Granulocyte-Macrophage Colony-Stimulating Factor, Interleukin-3 (IL-3), and IL-5 Greatly Enhance the Interaction of Human Eosinophils With Opsonized Particles by Changing the Affinity of Complement Receptor Type 3

By Michela Blom, Anton T.J. Tool, Paul T.M. Kok, Leo Koenderman, Dirk Roos, and Arthur J. Verhoeven

Eosinophil functions can be modulated by several cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5. We have investigated the modulatory role of these cytokines on the interaction of human eosinophils with opsonized particles (serum-treated zymosan [STZ]). Addition of STZ to eosinophils isolated from the peripheral blood of normal human donors resulted in an interaction of the STZ particles with only 15% to 25% of the cells. Treatment of the eosinophils with GM-CSF, IL-3, or IL-5 strongly enhanced both the rate of particle binding and the percentage of eosinophils binding STZ. The effect of the cytokines is most likely mediated by a change in affinity of the complement receptor type 3 (CR3) on the eosinophils for the complement fragment iC3b on the STZ particles. This is indicated by the observation that (1) the effect of the cytokines on STZ binding was prevented by a monoclonal antibody against the iC3b-binding site on CR3 and (2) the enhanced binding was already apparent before upregulation of CR3 on the cell surface was observed. In a previous study, similar results were obtained with platelet-activating factor (PAF)-primed eosinophils. Because we found that the cytokines strongly enhanced the STZ-induced PAF synthesis, we investigated the role of both released PAF and cell-associated PAF in the priming phenomenon by the cytokines. Cytokine priming appeared to be largely independent of the synthesis of PAF.

The development and function of eosinophils are regulated by a number of cytokines. Important cytokines during maturation and differentiation are granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and IL-5. In mature eosinophils, these cytokines prolong the in vitro survival. Moreover, they can potentiate ("prime") several functional responses of eosinophils, such as respiratory burst activity, antibody-dependent cytotoxicity, degranulation, and migratory responsiveness. The highly overlapping biologic activities of GM-CSF, IL-3, and IL-5 can be explained by the presence of a common β-chain in the receptors for these three cytokines. The α-chain determines the specificity of each receptor.

In this study, we have investigated the effect of GM-CSF, IL-3, and IL-5 on the interaction of opsonized particles (serum-treated zymosan [STZ]) with human eosinophils. Priming of eosinophils with GM-CSF, IL-3, or IL-5 strongly accelerated the velocity of particle binding and also more than doubled the percentage of eosinophils binding STZ particles. This enhanced binding is almost completely mediated by the complement receptor 3 (CR3) because the monoclonal antibody (MoAb) B2.12, directed against the iC3b-binding site on CR3, inhibited the binding of STZ particles to cytokine-primed eosinophils. Previously, we have shown that binding of STZ particles to eosinophils and subsequent activation of these cells is very sensitive for platelet-activating factor (PAF). Therefore, we investigated the influence of cytokine priming on the STZ-induced PAF synthesis to evaluate a possible role of endogenous PAF production in the enhancement of the percentage of eosinophils binding STZ caused by cytokines.

Materials and Methods

Materials. IL-3 and GM-CSF were obtained from Sandoz (Basel, Switzerland). IL-5 was obtained from Amersham (Amersham, UK). STZ was prepared as described before. Biotin-N-hydroxysuccinimide ester (BNHS) was purchased from Calbiochem (La Jolla, CA). Mepacrine was obtained from Sigma (St Louis, MO). Fluorescein isothiocyanate (FITC)-conjugated streptavidin was purchased from Caltech (San Francisco, CA). Hydroethidine (HE) was prepared from Polyscience (Warrington, PA). Stock solutions of HE (1 mg/mL, in Earles, 10% fetal calf serum [FCS]) were kept at 4°C for periods of up to 3 weeks. CD45 antibody (clone 3D3, IgG1 isotype) was a kind gift of Dr R. van Lier (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). All other chemicals were reagent grade. "Incubation medium" for the cell incubations contained 132 mmol/L NaCl, 6.0 mmol/L CaCl2, 1.0 mmol/L MgSO4, 1.2 mmol/L potassium phosphate, 20 mmol/L HEPES, 5.5 mmol/L glucose, and 0.5% (wt/vol) human albumin, pH 7.4. The MoAbs D3.J3 (IgG1 isotype), B2.12 (IgM isotype), VM-2 (IgM isotype), OKM-1 (IgG2b isotype), Bear-1 (IgG1 isotype), and IV.3 (IgG2b) have been described before.

Cell isolation. Blood was obtained from healthy volunteers. Eosinophils were isolated from the buffy coat of 500 mL of blood anticoagulated with 13 mmol/L trisodium citrate (pH 7.4) as described before, with slight modifications. In short, the mononuclear cells were removed by centrifugation of the blood over isocitrate Percoll (1.077 g/mL, pH 7.4). After isotonic lysis of erythrocytes in the pellet with an ice-cold solution containing 155 mmol/L NH4Cl, 10 mmol/L KHCO3, and 0.1 mmol/L EDTA (pH 7.2), the granulocytes were washed and suspended in incubation medium without
CaCl₂. Subsequently, the granulocytes were incubated for 30 minutes at 37°C to restore the initial density of the cells. After this incubation period, the cells were washed and resuspended in phosphate-buffered saline (PBS) supplemented with human serum albumin (0.5% wt/vol) and trisodium citrate (13 mmol/L). After preincubation of the granulocytes for 5 minutes at 37°C, FMLP (10 nmol/L) was added to the cell suspension, and the incubation was continued for 10 minutes. Subsequently, the eosinophils were purified by centrifugation (15 minutes at 1,000g) over isotonic Percoll (1.082 g/mL, pH 7.4). This purification method does not induce priming of the eosinophils. The eosinophils (purity, >95%) were washed and suspended in incubation medium.

**Measurement of STZ binding.** Binding of STZ particles was measured in a flow cytometer by a double-color assay, according to principles previously applied for cell-cell interaction. In short, STZ particles were biotinylated (30 μg/mg of STZ) for 2 hours at room temp. Subsequently, the particles were washed twice in PBS and incubated with streptavidin-FITC or (when indicated) with streptavidin–TRICOLOR for 30 minutes at room temperature. The fluorescent STZ particles retained the same potency to stimulate the respiratory burst of eosinophils as did the original STZ particles. Eosinophils (4 × 10⁶ cells/mL) were stained red by incubation for 60 minutes with HE (40 μmol/L). The cells were washed twice and were resuspended in incubation medium at 2 × 10⁶ cells/mL. After 5 minutes of preincubation of the eosinophils at 37°C in a reaction vessel that was thermostatted and equipped with a stirring magnet, either IL-3, IL-5, GM-CSF, or solvent was added. After cytokine treatment, FITC-conjugated STZ (FITC-STZ; 1 mg/mL) was added to the cells. At the times indicated, samples were taken and immediately fixed with ice-cold paraformaldehyde (PFA; 1% wt/vol in incubation medium). The samples were kept on ice and in the dark until analysis in a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) was performed. A "live" gate, analyzing the red fluorescent eosinophils and all double-colored events, was set to avoid measurement of the unbound particles. The results are expressed as double-colored events as a percentage of the total number of colored events and were corrected for spillover of green fluorescent particles (as determined by the addition of particles to PFA-treated eosinophils).

When added, mepacrine had a dual function: (1) to make the eosinophils green fluorescent and (2) to inhibit PAF synthesis. As a fluorescent dye, mepacrine (100 μmol/L) was added to the eosinophils 10 minutes after the addition of biotinylated, TRICOLOR-conjugated STZ. At this time, the eosinophils had phagocytosed the particles, so the influence of mepacrine as a phospholipase A₂ (PLA₂) inhibitor was bypassed. The percentage of PAF-treated eosinophils binding STZ particles determined in this way was similar to that found with the method in which HE was used as fluorescent dye. As a PLA₂ inhibitor, mepacrine (100 μmol/L) was added 5 minutes before the addition of STZ. Samples were taken and analyzed as described above.

**Receptor expression.** The expression of the surface antigen CR3 on human eosinophils was measured by indirect immunofluorescence and flow cytometry. In short, eosinophils were incubated with the cytokines (25 pg/mL) for various periods at 37°C and were fixed with 1% PFA before immunostaining. After the addition of the primary MoAb (B2.12, 10 μg/mL) for 30 minutes at 4°C and subsequent washes with ice-cold PBS containing 0.5% bovine serum albumin and 7.5 mmol/L sodium azide, fluoresceinated goat-antimouse-Ig was added and kept with the cells for another 30 minutes at 4°C. After washing, MoAb binding was quantitated for 5,000 cells with a FACScan flow cytometer (Becton Dickinson), and expressed as mean fluorescent intensity (MFI).

**Measurement of PAF.** PAF was measured with a commercially available competitive radioimmunoassay (New England Nuclear, Boston, MA). Samples were prepared as follows. After a preincubation of 5 minutes, eosinophils (2 × 10⁶/mL, 0.6 mL) were primed with GM-CSF, IL-3, or IL-5 (25 ng/mL) for 30 minutes. Mepacrine (100 μmol/L) or its solvent dimethylsulfoxide were added 5 minutes before the eosinophils were stimulated with 1 mg STZ/mL. The reaction was stopped 15 minutes after the addition of STZ by adding 3 mL of methanol/chloroform (2:1) with 2% (vol/vol) acetic acid included in the methanol (total PAF). To measure PAF release, the activated eosinophils were rapidly spun down (12,000g for 5 seconds) and the supernatant was then added to 3 mL of the methanol/chloroform mixture described above. After separation of the phases with 1 mL of chloroform and 1 mL of H₂O, the lower chloroform phase was stored at −70°C under nitrogen. Subsequently, the chloroform phases were evaporated by a stream of nitrogen and processed for radioimmunoassay according to the manufacturer’s instructions. The amounts of PAF in the samples were determined by comparison with a standard curve constructed with known amounts of PAF. Recovery of PAF during the whole procedure was more than 90%.

**RESULTS**

**Effect of GM-CSF, IL-3, and IL-5 on the interaction of human eosinophils with STZ.** The binding of STZ to human eosinophils kept in suspension was quantified by means of a double-color fluorescence-activated cell sorter (FACS) analysis. For this purpose, the STZ particles had been given a green fluorescent stain and the eosinophils a red fluorescent stain. Addition of STZ particles to eosinophils isolated from the peripheral blood of normal donors resulted in interaction of the STZ particles with only about 15% to 25% of the cells (as detected by double-colored events) 15 minutes after addition of STZ (Blom et al., see also Fig 1). Pretreatment of the eosinophils with GM-CSF, IL-3, or IL-5 for 30 minutes strongly enhanced the rate of particle binding and also increased the percentage of eosinophils binding STZ particles. As shown in Fig 1, this enhancement was dependent on the dose of the cytokines. Maximal enhancement was already present after priming for 5 minutes with 25 ng/mL of the cytokines (Table 1). The three cytokines tested showed a comparable ability to enhance the number of cells interacting with STZ.

Because the STZ particles used in these experiments are predominately coated with the complement fragment iC3b, we investigated the participation of iC3b in the STZ particle binding by using MoAb B2.12. This antibody is directed against the iC3b-binding site on CR3. The enhancement on STZ binding induced after GM-CSF priming was completely blocked when the eosinophils were preincubated with MoAb B2.12 (Table 2). Comparable effects of MoAb B2.12 were observed after priming with IL-3 or IL-5: the percentage of eosinophils binding STZ decreased from 60.5% ± 8.9% with IL-3 to 20.8% ± 4.5% and from 64.4% ± 6.9% with IL-5 to 25.6% ± 6.3%. A control IgM MoAb VIM-2 did not influence the interaction of STZ with unprimed or GM-CSF-primed eosinophils. Bear-1, an MoAb that recognizes the α-chain of CR3, also inhibited the GM-CSF-primed binding but not as completely as did MoAb B2.12. The observed decrease in the percentage of eosinophils binding STZ was 38%. Elevation of the concentration of Bear-1 to 50 μg/mL did not increase the extent of inhibition. Although complement fragment C3b was not detectable on the STZ particles, it was present at various levels on the STZ particles.
Fig 1. Dose-dependent cytokine priming of the binding of STZ particles to human eosinophils. After incubation of (red fluorescent) eosinophils without (C) or with different concentrations of (A) GM-CSF ([γ] 2.5 ng/mL, [β] 25 ng/mL, [α] 250 ng/mL), (B) IL-3 ([γ] 2.5 ng/mL, [β] 25 ng/mL, [α] 250 ng/mL), or (C) IL-5 ([γ] 2.5 ng/mL, [β] 25 ng/mL, [α] 250 ng/mL) for 30 minutes at 37°C, green fluorescent STZ particles (1 mg/mL) were added. Samples were taken at the times indicated in the figure and analyzed by flow cytometry. Double-colored events were scored and depicted as percentage of total number of red events. Results are expressed as means ± SD of three different experiments.

We investigated the involvement of the C3b/CR1 interaction in the particle binding by incubating the eosinophils with MoAb D3.53. This MoAb is directed against CR1 and has the capacity to block C3b binding. Incubation of unprimed or GM-CSF-primed eosinophils with MoAb D3.53 did not affect the percentage of eosinophils binding STZ (Table 2).

The iC3b on the STZ is fixed both to the particles and to the Fc-region of the IgG present on the zymosan particles.

Table 1. Time-Dependent Effect of Cytokine Priming on the Binding of STZ Particles to Human Eosinophils

<table>
<thead>
<tr>
<th>Priming (min)</th>
<th>Solvent</th>
<th>GM-CSF</th>
<th>IL-3</th>
<th>IL-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>23.1 ± 2.5</td>
<td>72.8 ± 12.3</td>
<td>70.8 ± 12.2</td>
<td>74.7 ± 7.6</td>
</tr>
<tr>
<td>30</td>
<td>24.2 ± 4.6</td>
<td>75.1 ± 11.9</td>
<td>72.9 ± 11.7</td>
<td>73.6 ± 7.8</td>
</tr>
<tr>
<td>45</td>
<td>26.0 ± 7.7</td>
<td>77.3 ± 9.0</td>
<td>69.5 ± 13.9</td>
<td>74.0 ± 10.2</td>
</tr>
<tr>
<td>60</td>
<td>25.8 ± 6.3</td>
<td>75.9 ± 7.0</td>
<td>67.6 ± 10.9</td>
<td>74.1 ± 11.5</td>
</tr>
</tbody>
</table>

Effect of different incubation times with cytokines on the percentage of eosinophils binding STZ. After incubation of the (red fluorescent) eosinophils for 5, 30, 45, or 60 minutes with GM-CSF, IL-3, or IL-5 (25 ng/mL) at 37°C, green fluorescent STZ particles were added. Samples were taken 15 minutes after the addition of STZ and analyzed by flow cytometry. Double-colored events were scored and depicted as a percentage of the total number of red events. Results are expressed as means ± SD of three different experiments.

Table 2. Involvement of CR1, CR3, and FcγRII in STZ Binding by Human Eosinophils

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Subclass</th>
<th>Receptor</th>
<th>Unprimed</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>59.7 ± 6.7</td>
<td></td>
<td>20.1 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>B2.12</td>
<td>IgM</td>
<td>CR3</td>
<td>18.8 ± 3.4</td>
<td>26.2 ± 3.8*</td>
</tr>
<tr>
<td>Bear-1</td>
<td>IgG1</td>
<td>CR3</td>
<td>19.3 ± 3.7</td>
<td>44.7 ± 6.7*</td>
</tr>
<tr>
<td>OMK-1</td>
<td>IgG2b</td>
<td>CR3</td>
<td>20.1 ± 6.0</td>
<td>64.6 ± 4.4*</td>
</tr>
<tr>
<td>D3.J3</td>
<td>IgG1</td>
<td>CR1</td>
<td>18.3 ± 4.5</td>
<td>57.3 ± 13.4</td>
</tr>
<tr>
<td>IV.3</td>
<td>IgG2b</td>
<td>FcγRII</td>
<td>17.5 ± 2.4</td>
<td>62.8 ± 6.1</td>
</tr>
<tr>
<td>VIM-2</td>
<td>IgM</td>
<td>CD66</td>
<td>23.2 ± 5.1</td>
<td>60.3 ± 3.9</td>
</tr>
<tr>
<td>3D3</td>
<td>IgG1</td>
<td>CD45</td>
<td>19.9 ± 3.9</td>
<td>61.8 ± 9.3</td>
</tr>
</tbody>
</table>

The effect of blockade of CR3, CR1, or FcγRII on the percentage of human eosinophils binding STZ particles. After 20 minutes of incubation with GM-CSF (25 ng/mL), optimal concentrations of MoAbs B2.12 (75 µg/mL), Bear-1 (25 µg/mL), OMK-1 (25 µg/mL), D3.J3 (25 µg/mL), or IV.3 (25 µg/mL) were added to the eosinophils. MoAbs VIM-2 (75 µg/mL) and 3D3 (25 µg/mL) were added as control MoAbs for mlgM and mlgG1, respectively. Ten minutes later, fluorescent STZ particles (1 mg/mL) were added and were analyzed by flow cytometry. Double-colored events were scored and depicted as a percentage of the total number of red events. Results are expressed as means ± SD of three different experiments.

* Significantly different (P < .05) from the control value (GM-CSF primed) as assessed by the Student’s t-test.
MoAb IV.3, which blocks the FcγR type II (CD32), was used to study the contribution of the Fc region of the IgG to the STZ binding. MoAb IV.3 did not inhibit the binding of STZ to either unprimed eosinophils or primed eosinophils (Table 2), and also not in the presence of MoAb B2.12 (results not shown). Hence, a significant fraction of the eosinophils (15% to 25%) was able to bind STZ particles without apparent involvement of iC3b, C3b, or IgG molecules.

To investigate the participation of the lectin components of zymosan in the particle binding, unopsonized zymosan as well as opsonized zymosan particles (STZ) were added to eosinophils incubated with OKM-1. As shown in Table 2, OKM-1 did not influence the percentage of primed eosinophils binding STZ. Furthermore, OKM-1 (like all other antibodies) failed to inhibit the interaction of the eosinophils with unopsonized zymosan (data not shown). The percentage of eosinophils (unprimed and primed) that bound unopsonized zymosan was never higher than 13% (after 15 minutes of incubation), indicating that for the enhanced binding after cytokine priming the presence of iC3b on the zymosan particles is required.

In a previous study with PAF-primed eosinophils, we concluded that the priming by PAF caused a change in the configuration of CR3, because treatment with PAF (1 μmol/L) for 2 minutes did not induce a major change in CR3 expression on the cell surface. To test the possibility that the same mechanism is involved in the priming by GM-CSF, IL-3, and IL-5, we investigated the effect of these cytokines on the surface expression of CR3. Figure 2 shows the CR3 expression at different times after cytokine addition. The only time at which a significant upregulation of CR3 was observed was after 45 minutes of incubation with IL-5 (P < .05). Stimulation with phorbol myristate acetate (PMA) was included as positive control. The percentage of eosinophils interacting with STZ (15 minutes after addition) was already enhanced after 5 minutes of cytokine treatment (25 ng/mL), despite the fact that no significant upregulation of CR3 was observed within this period (Fig 2). Taken together, these results indicate that priming of human eosinophils with these cytokines (as with PAF) results in an enhanced binding capacity for particle-bound iC3b of CR3 molecules already present on the eosinophil membrane.

**STZ-induced synthesis and release of PAF by cytokine-primed eosinophils.** Recently, we have shown that unprimed human eosinophils synthesize and release PAF upon stimulation with STZ. This release of PAF was found to be an important factor in the activation of the respiratory burst. The STZ-induced release of PAF by unprimed eosinophils also affected the extent of STZ binding after prolonged incubation: in the presence of the PAF receptor antagonist WEB 2086 the percentage of cells binding STZ was decreased from 23.4% ± 4.1% to 13.2% ± 3.8% (Fig 3). Hence, we had to consider the possibility that the cytokines affected the STZ binding by inducing an enhanced production and release of PAF, because it has been observed that GM-CSF, IL-3, and IL-5 treatment of eosinophils results in enhanced release of PAF after activation with several stimuli. Although the cytokines themselves did not induce PAF synthesis, treatment of the eosinophils with GM-CSF, IL-3, or IL-5 strongly enhanced the STZ-induced synthesis and release of PAF. Most of the PAF produced was released into the supernatant (Table 3). However, the enhanced binding of STZ to eosinophils primed with IL-3, IL-5, or GM-CSF was not affected by preincubation with WEB 2086 (Fig 3).

**Inhibition of the PAF synthesis by the PLA₂ inhibitor mepacrine.** Our data indicated that the PAF concentration in the incubation medium can become as high as 20 nmol/L under the experimental conditions used. Therefore, it seemed possible that WEB 2086 was not completely blocking the effect of extracellular PAF. We used the PLA₂ inhibitor mepacrine to exclude the possibility that the release of PAF
is responsible for the effect of cytokine priming. At the same time, we were able to evaluate whether intracellular PAF has a functional role in the cytokine effect on the percentage of eosinophils binding STZ. Indeed, mepacrine proved to be efficient in blocking the STZ-induced PAF synthesis in eosinophils, especially after cytokine priming (Table 3). However, addition of mepacrine did not (like WEB 2086) significantly influence the enhanced STZ binding of eosinophils when primed with the cytokines (Fig 4), indicating that priming by the cytokines is independent from PAF, either cell-associated or released from the cells.

### Table 3. PAF Generation and Release by Human Eosinophils

<table>
<thead>
<tr>
<th>Inhibitor:</th>
<th>STZ</th>
<th>Mepacrine STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprimed</td>
<td>150</td>
<td>260 (35)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>50</td>
<td>5,320 (75)</td>
</tr>
<tr>
<td>IL-3</td>
<td>20</td>
<td>5,610 (80)</td>
</tr>
<tr>
<td>IL-5</td>
<td>60</td>
<td>6,390 (70)</td>
</tr>
</tbody>
</table>

Synthesis of PAF was tested under three conditions: column 1, PAF synthesis by unprimed and cytokine-primed eosinophils (25 ng/mL for 30 minutes); column 2, PAF synthesis after additional stimulation with STZ (1 mg/mL) for 15 minutes; and column 3, PAF synthesis in the presence of mepacrine 100 μmol/L (mepacrine was added 5 minutes before the addition of STZ). PAF generation is expressed as picograms of PAF per 10⁶ eosinophils. The numbers in parentheses indicate the amount of PAF released into the supernatant, expressed as a percentage of the total PAF production. The enhancement of the PAF production by cytokine treatment and the inhibitory effect of mepacrine observed in this experiment was also observed in two other experiments performed with cells from different donors.

### DISCUSSION

This report describes an extension of the recognized effects of the cytokines GM-CSF, IL-3, and IL-5 on the function of mature eosinophils. We focused on an effect of these cytokines on the interaction of eosinophils with STZ particles. Treatment of the cells with GM-CSF, IL-3, or IL-5 accelerated the process of particle binding and also strongly enhanced the percentage of eosinophils binding STZ (Fig 1 and Table 1). Eosinophils that had bound STZ immediately started to phagocytose the particles (data not shown). This was established by trypan blue quenching experiments, in which a distinction can be made between bound and ingested particles.

In agreement with the observation that the main opsonin on our STZ particles is iC3b,19 we found that B2.12, an MoAb directed against the binding site on CR3 for this complement fragment, inhibited the increase in the percentage of eosinophils binding STZ upon cytokine priming (Table 2). In addition, Bear-1, an MoAb directed against the α-chain of CR3,24 partially inhibited STZ binding to eosinophils. An interaction of the STZ particles with CR1 is not contributing to the particle binding, because the blocking MoAb D3.J325 directed against CR1 did not influence the percentage of eosinophils binding STZ. Furthermore, MoAb IV.3, directed against FcγRII,25 did not influence the interaction of STZ with primed eosinophils. The absence of an effect of IV.3 might be explained by steric hindrance of complement fixed to the Fc region of the IgG molecules.

MoAbs B2.12 and Bear-1 did not inhibit the binding of STZ to unprimed eosinophils. To characterize this baseline binding, we also added unopsonized zymosan particles to the eosinophils. Yeast particles, like zymosan, can bind...
directly to CR3, and fixed iC3b is not required. The percentage of eosinophils (primed or unprimed) binding unopsonized zymosan particles was never higher than 13%. OKM-1, a CD11b MoAb that blocks yeast binding to neutrophils, failed to inhibit the interaction of unopsonized or serum-opsonized zymosan with eosinophils. Hence, a lectin-mediated interaction of zymosan with the eosinophils does not seem to be responsible for this baseline binding.

From the experiments discussed above we conclude that interaction of primed eosinophils with the STZ particles is mediated via CR3. Although surface expression of CR3 on eosinophils has been reported to change after treatment with GM-CSF, IL-3, or IL-5, enhancement of CR3 expression cannot be responsible for the enhanced binding observed in this study. In our experiments, we observed only a small upregulation of CR3 after 45 minutes of IL-5 treatment, whereas enhanced STZ binding was already present after 5 minutes of cytokine pretreatment. It is possible that the absence of a significant effect of the cytokines on CR3 expression in our experiments is caused by previous enhancement of CR3 expression during the isolation procedure, as has been described for neutrophils. In addition, mere incubation of control cells at 37°C induced upregulation of CR3 without a change in STZ-binding characteristics (data not shown). However, the STZ-binding function of the CR3 molecules on the membrane was clearly enhanced by cytokine priming without major changes in membrane surface expression, hence most likely caused by an affinity shift of the CR3 for particle-bound iC3b.

Previously, experiments with neutrophils have shown that the activation state of CR3, rather than the extent of expression of this receptor, is responsible for the enhanced binding to endothelium and ligands such as ICAM and iC3b. Neutrophils bind poorly to most ligands of CR3, but stimulation of these cells with a variety of agonists causes an increase in binding activity that peaks and returns to baseline levels within 1 hour. In our experiments with eosinophils, return to a state of low-affinity binding did not occur during prolonged incubation with the cytokines (up to 60 minutes); the STZ binding remained high (Table 1). The concept of a change in CR3 conformation induced by priming and/or activation has gained support from the binding characteristics of MoAb CBRM1/5. This MoAb only binds to a subpopulation of the CR3 on activated neutrophils and prevents stimulated binding of neutrophils to ICAM-1 and fibronectin, indicating the existence of a functional active form of CR3 expressed upon activation. Instead of a conformational change in CR3 underlying changes in iC3b binding, the effect of cytokine treatment might also be mediated by induction of CR3 clustering. Activation of CR3 is associated with clustering, because clustering of iC3b on erythrocytes is necessary for efficient recognition by CR3 on neutrophils.

In a previous study, we showed that the release of the lipid mediator PAF induced by stimulation with STZ particles is responsible for an increase in the percentage of (unprimed) eosinophils binding STZ and for an enhanced respiratory burst activity induced by STZ. Although the cytokines themselves do not induce PAF synthesis, the STZ-induced PAF synthesis is strongly enhanced after cytokine priming. The STZ-induced PAF synthesis by primed eosinophils, like the STZ binding, is inhibited by MoAb B2.12 (data not shown). The amount of PAF measured in the supernatant of cytokine-primed cells can become as high as 20 nmoVL, a concentration that is sufficient to enhance the percentage of eosinophils binding STZ. Therefore, the next step in unravelling the mechanism of cytokine priming was to investigate the involvement of PAF release in the cytokine-induced priming for iC3b binding. The PAF-receptor antagonist WEB 2086 as well as the PLA inhibitor mepacrine did not affect the percentage of eosinophils able to bind to STZ, although mepacrine totally blocked the PAF synthesis. This strongly indicates that the mechanism of cytokine priming is not dependent on PAF.

Taken together, our data indicate that the cytokines GM-CSF, IL-3, and IL-5 are able to enhance several functional properties of human eosinophils, eg, the iC3b-binding capacity of CR3 and the synthesis and release of PAF. Most likely, enhanced PLA activity is involved in the mechanism responsible for enhanced PAF synthesis in GM-CSF, IL-3, or IL-5 primed eosinophils because these cytokines also enhance the calcium ionophore-stimulated leukothriene C4 production. Because both eosinophils and PAF may play a crucial role in the pathogenesis of asthma, the possibility exists that cytokine-primed eosinophils are contributing to PAF formation in inflammatory reactions involving eosinophils.

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Granulocyte-macrophage colony-stimulating factor, interleukin-3 (IL-3), and IL-5 greatly enhance the interaction of human eosinophils with opsonized particles by changing the affinity of complement receptor type 3

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