ELSEVIER

European Journal of Pharmacology 297 (1996) 121–127

ejp

Induction of muscarinic receptor subtypes in monocytic/macrophagic cells differentiated from EoL-1 cells

Yoshinori Mita [a,*], Kunio Dobashi [a], Kouki Suzuki [a], Masatomo Mori [a], Tsugio Nakazawa [b]

[a] *First Department of Internal Medicine, Gunma University School of Medicine, 3-39 Shouwa-machi, Maebashi, Gunma 371, Japan*
[b] *College of Medical Care and Technology, Gunma University School of Medicine, 3-39 Shouwa-machi, Maebashi, Gunma 371, Japan*

Received 13 September 1995; accepted 24 October 1995

Abstract

We demonstrated that eosinophilic leukemia cell line-1 (EoL-1 cells) differentiated into monocytic/macrophagic cells by a treatment with interferon-γ. Muscarinic receptor mRNA was not detected in untreated EoL-1 cells, and the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_i$) did not rise either in these cells. Interestingly, when EoL-1 cells were treated with interferon-γ, mRNAs for muscarinic M_3 and M_5 receptors could be detected in these cells, along with an increase in $[Ca^{2+}]_i$ and chemotaxis induced by carbachol that could be blocked with 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) and pirenzepine. These data support the functional importance of muscarinic M_3 and M_5 receptors in monocytic/macrophagic cells differentiated from EoL-1 cells. This model also provides evidence of a significant functional interaction between muscarinic M_3 and M_5 receptors.

Keywords: Interferon-γ; Muscarinic receptor

1. Introduction

Pharmacologically distinct forms of the muscarinic receptors have been classified into muscarinic M_1, M_2 and M_3 receptor subtypes from the selectivity of novel antagonists, namely pirenzepine (Hammer and Giachetti, 1982), AF-DX 116 (11-[[2-[(dimethylamino) methyl]-1-piperidinyl]-acetyl]-5,11-dihydro-6-*H*-pyrido-[2,3-*b*][1,4]benzodiazepin-6-one) (Giachetti et al., 1986) and 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine methiodide) (Michel et al., 1989). Recently, genes for muscarinic receptor subtypes have been classified as muscarinic M_1 through M_5 receptors, based on results from complementary DNA (cDNA) cloning experiments (Bonner et al., 1987; Peralta et al., 1987).

It has been demonstrated that human phagocytic leukocytes have stereo-selective muscarinic receptor (Lopker et al., 1980), and that carbachol could induce polarization and chemotaxis (Stephens and Snyderman, 1982). However, neither are there reports about the characterization of muscarinic receptor subtypes expressed in monocytes, nor about the change of free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by carbachol in these cells. It is difficult to perform experiments using normal human monocytes because monocytes are a minor population of white blood cells and the contamination by other cells cannot be avoided. The contaminating cells, e.g., T-lymphocytes (Genaro et al., 1992), erythrocytes (Abood et al., 1976; Aronstam et al., 1977), and neutrophils (Dulis et al., 1979), express muscarinic receptors.

Eosinophilic leukemia cell line-1 (EoL-1) cells were recently established from a patient with eosinophilic leukemia (Saito et al., 1985). They have myeloblastic features with few eosinophilic granules (1–2%) under normal culture conditions. However EoL-1 cells differentiate into eosinophilic granule-containing cells when cultured in an alkaline medium, or after the addition of dimethyl sulphoxide (DMSO) (Saito et al., 1985), or tumor necrosis factor (Yoshie et al., 1989). Interferon-γ dramatically augmented 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-induced membrane oxidative metabolism of EoL-1 cells, but did not change the number of EoL-1 cells with distinct eosinophilic granules (2–3%)

* Corresponding author. Tel. 011-81-0272-20-7111; fax 011-81-272-20-8136.

SSDI 0014-2999(95)00722-9

(Yoshie et al., 1989). It was suggested that interferon-γ-treated EoL-1 cells did not differentiate into eosinophils, but differentiated into monocytes/macrophages.

In this study, we demonstrated that EoL-1 cells differentiated into monocytic/macrophagic cells by a treatment with interferon-γ, and analyzed the role of muscarinic receptor subtypes expressed in interferon-γ-treated EoL-1 cells.

2. Materials and methods

2.1. Materials

Recombinant interferon-γ and GIT medium were purchased from WAKO Pure Chemical Industries, Osaka, Japan. Esterase stain kit was obtained from Muto Pure Chemicals Co., Tokyo, Japan. Fura-2/acetoxymethyl ester (fura-2/AM), fura-2, atropine, carbachol, pirenzepine and 4-DAMP were purchased from Sigma, St. Louis, MO. Reverse transcription polymerase chain reaction kit was purchased from Takara, Tokyo, Japan. Quickprep micro messenger RNA (mRNA) purification kit was obtained from Pharmacia P-L Biochemicals, Milwaukee, WI. AF-DX 116 was purchased from Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT. Microchemotaxis chamber containing a micropore filter with a pore diameter of 8 μm was obtained from Kurabou Biomedicals Industries, Osaka, Japan.

2.2. Cells

EoL-1 cells were maintained at 37°C in GIT medium supplemented with 5% heat-inactived fetal calf serum (culture medium) in a humidified atmosphere of 5% CO_2. To allow the EoL-1 cells to differentiate into monocytic/macrophagic cells, the cells were cultured for 3 days in the culture medium containing 100 U/ml interferon-γ (interferon-γ medium) at an initial concentration of 10^5 cells/ml and cultured for another 3 days in the new interferon-γ medium. Cell viability was determined by trypan blue exclusion. In all cases, trypan blue exclusion indicated that $>95\%$ of cells remained viable.

2.3. Assay of differentiation marker in cells

Non-specific esterase activity, a typical marker for cells of monocyte/macrophage lineage, was determined in cytocentrifuged preparations using an esterase stain kit with α-naphthyl butyrate as substrate (pH 6.3) (Li et al., 1973). A strong non-specific esterase activity was detected only in monocytes while the activity was very weak or not demonstrable in lymphocytes, megakaryocytes, plasma cells, granulocytes and primitive myeloblasts.

2.4. Measurement of $[Ca^{2+}]_i$

The interferon-γ-treated EoL-1 cells were washed first with GIT medium and then with Ca^{2+}-free buffer consisting of (in mM) NaCl (125), KCl (2.5), $MgCl_2$ (0.7), Hepes (25), EDTA (0.5) and Glucose (10), pH 7.4. The cells were loaded with fura-2/AM dissolved in Ca^{2+}-free buffer at 37°C for 20 min. Then, the cells were washed twice with the same buffer and attached on the glass coverslips and placed in a 1 ml superfusion chamber and resuspended in Ca^{2+} buffer containing (in mM) NaCl (125), KCl (2.5), $MgCl_2$ (0.7), Hepes (25), $CaCl_2$ (1.0) and glucose (10), pH 7.4. Fura-2/AM-loaded cells were stimulated by carbachol (1 μM to 1 mM). Changes in fluorescence intensity of fura-2/AM loaded cells were determined at excitation wavelengths of 340 nm and 360 nm, using a fluorescence microscope (Nikon Instech Co., Tokyo, Japan) connected to a video-intensified microscope system (Hamamatsu Photonics Co., Hamamatsu, Japan) with an emission of 510 nm. The concentration of $[Ca^{2+}]_i$ was estimated from the ratio of fluorescence intensities at 340 nm and 360 nm. For calibration, we illuminated droplets of buffers containing fura-2 and the determined concentration of free Ca^{2+} (Calcium Calibration Buffer Kit No. 2, Molecular Probe, Eugene, USA).

2.5. Measurement of chemotactic activity

The inferferon-γ-treated cells were washed and resuspended in culture medium. The chemotaxis assay was performed in 24-well microchemotaxis chambers containing a micropore filter with a pore diameter of 8 μm. In the lower compartments of the chambers, carbachol was placed in duplicate. Cell suspensions of 0.5 ml (5×10^6 cells) was transferred into each of the upper compartments, and muscarinic receptor antagonists were added. After filling the chambers, they were incubated in humidified air in 5% CO_2 at 37°C for 60 min, and the filter was then fixed, stained with esterase stain kit and mounted on a glass slide. Non-specific esterase strong positive cells were counted in five high-power fields (×100) per well. Chemotactic response was expressed as the mean number of cells per high-power field.

2.6. Determination of mRNA levels by reverse transcription polymerase chain reaction

Total RNA was extracted from interferon-γ-treated EoL-1 cells according to the method of Quickprep micro mRNA purification kit. The purity and quantification were assessed from 260 nm/280 nm ratios. RNA (10 μg) was precipitated and dissolved in 40 mM Tris-HCl. The samples (0.1–1 μg) were denatured and reverse transcribed in 20 μl of buffer composed of 10

mM Tris-HCl, 50 mM KCl, 5 mM $MgCl_2$, 20 units of RNAase inhibitor, 50 units of reverse transcriptase, 2.5 μM of oligo $(dT)_{16}$ and 1 mM deoxynucleoside triphosphates. Incubation was carried out at 37°C for 60 min and was terminated by incubation at 80°C for 10 min. Polymerase chain reaction was carried out using the following 24-mers (synthesized by Genosys Biotechnologies, Woodlands, Texas): m_1-826s, m_1-1399a; m_2-911s, m_2-1170a; m_3-1400s, m_3-1765a; m_4-902s, m_4-1559a; m_5-654s, m_5-1265a; and human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) amplimers (Clontech Laboratories, Palo Alto, CA): 586-605s, 1018-1037a. Amplifications were carried out in 100 μl of buffer containing 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 150 pmol of primers, template corresponding to 10 ng of reverse-transcribed RNA and 2.5 units of *Themus aquaticus* polymerase. Reaction mixtures were covered with mineral oil and amplifications were carried out in 0.5 ml polypropylene tubes in a DNA Thermal Cycler (Perkin Elmer Cetus Instruments). The cycles were as follows: one cycle of 95°C for 5 min, 60°C for 30 s and 72°C for 1 min; followed by 35 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 1 min; and one cycle of 95°C for 30 s, 60°C for 30 s and 72°C for 5 min. The samples (20 μl) were electrophoresed in 3% NuSieve/1% agarose gels, containing 1 μg/ml ethidium bromide, and were viewed on a 300 nm ultraviolet transilluminator.

3. Results

3.1. Differentiation of EoL-1 cells into monocytic / macrophagic cells

The differentiation of EoL-1 cells cultured with interferon-γ into monocytic/macrophagic cells was examined by observing the appearance of non-specific esterase (a marker of monocytes and macrophages) (Li et al., 1973). The induction of the non-specific esterase could not be observed in EoL-1 cells cultured without interferon-γ for 6 days (data not shown). On the other hand, as shown in Fig. 1, interferon-γ strongly induced the non-specific esterase in EoL-1 cells in a time-dependent manner, whereas weakly positive cells were reduced in number. The appearance of non-specific esterase activity was observed in about 85% of the cells cultured with 100 U/ml interferon-γ for 6 days, indicating that about 85% of EoL-1 cells were differentiated into monocytic/macrophagic cells during the treatment.

3.2. Carbachol-induced rise in $[Ca^{2+}]_i$

Carbachol at various concentrations (1–100 μM) had no effect on the rise in $[Ca^{2+}]_i$ in untreated EoL-1 cells (data not shown), but caused a dose-dependent rise in $[Ca^{2+}]_i$ in EoL-1 cells cultured with 100 U/ml interferon-γ for 6 days (Fig. 2). Atropine, a non-selective muscarinic receptor antagonist, inhibited 100 μM carbachol-induced increments in $[Ca^{2+}]_i$ in a dose-dependent manner with an IC_{50} value of 12.9 nM (Fig. 3). To characterize the subtypes of muscarinic receptors mediating $[Ca^{2+}]_i$ elevation, we examined the inhibitory effects of muscarinic receptor antagonists, pirenzepine ($M_1 > M_3 > M_2$), AF-DX 116 ($M_2 > M_1 > M_3$) and 4-DAMP ($M_3 \geqq M_1 > M_2$), on 100 μM carbachol-induced rise in $[Ca^{2+}]_i$. The order of inhibitory potency of muscarinic receptor antagonists for carbachol-induced $[Ca^{2+}]_i$ rise was 4-DAMP $\geqq$ pirenzepine $\gg$ AF-DX 116 (Fig. 4). IC_{50} values for the inhibitory effects on the 100 μM carbachol-induced

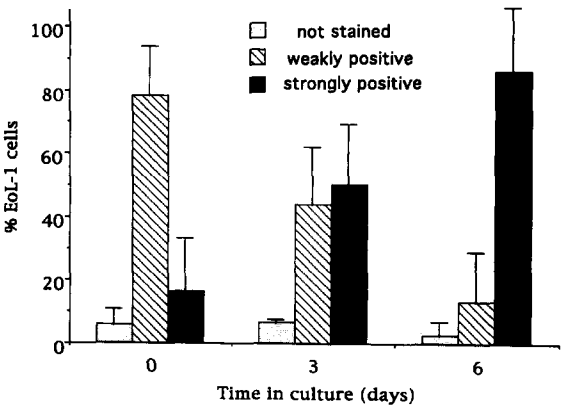

Fig. 1. Differentiation of EoL-1 cells into monocytic/macrophagic cells by treatment with interferon-γ. EoL-1 cells were cultured with 100 U/ml interferon-γ for various time periods. After each incubation period, nonspecific esterase activity was determined as the differentiation marker. Results are shown as means ± S.D. of three different experiments.

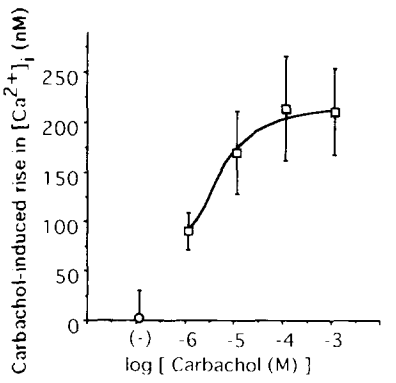

Fig. 2. Carbachol-induced rises in $[Ca^{2+}]_i$ in EoL-1 cells cultured with 100 U/ml interferon-γ for 6 days. Increments in $[Ca^{2+}]_i$ were examined for various concentrations of carbachol, according to the methods described in Materials and methods. Results are shown as means ± S.D. of measurements in ten cells. Basal value of in interferon-γ-treated EoL-1 cells was 135.0 ± 27.3 nM.

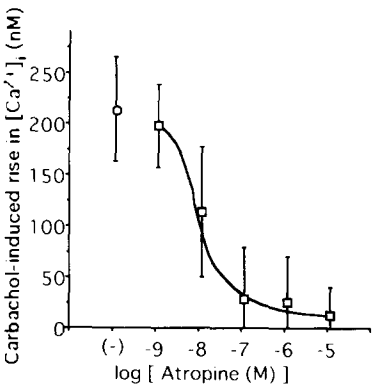

Fig. 3. Inhibitory effects of atropine on carbachol-induced rises in $[Ca^{2+}]_i$ in EoL-1 cells cultured with 100 U/ml interferon-γ for 6 days. After a pretreatment with various concentrations (1 nM to 10 mM) of atropine for 100 s, the effects of 100 μM carbachol were examined. Rises in $[Ca^{2+}]_i$ were measured by the methods described in Materials and methods. Results are shown as means ± S.D. of values obtained from ten cells.

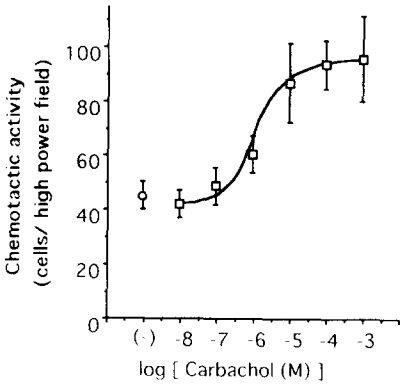

Fig. 5. Carbachol-induced chemotactic activity in interferon-γ-treated EoL-1 cells. The wells at the bottom of the chamber contained various concentration (10 nM to 1 mM) of carbachol and the upper piece contained EoL-1 cells cultured with 100 U/ml interferon-γ for 6 days. Chemotactic activity was measured according to the methods described in Materials and methods. Results are shown as means ± S.D. of values obtained from experiments performed in duplicate.

rise in $[Ca^{2+}]_i$ were 65.0 nM for pirenzepine, > 10000 nM for AF-DX 116 and 54.4 nM for 4-DAMP.

3.3. Chemotactic activity induced by carbachol

Carbachol had no effect on chemotactic activities in untreated EoL-1 cells (data not shown). On the other hand, chemotactic activities were induced in interferon-γ-treated EoL-1 cells in response to various concentrations (10 nM to 1 mM) of carbachol in a dose-dependent manner (Fig. 5). To determine the subtypes of muscarinic receptors mediating the chemotactic activity, the inhibitory effects of muscarinic receptor antagonists at various concentrations (100 nM to 100 μM) on 100 μM carbachol-induced chemotactic activity were compared. The order of inhibitory potency of muscarinic receptor antagonists for carbachol-induced chemotactic activity was 4-DAMP ≧ pirenzepine ≫ AF-DX 116 (Fig. 6). These pharmacological findings indicated that 4-DAMP and pirenzepine preferentially antagonized muscarinic M_1 and/or M_3 receptor on the surface of interferon-γ-treated EoL-1 cells, resulting in the inhibition of chemotactic activity.

3.4. The muscarinic receptor mRNA expression in EoL-1 cells

We also examined the expression of mRNA of muscarinic receptor subtypes. RNAs were extracted from

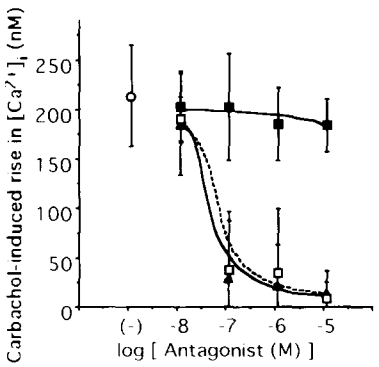

Fig. 4. Inhibitory effects of muscarinic antagonists on carbachol-induced rises in $[Ca^{2+}]_i$ in EoL-1 cells cultured with 100 U/ml interferon-γ for 6 days. After a pretreatment with pirenzepine (□), AF-DX 116 (■) or 4-DAMP (▲) at various concentrations (10 nM to 10 μM) for 100 s, the effects of 100 μM carbachol were examined. Rises in $[Ca^{2+}]_i$ were measured by the methods described in Materials and methods. Results are shown as means ± S.D. of measurements in six cells.

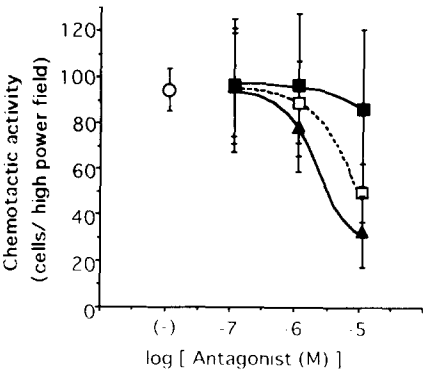

Fig. 6. Inhibitory effect of pirenzepine (□), AF-DX 116 (■) or 4-DAMP (▲) on carbachol-induced chemotactic activity in interferon-γ-treated EoL-1 cells. The wells at the bottom of the chamber contained 100 μM carbachol, and the upper piece contained various concentrations (100 nM to 10 μM) of muscarinic antagonists and EoL-1 cells cultured with 100 U/ml interferon-γ for 6 days. Chemotactic activity was measured by the methods described in Materials and methods. Results are shown as means ± S.D. of values obtained from experiments performed in duplicate.

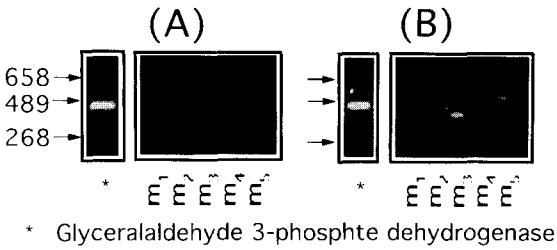

Fig. 7. Expression of muscarinic receptor mRNA in EoL-1 cells. Total RNA was prepared from untreated EoL-1 cells (A), or EoL-1 cells treated with 100 U/ml interferon-γ for 6 days (B), and reverse transcription and polymerase chain were then performed as described in Materials and methods.

untreated and interferon-γ-treated EoL-1 cells and measured using the reverse transcription polymerase chain reaction method. Untreated EoL-1 cells did not express muscarinic receptor subtypes while interferon-γ-treated EoL-1 cells expressed muscarinic M_3 and M_5 receptor subtypes. The mRNAs for muscarinic M_1, M_2 and M_4 receptor subtypes were not detected either in untreated or interferon-γ-treated EoL-1 cells (Fig. 7). In the absence of reverse transcriptase, these muscarinic receptor subtypes were not detected in untreated or interferon-γ-treated EoL-1 cells (data not shown), indicating that there was no contamination from cellular DNA or amplified products.

4. Discussion

The purpose of our present study was to determine the subtypes of muscarinic receptors expressed on monocytic/macrophagic cells by using interferon-γ-treated EoL-1 cells, and establish the functional significance of these receptors.

EoL-1 cells were originally established from a patient with eosinophilic leukemia. They have the unique property to differentiate into eosinophilic granule-containing cells when cultured with DMSO or tumor necrosis factor (Yoshie et al., 1989). Interferon-γ augmented TPA-induced membrane oxidative metabolism of EoL-1 cells, but did not change the number of EoL-1 cells with distinct eosinophilic granules (2–3%) (Yoshie et al., 1989). Interferon-γ was also shown to inhibited interleukin-3-dependent differentiation of eosinophils in a bone marrow suspension culture system (Gajewski et al., 1988; Sillaber et al., 1992). These data suggested that interferon-γ treatment did not cause differentiation of EoL-1 cells into eosinophils.

It has also been reported that HL-60 cells (human promyelocytic leukemia cell line) also differentiated into monocytic/macrophagic cells when treated with 1α, 25 $(OH)_2$ D_3, TPA (Kizaki et al., 1993) or interferon-γ (Ball et al., 1984). Interferon-γ activated oxidative metabolism and antimicrobial activity in human macrophages (Nathan et al., 1983). These results suggested that interferon-γ could induce differentiation of EoL-1 cells into monocytic/macrophagic cells. To ascertain whether interferon-γ can cause differentiation of EoL-1 cells to monocytic/macrophagic cells, we examined non-specific esterase activity in human leukocytes as a marker for differentiation into monocytic/macrophagic cells. When α-naphthyl butyrate was used as a substrate, non-specific esterase activity was strong in monocytes but very weak or not demonstrable in other blood cells (Li et al., 1973). Non-specific esterase activity of HL-60 cells was increased as they differentiated into monocytic/macrophagic cells (Kizaki et al., 1993). As shown in Fig. 1, the non-specific esterase activity was increased in EoL-1 cells after by the treatment with interferon-γ when more than 80% of the cells became strongly positive. In other words, it was shown that interferon-γ-treated EoL-1 cells became monocytic/macrophagic cells.

Human phagocytic leukocytes have been shown to possess muscarinic receptors on their surface (Lopker et al., 1980). Human monocyte chemotaxis was induced by carbachol through the activation of muscarinic receptors. It has been observed that the rounded cells change into triangular (polarized) cells before the directional movement. This polarization by the pharmacological agents (phenylephrine, carbachol and serotonin) required extracellular Ca^{2+} (Stephens and Snyderman, 1982). Therefore, we examined changes in $[Ca^{2+}]_i$ and chemotaxis induced by carbachol in interferon-γ-treated and untreated EoL-1 cells. As shown in Figs. 2 and 5, carbachol could dose-dependently induce a rise in $[Ca^{2+}]_i$ and chemotaxis only in interferon-γ-treated EoL-1 cells. By using muscarinic receptor antagonists, we tried to pharmacologically determine the muscarinic receptor subtypes that mediated the carbachol-induced increase in $[Ca^{2+}]_i$ and chemotaxis in interferon-γ-treated EoL-1 cells. Pharmacologically distinct forms of the muscarinic receptor have been classified into muscarinic M_1, M_2 and M_3 receptor subtypes from the selectivity of novel antagonists, namely pirenzepine (Hammer and Giachetti, 1982), AF-DX 116 (Giachetti et al., 1986) and 4-DAMP (Michel et al., 1989). The carbachol-induced rise in $[Ca^{2+}]_i$ and chemotaxis were inhibited by these antagonists in the order of 4-DAMP ≧ pirenzepine ≫ AF-DX 116 in interferon-γ-treated EoL-1 cells. These data suggested that the muscarinic M_2 receptor did not mediate carbachol-induced increase in $[Ca^{2+}]_i$ or chemotaxis. However, pharmacologically, it was difficult to determine whether the muscarinic M_3 or M_1 receptor subtype was mainly concerned with the activation of interferon-γ-treated EoL-1 cells by carbachol.

Genes for muscarinic receptor subtypes have recently been classified as muscarinic m_1 through m_5

receptor genes, based on results from cDNA cloning experiments (Bonner et al., 1987; Peralta et al., 1987). We examined muscarinic receptor mRNA expressed in interferon-γ-treated or untreated EoL-1 cells, to clarify which subtype of muscarinic receptor mediate the elevation of $[Ca^{2+}]_i$ and chemotaxis. As shown in Fig. 7, muscarinic receptor mRNA was not detected in EoL-1 cells not treated with interferon-γ. On the other hand, interferon-γ-treated EoL-1 cells expressed the muscarinic m_3 and m_5 receptor genes. These results showed that muscarinic m_3 and m_5 receptor mRNAs were induced as the EoL-1 cells differentiated to monocytic/macrophagic cells. Furthermore, these results also suggested that carbachol did not activate interferon-γ-treated EoL-1 cells through the muscarinic M_1 receptor. It has been reported that Jurkat cells (human leukemic helper T lymphoma cell line) express mRNA of muscarinic M_3 receptor subtype only and the rise in $[Ca^{2+}]_i$ induced by oxotremorine-M (an agonist of muscarinic receptor) was inhibited by 4-DAMP > atropine ≫ pirenzepine > AF-DX 116 in Jurkat cells (Kaneda et al., 1993). In our experiments, carbachol-induced rise in $[Ca^{2+}]_i$ and chemotaxis were inhibited by 4-DAMP = pirenzepine ≫ AF-DX 116 in interferon-γ-treated EoL-1 cells. The blocking effect of pirenzepine in our study was different from that observed by Kaneda et al. (1993). To explain this difference, the expression of muscarinic M_5 receptor may be important. Allaoua et al. (1993) reported that dopamine neurons were selectively enriched with the mRNA for the muscarinic M_5 receptor subtypes. Potentiation of *N*-methyl-D-aspartate-stimulated dopamine release by oxotremorine-M was inhibited by pirenzepine > AF-DX 384 ((±)-5,11-dihydro-11-[[[2-[2-[(dipropylamino) methyl]-1-piperidinyl]ethyl]amino]carbonyl]-6*H*-pyrido-[2,3-*b*][1,4]benzodiazepine-6-one; muscarinic M_2 receptor antagonist) ≫ 4-DAMP (Allaoua et al., 1993). It may therefore be suggested that the muscarinic M_5 receptor functionally affects the carbachol-induced rise in $[Ca^{2+}]_i$ and chemotaxis. It is expected that further analysis of interferon-γ-treated EoL-1 cells will provide much information about the role of muscarinic M_5 receptor and the interaction between muscarinic M_3 and M_5 receptors.

Generally, muscarinic M_2 and M_4 receptors inhibit the activity of adenylyl cyclase (Fukuda et al., 1989; Ashkenazi et al., 1989), while muscarinic M_1, M_3 and M_5 receptors activate the phosphatidylinositol turnover. Interestingly, interferon-γ induced the expression of muscarinic receptor subtypes that activate phosphatidylinositol turnover. Further analysis will demonstrate the role of muscarinic receptor subtypes in interferon-γ-treated EoL-1 cells.

The present study is the first to demonstrate the differentiation of EoL-1 cells into monocytic/macrophagic cells by the treatment with interferon-γ. This may provide valuable future directions to investigate the mechanisms regulating the induction and function of muscarinic receptor subtypes in human monocytes or macrophages.

References

Abood, L.G., H.G. Atkinson and M.K. MacNeil, 1976, Stereospecific opiate binding in human erythrocyte membranes and changes in heroin addicts, J. Neurosci. Res. 2, 427.

Allaoua, H., I. Chaudien, R. Alonso, R. Quirion and P. Boksa, 1993, Muscarinic potentiation of excitatory amino acid-evoked dopamine release in mesencephalic cells: specificity for the NMDA response and role of intracellular messengers, Synapse 15 (1), 39.

Aronstam, R.S., L.G. Abood and M.K. MacNeil, 1977, Muscarinic cholinergic binding in human erythrocyte membranes, Life Sci. 20, 1175.

Ashkenazi, A., E.G. Peralta, J.W. Winslow, J. Ramachandran and D.J. Capon, 1989, Functional diversity of muscarinic receptor subtypes in cellular signal transduction and growth, Trends Pharmacol. Sci. Suppl. 16.

Ball, E.D., P.M. Guyre, L. Shen, J.M. Glynn, C.R. Maliszewski, P.E. Baker and M.W. Fanger, 1984, Gamma interferon induces monocytoid differentiation in the HL-60 cell line, J. Clin. Invest. 73 (4), 1072.

Bonner, T.I., N.J. Buckley, A.C. Young and M.R. Brann, 1987, Identification of a family of muscarinic acetylcholine receptor genes, Science 237, 527.

Dulis, B.H., M.A. Gordon and I.B. Wilson, 1979, Identification of muscarinic binding sites in human neutrophils by direct binding, Mol. Pharmacol. 15, 28.

Fukuda, K., T. Kubo, A. Maeda, I. Akiba, H. Bujo, J. Nakai and M. Mishina, 1989, Selective effector coupling of muscarinic acetylcholine receptor subtypes, Trends Pharmacol. Sci. Suppl. 4.

Gajewski, T.F., E. Goldwasser and F.W. Fitch, 1988, Antiproliferative effect of IFN-γ in immune regulation. II. IFN-γ inhibits the proliferation of murine bone marrow cells stimulated with IL-3, IL-4, or granulocyte-macrophage colony-stimulating factor, J. Immumol. 141, 2635.

Genaro, A.M., L. Sterin-Borda, G. Gorelik and E. Borda, 1992, Prostanoids synthesis in lymphocyte subpopulations by adrenergic and cholinergic receptor stimulation, Int. J. Immunopharmacol. 14 (7), 1145.

Giachetti, A., R. Micheletti and E. Montagna, 1986, Cardioselective profile of AF-DX 116, a muscarinic M_2 receptor antagonist, Life Sci. 38, 1663.

Hammer, R. and A. Giachetti, 1982, Muscarinic receptor subtypes: M_1 and M_2 biochemical and functional characterization, Life Sci. 31, 2991.

Kaneda, T., Y. Kitamura and Y. Nomura, 1993, Presence of m_3 subtype muscarinic acetylcholine receptors and receptor mediated increases in the cytoplasmic concentration of Ca^{2+} in Jurkat, a human leukemic helper T lymphocyte line, Mol. Pharmacol. 43 (3) 356.

Kizaki, K., S. Naito, S. Horie, H. Ishii and M. Kazama, 1993, Different thrombomodulin induction in monocytic, macrophagic and neutrophilic cells differentiated from HL-60 cells, Biochem. Biophys. Res. Commun. 193 (1), 175.

Li, C.Y., K.W. Lam and L.T. Yam, 1973, Esterases in human leukocytes, J. Histochem. Cytochem. 21 (1), 1.

Lopker, A., L.G. Abood, W. Hoss and F.J. Lionetti, 1980, Stereoselective muscarinic acetylcholine and opiate receptors in human phagocytic leukocytes, Biochem. Pharmacol. 29, 1361.

Michel, A.D., E. Stefanich and R.L. Whiting, 1989, Direct labeling of rat M_3-muscarinic receptors by [^{3}H]-4-DAMP, Eur. J. Pharmacol. 166, 459.

Nathan, C.F., H.W. Murray, M.E. Wiebe and B.Y. Rubin, 1983, Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity, J. Exp. Med. 158, 670.

Peralta, E.G., A. Ashkenazi, J.W. Winslow, D.H. Smith, J. Ramachandran and D.J. Capon, 1987, Distinct primary structures, ligand-binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors, EMBO J. 6 (13), 3923.

Saito, H., A. Bourinbaiar, M. Ginsburg, K. Minato, E. Ceresi, K. Yamada, D. Machover, J. Bréard and G. Mathé, 1985, Establishment and characterization of a new human eosinophilic leukemia cell line, Blood 66 (6), 1233.

Sillaber, C., K. Geissler, R. Scherrer, R. Kaltenbrunner, P. Bettelheim, K. Lechner and P. Valent, 1992, Type β transforming growth factors promote interleukin-3 (IL-3)-dependent differentiation of human basophils but inhibit IL-3-dependent differentiation of human eosinophils, Blood 80, 634.

Stephens, C.G. and R. Snyderman, 1982, Cyclic nucleotides regulate the morphologic alterations required for chemotaxis in monocytes, J. Immunol. 128 (3), 1192.

Yoshie, O., T. Majima and H. Saito, 1989, Membrane oxidative metabolism of human eosinophilic cell line EoL-1 in response to phorbol diester and formyl peptide: synergistic augmentation by interferon-γ and tumor necrosis factor, J. Leuk. Biol. 45, 10.