac puncture under deep anesthesia with ether. Serum was separated by centrifugation and stored in a deep freezer until assayed.

2.3. Determination of serum IgG_1 and IgM

2.3.1. Total IgG_1 and anti-OVA-specific IgG_1

Total serum IgG_1 levels were quantified by ELISA using 96-well microtiter plates coated with goat anti-mouse IgG_1 (Cat # A90-105A, Bethyl, Montgomery, TX, USA) and goat anti-mouse IgG_1 conjugated with horseradish peroxidase (Cat # A90-105P, Bethyl, Montgomery, TX, USA). Mouse monoclonal anti-OVA-specific IgG_1 (HYB 099-01, Antibodyshop, Gentofte, Denmark) was used as the standard.

Serum levels of anti-OVA-specific IgG_1 were determined by ELISA using 96-well microtiter plates coated with OVA and goat anti-mouse IgG_1 conjugated with horseradish peroxidase (Cat # A90-105P, Bethyl, Montgomery, TX, USA). Mouse monoclonal anti-OVA-specific IgG_1 (HYB 099-01, Antibodyshop, Gentofte, Denmark) served as the standard.

2.3.2. Total IgM and anti-OVA-specific IgM

Serum concentrations of total IgM and anti-OVA-specific IgM were determined using a commercially available Mouse IgM ELISA Quantitation Kit (Bethyl, Montgomery, TX, USA) and a Mouse Anti-Ovalbumin IgM ELISA Kit (Cat. # 600-170-OGM, Alpha Diagnostic, San Antonio, TX, USA), respectively, according to manufacturer's instructions.

2.4. Secretion of TNF-α, IFN-γ and IL-6 and expression of ChAT and AChE mRNAs in spleen cells

2.4.1. Preparation of spleen cells

Mice were sacrificed by deep anesthesia with ether, after which the spleen was dissected out. Suspensions of single spleen cells were prepared by passing the cells through a nylon mesh, after which contaminating erythrocytes were removed by treatment with ammonium chloride lysis buffer. Cell surface expression of antigens was identified by monoclonal antibody staining, followed by flow cytometry using a FACSCalibur (Becton-Dickinson, Palo Alto, CA, USA) with analysis using CellQuest software (Becton-Dickinson). Spleen cells thus prepared consisted of 90% lymphocytes (30% $CD4^+$ and 15% $CD8^+$ T cells; 45% $CD45^+$ B cells) and 10% monocytes ($CD14^+$ cells).

2.4.2. Determination of TNF-α, IFN-γ and IL-6 secretion in spleen cells

Spleen cell samples were suspended in a 10% fetus bovine serum/RPMI 1640 culture medium (2×10^6 cells/mL) and then incubated with 100 μg/mL OVA at 37 °C in a CO_2 incubator. The conditioned media were collected after 24 h or 48 h of culture to assess secretion of IL-6 or TNF-α and IFN-γ, respectively. The levels of these cytokines were determined using commercially available ELISA kits (IFN-γ, BD Sciences, San Diego, CA, USA; TNF-α and IL-6, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.4.3. Expression of AChE and ChAT mRNAs in spleen cells

2.4.3.1. Extraction of total RNA from spleen cells. After incubating spleen cells for 48 h in the presence of 100 μg/mL