Differential Activation of Dibutyryl cAMP-Differentiated HL-60 Human Leukemia Cells by Chemoattractants

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Abstract—Dibutyryl cAMP-differentiated HL-60 human leukemia cells possess receptors for the chemoattractants N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), C5a and leukotriene B4 (LTB4). We compared the effects of these chemoattractants in HL-60 membranes and in intact HL-60 cells. fMLP, C5a and LTB4, stimulated GTP hydrolysis and guanosine 5'-O-[3-thio]triphosphate (GTP[yS]) binding in HL-60 membranes with similar effectiveness and in a pertussis toxin (PTX)-sensitive manner. They also stimulated photolabeling of the α-subunits of the guanine nucleotide-binding proteins (G-proteins), Gα2 and Gα3 with similar effectiveness. Chloride salts of monovalent cations differentially enhanced and inhibited chemoattractant-induced GTP hydrolyses. C5a was less effective than fMLP in enhancing cholera toxin-catalysed ADP-ribosylation of Gα2 and Gα3, and LTB4 was ineffective. fMLP was more effective than C5a and LTB4 in stimulating Ca2+ influx in HL-60 cells. C5a- and LTB4-induced rises in cytosolic Ca2+ concentration ([Ca2+]i) were PTX-sensitive, whereas the effect of fMLP was partially PTX-insensitive. LTB4-induced rises in [Ca2+]i were more sensitive towards homologous desensitization than those induced by C5a, and the effect of fMLP was resistant in this regard. C5a was considerably less effective than fMLP in activating superoxide anion formation and azurophilic granule release, and LTB4 was ineffective. Our data suggest that fMLP, C5a and LTB4 effectively activate the G-proteins, Gα2 and Gα3, in HL-60 cells and that fMLP may additionally activate PTX-insensitive G-proteins. fMLP, C5a and LTB4 are full, partial and incomplete secretagogues, respectively, and these differences may be due to differences in homologous receptor desensitization and qualitative G-protein activation.

Key words: HL-60 cells; formyl peptides; complement C5a; leukotriene B4; chemoattractant receptors; G-proteins

Human neutrophils and differentiated HL-60 human leukemia cells possess receptors for the chemoattractants N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), C5a and leukotriene B4 (LTB4). We compared the effects of these chemoattractants in HL-60 membranes and in intact HL-60 cells. fMLP, C5a and LTB4, stimulated GTP hydrolysis and guanosine 5'-O-[3-thio]triphosphate (GTP[yS]) binding in HL-60 membranes with similar effectiveness and in a pertussis toxin (PTX)-sensitive manner. They also stimulated photolabeling of the α-subunits of the guanine nucleotide-binding proteins (G-proteins), Gα2 and Gα3 with similar effectiveness. Chloride salts of monovalent cations differentially enhanced and inhibited chemoattractant-induced GTP hydrolyses. C5a was less effective than fMLP in enhancing cholera toxin-catalysed ADP-ribosylation of Gα2 and Gα3, and LTB4 was ineffective. fMLP was more effective than C5a and LTB4 in stimulating Ca2+ influx in HL-60 cells. C5a- and LTB4-induced rises in cytosolic Ca2+ concentration ([Ca2+]i) were PTX-sensitive, whereas the effect of fMLP was partially PTX-insensitive. LTB4-induced rises in [Ca2+]i were more sensitive towards homologous desensitization than those induced by C5a, and the effect of fMLP was resistant in this regard. C5a was considerably less effective than fMLP in activating superoxide anion formation and azurophilic granule release, and LTB4 was ineffective. Our data suggest that fMLP, C5a and LTB4 effectively activate the G-proteins, Gα2 and Gα3, in HL-60 cells and that fMLP may additionally activate PTX-insensitive G-proteins. fMLP, C5a and LTB4 are full, partial and incomplete secretagogues, respectively, and these differences may be due to differences in homologous receptor desensitization and qualitative G-protein activation.

Key words: HL-60 cells; formyl peptides; complement C5a; leukotriene B4; chemoattractant receptors; G-proteins
have been described elsewhere [12-20]. Virtually all Gi-protein cr-subunits were ADP-ribosylated as assessed by ADP-ribosylation was determined as described [16]. In brief, reaction mixtures (100 µL) contained HL-60 membranes (3.0-7.0 µg of protein/tube), 0.5 µM [γ-32P]GTP (3.5 µCi/tube), 3 mM MgCl2, 1 mM ATP, 10 mM thymidine, 0.2% (w/v) bovine serum albumin and 2 µg of activated CTX and 0.1 M potassium phosphate, pH 7.4, in the presence of various chemoattractants. Reactions were conducted for 60 min at 30°C.

**Assay for CTX-catalysed ADP-ribosylation of membrane proteins.** ADP-ribosylation was determined according to Klinker et al. [16]. In brief, reaction mixtures (50 µL) contained HL-60 membranes (50 µg of protein/tube), 3 µM [32P]NAD (5 µCi/tube), 3 mM MgCl2, 1 mM ATP, 10 mM thymidine, 0.2% (w/v) bovine serum albumin, 2 µg of activated CTX and 0.1 M potassium phosphate, pH 7.4, in the presence of various chemoattractants. Reactions were conducted for 60 min at 30°C.

**Miscellaneous** Protein was determined according to Lowry et al. [21]. [α-32P]GTP was prepared as described [22]. [α-32P]GTP azidoanilide was prepared according to Offermanns et al. [19]. [32P]NAD was synthesized according to Cassel and Pfeuffer [23]. SDS–PAGE, immunoblotting and autoradiography were performed as described by Rosenthal et al. [18]. Immunoblotting studies using anti-peptide antisera, αi-common (AS 266), αi (AS 269) and αi (AS 105) [20], revealed that photolabeled and ADP-ribosylated 40 and 41 kDa proteins in membranes of Bt2cAMP-differentiated HL-60 cells corresponded to Gβo and Gαi, respectively (data not shown). [Ca2+]i was determined using the fluorescent dye, Fura-2, as described [13]. O2- formation was monitored at 550 nm by continuous measurement of ferricytochrome C reduction inhibitable by superoxide dismutase [EC 1.15.1.1], using an Uvikon 810 dual beam spectrophotometer (Kontron, Eching, Germany) [15]. The absolute amounts of O2- generated within 10 min were calculated. β-Glucuronidase release was determined as described by Wenzel-Seifert and Seifert [24].

**Materials and Methods**

**Materials.** Human recombinant C5a, fMLP and LTB4 were obtained from Sigma Chemie (Deisenhofen, Germany). PTX was obtained from List Biological Laboratories (Campbell, CA, U.S.A.). [35S]GTP[yS] (1000-1500 Ci/mmol) was obtained from Dupont/New England Nuclear (Bad Homburg, Germany). PTX was obtained from Pfeuffer [23]. SDS-PAGE, immunoblotting and autoradiography were performed as described by Rosenthal et al. [18]. Immunoblotting studies using anti-peptide antisera, αi-common (AS 266), αi (AS 269) and αi (AS 105) [20], revealed that photolabeled and ADP-ribosylated 40 and 41 kDa proteins in membranes of Bt2cAMP-differentiated HL-60 cells corresponded to Gβo and Gαi, respectively (data not shown). [Ca2+]i was determined using the fluorescent dye, Fura-2, as described [13]. O2- formation was monitored at 550 nm by continuous measurement of ferricytochrome C reduction inhibitable by superoxide dismutase [EC 1.15.1.1], using an Uvikon 810 dual beam spectrophotometer (Kontron, Eching, Germany) [15]. The absolute amounts of O2- generated within 10 min were calculated. β-Glucuronidase release was determined as described by Wenzel-Seifert and Seifert [24].

**Fig. 1.** Concentration–response curves for chemoattractants on high-affinity GTP hydrolysis in HL-60 membranes. High-affinity GTPase activity in HL-60 membranes was determined as described in Materials and Methods in the presence of fMLP (●), C5a (▲) and LTB4 (▼) at the indicated concentrations. Data shown are the means of assay quadruplicates. The SD values were generally less than 5% of the means. Basal GTP hydrolysis amounted to 24.4 ± 0.5 pmol/mg/min. Similar results as those shown were obtained in three independent experiments.

Pathways activated by C5a, we studied the effects of C5a in comparison to those of fMLP and LTB4 in intact Bt2cAMP-differentiated HL-60 cells and in membranes of these cells. Here, we show that there are not only differences in the state of Gi-protein activation induced by fMLP and LTB4, but also between fMLP and C5a as well as between C5a and LTB4.
Chemoattractant-induced activation of HL-60 cells

Table 1. Chemoattractant-stimulated high-affinity GTP hydrolysis and GTP[γS] binding in HL-60 membranes: effect of PTX

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Control</th>
<th>PTX</th>
<th>Control</th>
<th>PTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O (solvent)</td>
<td>26.0 ± 0.9</td>
<td>15.4 ± 0.8</td>
<td>1.09 ± 0.05</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>fMLP</td>
<td>47.7 ± 2.1</td>
<td>14.8 ± 0.5</td>
<td>2.71 ± 0.13</td>
<td>0.63 ± 0.02</td>
</tr>
<tr>
<td>C5a</td>
<td>48.9 ± 1.0</td>
<td>14.2 ± 0.4</td>
<td>2.65 ± 0.07</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>LTB4</td>
<td>44.7 ± 0.7</td>
<td>15.5 ± 1.1</td>
<td>2.81 ± 0.10</td>
<td>0.64 ± 0.03</td>
</tr>
</tbody>
</table>

Treatments with carrier (control) and PTX were performed as described in Materials and Methods. High-affinity GTPase activity and GTP[γS] binding in HL-60 membranes were determined as described in “Materials and Methods”. The concentrations of fMLP, C5a and LTB4 were 10 μM, 10 nM and 1 μM, respectively. For determination of basal GTP hydrolysis, H2O (solvent) was added instead of stimulus. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in four independent experiments.

Table 2. Effects of chloride salts of monovalent cations on chemoattractant-stimulated high-affinity GTP hydrolysis in HL-60 membranes

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Solvent (H2O)</th>
<th>NaCl</th>
<th>KCl</th>
<th>LiCl</th>
<th>Choline chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>fMLP</td>
<td>80</td>
<td>123</td>
<td>160</td>
<td>118</td>
<td>127</td>
</tr>
<tr>
<td>C5a</td>
<td>84</td>
<td>57</td>
<td>71</td>
<td>45</td>
<td>77</td>
</tr>
<tr>
<td>LTB4</td>
<td>65</td>
<td>145</td>
<td>146</td>
<td>116</td>
<td>84</td>
</tr>
</tbody>
</table>

High-affinity GTPase activity in HL-60 membranes was determined in the presence of various chloride salts of monovalent cations (150 mM each) or solvent as described in Materials and Methods. The concentrations of fMLP, C5a and LTB4 were 10 μM, 10 nM and 1 μM, respectively. Data shown are the means of assay quadruplicates. The SD values were generally less than 5% of the means. Basal GTP hydrolyses in the presence of solvent, NaCl, KCl, LiCl and choline chloride were 20.2 ± 0.6, 20.3 ± 0.5, 19.6 ± 0.3, 25.3 ± 0.4 and 19.0 ± 0.5 pmol/mg/min, respectively. The stimulatory effects of chemoattractants are referred to these basal GTP hydrolyses. Similar results as those shown were obtained in three independent experiments.

RESULTS

We first studied the effects of chemoattractants on high-affinity GTPase activity (EC 3.6.1.-) in HL-60 membranes. fMLP activated GTP hydrolysis with an EC50 of 190 nM and a plateau at 10–30 μM (Fig. 1). The corresponding values for C5a were 300 pM and 10–100 nM, respectively. The EC50 of LTB4 for GTPase stimulation was 60 nM, and a plateau was reached at 1–3 μM. At maximally stimulatory concentrations, the chemoattractants were similarly effective in activating GTP hydrolysis. As has been shown for fMLP in HL-60 membranes [16], C5a and LTB4 also increased Vmax of GTP hydrolysis (data not shown).

Table 1 compares the effects of chemoattractants at maximally stimulatory concentrations on GTP hydrolysis and GTP[γS] binding in control membranes and in membranes of PTX-treated HL-60 cells. As was the case for GTP hydrolysis, fMLP, C5a and LTB4 were similarly effective in stimulating GTP[γS] binding. PTX abolished the stimulatory effects of chemoattractants on GTPase and GTP[γS] binding.

Chloride salts of monovalent cations enhance fMLP-induced GTPase activation in HL-60 membranes, presumably through an alteration of receptor/G-protein interaction [3, 25]. NaCl, KCl, LiCl and choline chloride (150 mM each) enhanced the relative stimulatory effects of fMLP on GTPase in HL-60 membranes by 54, 100, 48 and 59%, respectively (Table 2). The corresponding values for LTB4 were 123, 125, 78 and 29%, respectively. In contrast, NaCl, KCl, LiCl and choline chloride diminished C5a-stimulated GTP hydrolysis by 32, 15, 46 and 8%, respectively.

Next, we assessed the effects of fMLP, C5a and LTB4 on photolabeling of G-protein α-subunits with GTP azidoanilide. After photolabeling, G-protein α-subunits were immunoprecipitated with the αcommon antisera and then subjected to SDS-PAGE and autoradiography. fMLP, C5a and LTB4 were similarly effective in enhancing incorporation of GTP azidoanilide into 40 kDa proteins (Gia2) and into 41 kDa proteins (Gia3) (Fig. 2). The abundance of Gia2 in HL-60 cells is much higher than that of Gia3 [6, 26], so that the intensity of labeling of the latter protein is considerably lower than that of the former.
Fig. 2. Effects of chemoattractants on incorporation of GTP azidoanilide into G-protein α-subunits in HL-60 membranes. Photolabeling and immunoprecipitation were performed as described in Materials and Methods. The autoradiogram of an SDS gel containing 4.3 M urea and 8% (w/v) acrylamide is shown. Lane 1, fMLP (10 μM); lane 2, H2O added instead of stimulus (control); lane 3, LTB4 (1 μM); lane 4, C5a (1 μM). Numbers on the left, molecular masses of marker proteins (kDa). 40 kDa proteins represent Gia2, and 41 kDa proteins represent Gia3. The autoradiogram shown is representative of four independent experiments.

Fig. 3. Effects of fMLP and C5a on CTX-catalysed ADP-ribosylation of G-protein α-subunits in HL-60 membranes. CTX-catalysed ADP-ribosylation was performed as described in Materials and Methods. The autoradiogram of an SDS gel containing 4.3 M urea and 9% (w/v) acrylamide is shown. Lane 1, H2O added instead of stimulus (control); lane 2, C5a (10 nM); lane 3, C5a (100 nM); lane 4, C5a (1 μM); lane 5, fMLP (1 μM). Numbers on the left, molecular masses of marker proteins (kDa). DF, dye front. 40 kDa proteins represent Gia2, and 41 kDa proteins represent Gia3. The autoradiogram shown is representative of three independent experiments.

Labeling of a 31 kDa protein with GTP azidoanilide was observed as well (see Fig. 2). Unlike 40 and 41 kDa proteins, chemoattractants did not enhance labeling of the 31 kDa protein. Thus, the 31 kDa protein may represent a proteolytic fragment of Gia2 and/or Gia3 which still binds GTP azidoanilide but no longer interacts with chemoattractant receptors.

The effects of chemoattractants on CTX-catalysed ADP-ribosylation of G-protein α-subunits were studied as well. As has been shown for membranes of DMSO-differentiated HL-60 cells [7], LTB4 (1 μM) did not enhance ADP-ribosylation of G-protein α-subunits in membranes of Bt2cAMP-differentiated HL-60 cells (data not shown). In contrast, fMLP (1 μM) effectively stimulated CTX-catalysed ADP-ribosylation of Gia2 and Gia3 (Fig. 3). By comparison to fMLP, C5a (10 nM–1 μM) was considerably less effective in enhancing ADP-ribosylation of Gia2 and, specifically, of Gia3.

Time courses of the rises in [Ca2+]i caused by chemoattractants at maximally stimulatory concentrations [13] are shown in Fig. 4. fMLP, C5a and LTB4 induced rapid rises in [Ca2+]i. The peak [Ca2+]i values stimulated by fMLP and C5a were higher than those induced by LTB4. In the absence of extracellular Ca2+, chemoattractant-induced rises in [Ca2+]i were shorter than in its presence. These data indicate that fMLP, C5a and LTB4 induced both Ca2+ mobilization from intracellular stores and Ca2+ influx from the extracellular space. The imidazole, 1-β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl-1H-imidazole hydrochloride (SK&F 96365), reduces fMLP-induced Ca2+ influx through non-selective cation channels in Bt2cAMP-differentiated HL-60 cells [3]. SK&F 96365 (30 μM) inhibited C5a- and LTB4-induced Ca2+ influxes to the same extent as that induced by fMLP (data not shown), indicating that they were mediated through non-selective cation channels as well. C5a- and LTB4-induced Ca2+ influxes declined more rapidly than fMLP-induced Ca2+ influx. Chemoattractant-induced Ca2+ mobilizations were blocked by PTX. With respect to Ca2+ influx, approx 20% of the effect of fMLP was PTX-insensitive. In comparison, PTX inhibited C5a- and LTB4-induced Ca2+ influxes almost completely.
Fig. 4. Chemoattractant-induced rises in \([\text{Ca}^{2+}]_i\) in HL-60 cells: effect of PTX. Determinations of \([\text{Ca}^{2+}]_i\) were performed as described in Materials and Methods. The increases in \([\text{Ca}^{2+}]_i\), induced by fMLP (1 nM), C5a (10 nM) and LTB4 (10 nM) in the presence of 1 mM extracellular CaCl\(_2\) (A–C) or 1 mM extracellular EGTA (D–F) were assessed. Treatments with carrier (control) (traces 1) and PTX (traces 2) were performed as described in Materials and Methods. Arrows indicate the addition of chemoattractants. Superimposed original fluorescence tracings are shown. Similar results were obtained in four experiments with different preparations of HL-60 cells.

Fig. 5. Homologous desensitization of chemoattractant-induced rises in \([\text{Ca}^{2+}]_i\) in HL-60 cells. Determinations of \([\text{Ca}^{2+}]_i\), were performed as described in Materials and Methods. The increases in \([\text{Ca}^{2+}]_i\), induced by chemoattractants in the presence of 1 mM extracellular CaCl\(_2\) were assessed. The arrows on the left indicate the additions of fMLP (2 nM), C5a (0.3 nM) and LTB4 (3 nM), respectively. The arrows on the right indicate the repeated additions of chemoattractants at the same concentrations. Superimposed original fluorescence tracings are shown. Similar results were obtained in three experiments with different preparations of HL-60 cells.

Table 3. Effects of chemoattractants on \(O_2^-\) formation and \(\beta\)-glucuronidase release in HL-60 cells

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>(O_2^-) formation (nmol/10(^6) cells)</th>
<th>(\beta)-glucuronidase release (% of cellular content)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fMLP</td>
<td>2.5 ± 0.3</td>
<td>34.1 ± 3.3</td>
</tr>
<tr>
<td>C5a</td>
<td>1.3 ± 0.2</td>
<td>21.3 ± 2.0</td>
</tr>
<tr>
<td>LTB4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

O\(_2^-\) formation and \(\beta\)-glucuronidase release in HL-60 cells were determined as described in Materials and Methods. The concentrations of fMLP, C5a and LTB4 were 1 nM, 100 nM and 1 nM, respectively. In the absence of stimuli, HL-60 cells did not generate \(O_2^-\). Basal \(\beta\)-glucuronidase release in the absence of stimuli was 5.5 ± 0.6% of the cellular content. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.
DISCUSSION

The aim of our study was to characterize C5a-mediated signal transduction pathways in Bt2cAMP-differentiated HL-60 cells. Specifically, we were interested in determining whether there are differences between fMLP and C5a and between C5a and LTB4. The three chemoattractants were similarly effective in activating GTP hydrolysis, GTP[γS] binding and Ca²⁺ mobilization, the latter parameter reflecting phospholipase C activation, and their effects were completely PTX-sensitive (see Table 1 and Fig. 4). Thus, fMLP, C5a and LTB4 activate G-proteins in Bt2cAMP-differentiated HL-60 cells. Similar data have been obtained for human neutrophils and DMSO-differentiated HL-60 cells [4, 5, 7].

In order to ascertain which G-protein subtypes are activated by chemoattractants, we photolabeled G-protein α-subunits with GTP azidoanilide. In agreement with the data obtained for membranes of DMSO-differentiated HL-60 cells, we found that chemoattractants were similarly effective in activating Gia2 in membranes of Bt2cAMP-differentiated HL-60 membranes (see Fig. 2) [6]. In our previous study [6], we were unable to determine whether fMLP, C5a and LTB4 activate Gia2 in HL-60 membranes. This could have been due to the lack of resolution of Gia2 and Gia3 on SDS-PAGE [6]. Another reason could have been that the concentration ratio Gia2/Gia3 in HL-60 membranes is high [6, 25], so that the strong labeling of a Gia2 masked that of Gia3. Therefore, we increased the sensitivity of the method by immunoprecipitating G-protein α-subunits with the anti-peptide antiserum, α common, before performing SDS-PAGE and by including urea into the gel. Under these conditions, labeling of Gia3 was, in fact, evident. As was the case for Gia2, chemoattractants were similarly effective in increasing photolabeling of Gia3 (see Fig. 2).

However, when CTX-catalysed ADP-ribosylation is considered, substantial differences between fMLP, C5a and LTB4 become apparent. Specifically, C5a activated ADP-ribosylation of Gia2 and, particularly, of Gia3 much less effectively than fMLP, and LTB4 was ineffective (see Fig. 3). Intriguingly, there was no difference in the extent of ADP-ribosylation of Gia2 and Gia3 in the presence of MFLP, whereas with respect to incorporation of GTP azidoanilide, labeling of Gia3 was much more prominent than that of Gia2 (compare lane 1 in Fig. 2 with lane 5 in Fig. 3). These differences between CTX-catalysed ADP-ribosylation and photolabeling and the data concerning GTP hydrolysis and GTP[γS] binding (see Table 1) indicate that the former parameter is not suitable to assess quantitatively G-protein activation by chemoattractants. Rather, CTX-catalysed ADP-ribosylation is useful in revealing qualitative differences in the G-protein activation state caused by fMLP, C5a and LTB4.

Differences in the effects of chemoattractants on G-protein activation were not only apparent with respect to CTX-catalysed ADP-ribosylation but also with regard to the effects of chloride salts of monovalent cations on GTP hydrolysis. Specifically, cations enhanced the effectiveness of fMLP and LTB4 in activating GTP hydrolysis, whereas they decreased that of C5a (see Table 2). It is noteworthy that NaCl and KCl were similarly effective with respect to LTB4 but with respect to fMLP, KCl was more effective than NaCl (see Table 2). When C5a is considered, LiCl was the most effective inhibitor (see Table 2). Thus, the differential effects of salts on chemoattractant GTP hydrolyses support our assumption that there are not only qualitative differences in the G-protein activation state induced by fMLP and LTB4, but also between fMLP and C5a and between C5a and LTB4.

On the level of intact cells, we observed differences between the three chemoattractants as well. Specifically, the order of effectiveness of agonists in inducing Ca²⁺ influx was fMLP > C5a > LTB4 (see Fig. 4). In a recent study, we showed that Ca²⁺ influx is required for activation of O²⁻ formation and β-glucuronidase release [2]. In accordance with the aforementioned data, the order of effectiveness of chemoattractants in activating O²⁻ formation and exocytosis was found to be fMLP > C5a > LTB4 (ineffective) (see Table 3). In agreement with our results, Stutchfield and Cockcroft [27] reported that C5a is less effective than fMLP in activating phosphatidic acid formation in Bt2cAMP-differentiated HL-60 cells. Thus, fMLP is a full secretagogue, C5a is a partial secretagogue and LTB4 is an incomplete secretagogue (as it induces releases in [Ca²⁺] but not O²⁻ formation or exocytosis). Histamine, acting via H₂-receptors, is also an incomplete secretagogue in Bt2cAMP-differentiated HL-60 cells [28]. However, unlike the effects of LTB4 on [Ca²⁺], those of histamine show substantial PTX-insensitivity (see Fig. 4) [28].

Differences in receptor desensitization may account, at least in part, for the differential effectiveness of chemoattractants in activating C5a²⁺ influx, O²⁻ formation and β-glucuronidase release. Indeed, the order of sensitivity of chemoattractants towards homologous desensitization was LTB4 > C5a > fMLP (resistant) (see Fig. 5). This order correlates inversely with that concerning activation of C5a²⁺ influx, O²⁻ formation and exocytosis (compare Fig. 5 with Fig. 4 and Table 3). Like LTB4-induced rises in [Ca²⁺], those induced by histamine in Bt2cAMP-differentiated HL-60 cells are very sensitive to homologous desensitization [28]. Differences in homologous desensitization of receptors could also provide an explanation for the findings that C5a-induced rises in [Ca²⁺], and NADPH oxidase activation are shorter than those induced by fMLP (see Fig. 4) [10, 11]. Interestingly, C5a receptors are also more sensitive to desensitization via protein kinase C than formyl peptide receptors [29]. In contrast, cAMP-increasing substances inhibit fMLP-induced exocytosis, whereas C5a-induced β-glucuronidase release is unaffected [12].

Quantitative differences in activation of G-protein α-subunits do not contribute to the differential effects of fMLP, C5a and LTB4 on C5a²⁺ influx, O²⁻ formation and exocytosis as they showed very similar effects on GTP hydrolysis, GTP[γS] binding and photolabeling (see Table 1 and Fig. 2). However, the differences between the chemoattractants
concerning CTX-catalysed ADP-ribosylation (see Fig. 3) and regulation by chloride salts of monovalent cations (see Table 2) of GTP hydrolysis suggest that qualitative differences in G-protein activation states finally result in differential cellular effects of fMLP, C5a and LTB4. The molecular basis for this differential G-protein activation remains to be determined.

Recently, Amatruda et al. [30] reported that in transfected COS-7 cells, C5a receptors mediate activation of phospholipase C through the PTX-insensitive G-protein, Gαs, a member of the Gq-family. Moreover, C5a activates phospholipase C through a partially PTX-insensitive mechanism in the monocytic cell line, THP-1, which expresses Gαs at high concentrations [30]. Furthermore, C5a mediates Ca2+ influx in Bt2cAMP-differentiated U937 cells through a PTX-insensitive mechanism [31].

Evidently, activation by C5a of phospholipase C via Gαs or another PTX-insensitive G-protein of the Gq-family is not of relevance in Bt2cAMP-differentiated HL-60 cells, as C5α-induced Ca2+ mobilization was abolished by PTX (see Fig. 4). Additionally, PTX-insensitive G-proteins are also not of particular importance for mediation of C5α-induced Ca2+ influx in Bt2cAMP-differentiated HL-60 cells (see Fig. 4). In human neutrophils, C5α-induced Ca2+- influx is PTX-sensitive as well [31]. Taken together, these findings show that the coupling of C5a receptors to Gi-proteins and PTX-insensitive G-proteins in myeloid cells shows substantial cell type specificity.

Compared to C5α, fMLP-induced Ca2+ influx in Bt2cAMP-differentiated HL-60 cells shows substantial PTX-insensitivity (see Fig. 4). However, fMLP did not show any stimulatory effect on GTPase and GTP[yS] binding in membranes of PTX-treated cells (see Table 1). An explanation for this apparent discrepancy could be the fact that the guanine nucleotide exchange rates of PTX-insensitive G-proteins of the Gq-family are low [32] so that their activation in membranes is masked by the rapidly exchanging G-proteins.

In conclusion, fMLP, C5a and LTB4 activate the G-proteins, Gαq and Gα12, in Bt2cAMP-differentiated HL-60 cells, but they do so in different manners. Our data suggest that Gα-proteins are not static signal amplifiers, but that G-proteins transduce and enhance signals differentially, depending on the type of chemoattractant receptor being activated. Unlike fMLP, C5a is only a partial secretagogue in Bt2cAMP-differentiated HL-60 cells and this may be due to differences in homologous receptor desensitization and qualitative G-protein activation.

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