FORUM MINIREVIEW

The Non-neuronal Cholinergic System

Roles of Muscarinic Acetylcholine Receptors in Interleukin-2
Synthesis in Lymphocytes

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ABSTRACT—Receptors for many neurotransmitters including catecholamines and acetylcholine (ACh) have been detected on the cell surface of lymphocytes. It has been demonstrated that a human T cell line synthesizes ACh and suggested that ACh may be an autacoid modulating T cell-dependent immune responses. However, the biochemical interactions of the ACh system with the immune system have not been elucidated in detail. We have shown that m1 and m2 muscarinic receptor mRNAs are expressed in human peripheral blood lymphocytes and in human T cell line Jurkat cells and that pretreatment of these cells with a muscarinic receptor agonist enhances interleukin-2 (IL-2) production. We also postulated possible intracellular signaling pathways via which muscarinic receptors regulate IL-2 production in Jurkat cells. The findings suggest that M₁ muscarinic receptors are involved in muscarinic receptor-mediated enhancement of IL-2 production in Jurkat cells and that the transcription factor AP-1 and pathways via mitogen-activated protein kinase (MAPK) / extracellular signal regulated protein kinase and c-Jun N-terminal kinase, but not via p38 MAPK, may be involved in the muscarinic receptor-mediated enhancement of IL-2 production. Our findings demonstrate a neuro-immune interaction through muscarinic receptor signaling in immune cells.

Keywords: Neuroimmune interaction, Muscarinic receptor, Interleukin-2, Jurkat cell

Several studies suggest that the nervous system regulates immune function. Electron microscopic studies have demonstrated that vagus nerves are present in lymphoid tissues such as the spleen and thymus and that nerve terminals form synaptic contacts with lymphocytes (1). In addition, lymphocytes possess various neurotransmitters and neuropeptide receptors such as β -adrenoceptors, muscarinic acetylcholine (ACh) receptors, 5-hydroxytryptamine-1 receptors and type B cholecystokinin receptors. It is known that the β -adrenoceptor-G_s-adenylyl cyclase system is involved in the inhibitory regulation of interleukin (IL)-2 production and thus the proliferation of T cells (2). Despite several studies on the functional role of the β -adrenergic system, details of the biochemical interactions of the muscarinic ACh system with the immune system (3) have not been elucidated. Lymphocytes have been shown to synthesize ACh (4), and nicotinic and muscarinic ACh receptors

are present on the surface of lymphocytes (5, 6). We demonstrated that the activation of muscarinic ACh receptors increases at the cytoplasmic concentration of Ca²⁺ in Jurkat cells, a human leukemic helper T lymphocyte line (7). Recently, it has been reported that ACh attenuated the release of cytokines tumor necrosis factor (TNF), IL-1 β , IL-6 and IL-18 in lipopolysaccharide-stimulated macrophages and that the vagal stimulation inhibited TNF synthesis in the liver (8). These reports suggest that ACh may be an autacoid modulating immune responses.

IL-2 is the first in a series of lymphocytotropic hormones, and it has a pivotal role in the generation and regulation of immune responses. Activation of TCR/CD3 complexes induces IL-2 production in T cells, and some immunosuppressants, such as cyclosporin A and FK-506, inhibit this IL-2 production (9). Cyclosporin A- and FK-506-binding proteins, identified as immunophilins (cyclophilins and FKBP, respectively) (10), are targets for Ca²⁺/CaM-dependent phosphatase 2B, also known as calcineurin (11). However, the detailed mechanisms of IL-2 production

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and regulation are not clear.

Stimulatory role of muscarinic ACh receptors in T cell antigen receptor-mediated IL-2 production in human peripheral blood lymphocytes (hPBL)

Pretreatment of hPBL with ACh (in the presence of physostigmine) or oxotremorine-M (muscarinic agonist, Oxo-M) but not nicotine for 24 h enhanced phytohemagglutinin (PHA)-induced IL-2 production (Fig. 1). On the other hand, ACh or Oxo-M alone did not affect IL-2 production. Anti-CD3 mAb-induced IL-2 production was also enhanced by Oxo-M (62%), so that it was similar to PHA-induced IL-2 production (69%). Using CD3-positive T cells prepared by a positive panning selection from hPBL, pretreatment with Oxo-M enhanced PHA-induced IL-2 production (5). These findings suggest that activation of muscarinic receptors enhances T cell antigen receptor/CD3-induced IL-2 production.

Possible intracellular signaling pathways of muscarinic receptors that regulate IL-2 production in human T cell line Jurkat cells

The presence of muscarinic receptors on human lymphocytes has been demonstrated by several studies using radioligand binding assays (12, 13). We detected m1 and m2 muscarinic receptor mRNA subtypes in Jurkat cells as well as in hPBL (5). On the other hand, Ewa and Nordberg (14) showed that m3, m4 and m5 muscarinic receptor mRNAs were expressed in purified human T cells, while m1 and m2 subtypes were not detected. In addition, they reported that both m3 and m5 subtypes were expressed in Peer cells, a

human leukemic T cell line. Although Kawashima et al. (15) showed the expression of m4 and m5 subtype mRNAs in the CEM, MOLT-3 and Jurkat cells, m3 subtype mRNA expression was detected in both CEM and MOLT-3, but not in Jurkat cells. These differences are probably due to the experimental conditions of the T cells in the laboratory (16).

We detected m1 and m2 receptors in Jurkat cells and found that stimulation of these receptors with Oxo-M enhanced both IL-2 production and promoter activity induced by phorbol 12-myristate 13-acetate (PMA)/A23187 (calcium ionophore). This enhancement was inhibited by 4-DAMP (M_1 - and M_3 -receptor antagonist) and atropine (non-selective muscarinic receptor antagonist), while AF-DX116 (M_2 -receptor antagonist) further enhanced IL-2 production (16). Therefore, stimulation of M_1 receptors alone enhanced IL-2 production and stimulation of M_2 receptors alone may have inhibited it, although stimulation of both types of M_1 and M_2 receptors together appeared to enhance it. The IL-2 promoter activity is regulated by the 300-bp region adjacent to the transcription initiation site (17). This region contains specific binding sites for various transcription factors including activation protein (AP)-1, nuclear factor (NF)- κ B, NF-AT and Oct-1, and cooperation of these factors is required for maximal activation of the IL-2 promoter (18). The stimulation of M_1 receptors (with Oxo-M in the presence of M_2 antagonist AF-DX116) enhanced the PMA/A23187-induced binding activity to AP-1 consensus sequences in the IL-2 promoter (Fig. 2) (16). The stimulation of M_1 receptors did not modify the DNA binding of NF- κ B, NF-AT or Oct-1. The findings suggest that the transcription factor AP-1 is involved in M_1 -receptor-mediated enhancement of IL-2 transcription in Jurkat cells.

It has been reported that signals from muscarinic receptors affect the mitogen-activated protein kinases (MAPKs) in various cells (19, 20). However, the effects of muscarinic receptor stimulation on the MAPKs cascades in lymphocytes have not been established. When M_1 receptors were stimulated, the activities of MAPK/extracellular signal regulated protein kinase (ERK) and c-Jun N-terminal kinase (JNK) were increased, while p38 MAPK was not affected (16). The findings of this study indicate that ERK and JNK, but not p38 kinase, are involved in the M_1 -receptor-mediated enhancement of IL-2 production by stimulating AP-1 activity. There are some reports that MAPK signal transduction pathways are involved in IL-2 gene transcription in T cells: Ras, the JNK cascade, and one or more of AP-1 (21), raf-1, MAPK-extracellular signal-regulated kinase (MEK) 1 and ERK 1/2 (22). Therefore, ERK and JNK may activate AP-1 proteins via the activation of c-Fos and c-Jun, respectively.

In conclusion, muscarinic receptors in T cells play func-

Fig. 1. Effects of cholinergic agonists on PHA-induced IL-2 production in hPBL. hPBL were incubated with cholinergic agonists (1 μ M ACh in the presence of 100 μ M physostigmine, 1 μ M Oxo-M, 1 μ M nicotine) for 24 h. Then 10 μ g/ml PHA was added and cells were further incubated for 24 h. The amounts of IL-2 released into the medium were analyzed by ELISA. Results are expressed as the mean $\pm$ S.E.M. in all four experiments. * P <0.05 vs none.

Fig. 2. Effect of M_1 -receptor stimulation on PMA/A23187-induced AP-1, NF- κ B, NF-AT and Oct-1 consensus sequence binding proteins in Jurkat cells. Jurkat cells were treated with 100 μ M Oxo-M in the presence of 1 μ M AF-DX116, an M_2 antagonist, for 1 h prior to treatment with 1 nM PMA plus 500 nM A23187. The band shift assays were performed using each nuclear extract. The quantities of each band are shown in a bar chart. * $P < 0.05$ vs none.

Fig. 3. Proposed role of muscarinic ACh receptors in the regulation of IL-2 production. Jurkat cells express m_1 and m_2 receptor mRNAs. Stimulation of M_1 receptors enhances PMA/A23187-induced IL-2 production. AP-1 may be involved in this enhancement, and both MAPK and JNK also seem to be involved. M_2 receptors may function to decrease the IL-2 production. SAPK: stress-activated protein kinase.

tional roles in regulation of IL-2 synthesis. The transcription factor AP-1 and MAPK signal transduction pathways may be involved in the M_1 -receptor-mediated enhancement of IL-2 synthesis in Jurkat cells (Fig. 3). These findings show the existence of neuroimmune interactions through muscarinic receptor signaling in immune cells.

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