Human Blood Basophils Synthesize Interleukin-2 Binding Sites

By H. Stockinger, P. Valent, O. Majdic, P. Bettelheim, and W. Knapp

Recent data suggest that basophils express receptors for a variety of lymphokines. In this study we present the biochemical characterization of the interleukin-2 (IL-2) receptor on the basophil surface membrane. Highly enriched populations (purity: 92% to 99%) of blood basophils were obtained from chronic granulocytic leukemia (CGL) patients (n = 3) by negative selection using monoclonal antibodies (MoAbs) and complement. CGL basophils were found to bind CD25 MoAbs (n = 4) directed against different epitopes of the 55- to 60-Kd subunit of the IL-2 receptor (= Tac peptide). Immunoprecipitation experiments with lysates of purified CGL basophils and CD25 MoAbs showed a protein with a molecular weight of 60 Kd, equivalent to the Tac peptide on human T blasts. Quantitative binding studies and Scatchard plot analysis using radiolabeled recombinant human (rh) IL-2 indicated the presence of 12,000 ± 4,700 low affinity IL-2 binding sites (kd = 66 nmol/L) per purified CGL basophil. Northern blot analysis with enriched CGL basophils showed two messenger RNA bands of 3.5 and 1.5 kilobases hybridizing to radiolabeled Tac cDNA. Immunoprecipitation of the Tac peptide from enriched basophils metabolically labeled with 35S-methionine showed active synthesis of the IL-2 receptor. Our results show that human blood basophils synthesize and express receptors for IL-2.

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nosorbents at 4°C for 3 hours. For nonreducing conditions, bound material was eluted by boiling in gel buffer (0.1 mol/L Tris-HCl, 1 mmol/L phenylmethylsulfonyl fluoride, 0.2% deoxycholate sodium salt). For metabolic protein labeling, 4% 35S-methionine (New England Nuclear). Afterward, 2 mmol/L of 2-mercaptoethanol was added to the gel buffer and the samples were boiled for 5 minutes. Immunosorbents were prepared by incubating 25 μL hybridoma ascites with 100 μL protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) at room temperature for 1 hour. Samples were analyzed by SDS in a discontinuous buffer system on 12% polyacrylamide slab gels. Dried gels were fluorographed on Kodak XS-5 films (Kodak, Rochester, NY) at −70°C.

**Binding Assay**

The binding of radiolabeled IL-2 was basically assessed according to the method described by Tsudo et al.19 125I-Iodine-labeled rhIL-2 was purchased from New England Nuclear (specific activity: 20 to 50 μCi/μg). For detection of high affinity binding, we started with a serial dilution of 800 pmol/L (final concentration). For detection of low affinity binding sites, unlabeled rhIL-2 (Sandoz) was combined with labeled rhIL-2 to obtain final concentrations of 75 nmol/L (final specific activity: 1.5 to 3.75 μCi/μg) and 50 nmol/L (final specific activity: 2 to 5 μCi/μg) as diluting starting points. Cells (1 to 2 × 10^6) were incubated with serial dilutions of iodinated rhIL-2 at 4°C for 1 hour in a total volume of 200 μL with RPMI 1640 medium containing 1% BSA, 20 mmol/L Hepes (pH 7.3), and 0.1% NaN3. Cells were centrifuged at 10,000 × g for 15 seconds and resuspended in 150 μL of the same medium. The cell suspension was subsequently centrifuged through a 150-μL layer of a mixture of 20% olive oil/80% di-n-butyl phthalate (Sigma Chemical Co, St Louis, MO) at 10,000 × g for 3 minutes. The tips of the tubes were cut off and cell-bound radioactivity was measured in a γ-counter. Nonspecific binding was determined in the presence of a 500-fold excess of unlabeled recombinant IL-2. The number of binding sites and their dissociation constants (kd) were calculated by using Scatchard transformation with the LIGAND program (P.J. Munson and D. Rodbard, National Institutes of Health, Bethesda, MD).

**Northern Blot Analysis**

Total cellular RNA was isolated from 1 × 10^6 cells by solubilization in 3 mol/L LiCl/6 mol/L urea according to the method of Auffray and Rougeon.20 RNA samples (30 μg/lane) were size-fractionized on formamide containing 1% agarose gels, and transferred to Hybond-N (Amersham International, Amersham, Institute, Vienna, Austria), rhIL-3 (Sandoz), or rhIFNγ (Interferon Science, New Brunswick, NJ). Basophils were analyzed at various times of culture as indicated.

**Immunofluorescence Analysis**

The binding of antibodies was assessed by indirect immunofluorescence with fluoresceinated Goat F(ab') anti-mouse Ig antibodies (Grub, Scandic, Vienna). As negative control, a mixture of irrelevant antibodies was included in each experiment. Membrane fluorescence was analyzed by flow cytometry (FACS 440, Becton Dickinson). The percentage of positivity was calculated with the arbitrary 1% cutoff channel position of the negative control. For quantitative evaluation we calculated the mean fluorescence channel number and referred to a standard curve obtained with a series of Quantitative FLuorescence with fluoresceinated goat F(ab'), anti-mouse Ig antibodies (Becton Dickinson). The binding of radiolabeled IL-2 was basically assessed according to the method described by Tsudo et al.19 125I-Iodine-labeled rhIL-2 was purchased from New England Nuclear (specific activity: 20 to 50 μCi/μg). For detection of high affinity binding, we started with a serial dilution of 800 pmol/L (final concentration). For detection of low affinity binding sites, unlabeled rhIL-2 (Sandoz) was combined with labeled rhIL-2 to obtain final concentrations of 75 nmol/L (final specific activity: 1.5 to 3.75 μCi/μg) and 50 nmol/L (final specific activity: 2 to 5 μCi/μg) as diluting starting points. Cells (1 to 2 × 10^6) were incubated with serial dilutions of iodinated rhIL-2 at 4°C for 1 hour in a total volume of 200 μL with RPMI 1640 medium containing 1% BSA, 20 mmol/L Hepes (pH 7.3), and 0.1% NaN3. Cells were centrifuged at 10,000 × g for 15 seconds and resuspended in 150 μL of the same medium. The cell suspension was subsequently centrifuged through a 150-μL layer of a mixture of 20% olive oil/80% di-n-butyl phthalate (Sigma Chemical Co, St Louis, MO) at 10,000 × g for 3 minutes. The tips of the tubes were cut off and cell-bound radioactivity was measured in a γ-counter. Nonspecific binding was determined in the presence of a 500-fold excess of unlabeled recombinant IL-2. The number of binding sites and their dissociation constants (kd) were calculated by using Scatchard transformation with the LIGAND program (P.J. Munson and D. Rodbard, National Institutes of Health, Bethesda, MD).

**Table 1. Specification of MoAbs Used in This Study**

<table>
<thead>
<tr>
<th>MoAb Name</th>
<th>Isotype</th>
<th>CD</th>
<th>Reactive Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Tac</td>
<td>IgG2a</td>
<td>CD25</td>
<td>IL-2 receptor α chain</td>
</tr>
<tr>
<td>CD25-3G10</td>
<td>IgG1</td>
<td>CD25</td>
<td>IL-2 receptor α chain</td>
</tr>
<tr>
<td>CD25-4E3</td>
<td>IgG2b</td>
<td>CD25</td>
<td>IL-2 receptor α chain</td>
</tr>
<tr>
<td>CD25-8G8</td>
<td>IgG1</td>
<td>CD25</td>
<td>IL-2 receptor α chain</td>
</tr>
<tr>
<td>Leu1</td>
<td>IgG2a</td>
<td>CD5</td>
<td>gp67</td>
</tr>
<tr>
<td>Leu7</td>
<td>IgM</td>
<td>CD57</td>
<td>gp110, HNK 1</td>
</tr>
<tr>
<td>Leu9</td>
<td>IgG2a</td>
<td>CD7</td>
<td>gp40</td>
</tr>
<tr>
<td>BMA-022</td>
<td>IgG2a</td>
<td>CD2</td>
<td>LFA3-receptor</td>
</tr>
<tr>
<td>BMA-O110</td>
<td>IgG2b</td>
<td>CD9</td>
<td>gp24</td>
</tr>
<tr>
<td>BA-2</td>
<td>IgG1</td>
<td>CD14</td>
<td>gp55</td>
</tr>
<tr>
<td>VIM13</td>
<td>IgM</td>
<td>CD15</td>
<td>3-FAL, X-hapten</td>
</tr>
<tr>
<td>VIPC5</td>
<td>IgM</td>
<td>CD24</td>
<td>gp42</td>
</tr>
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<td>VIT3</td>
<td>IgM</td>
<td>CD3</td>
<td>CD3-complex</td>
</tr>
<tr>
<td>VIMD5</td>
<td>IgM</td>
<td>—</td>
<td>glycoporphin-A</td>
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<td>IgM</td>
<td>CD1</td>
<td>gp49</td>
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<td>IgG2a</td>
<td>—</td>
<td>HLA-DR</td>
</tr>
<tr>
<td>VDD1</td>
<td>IgG1</td>
<td>—</td>
<td>Blood group H antigen</td>
</tr>
<tr>
<td>CLB-Env3</td>
<td>IgM</td>
<td>—</td>
<td>igE</td>
</tr>
</tbody>
</table>

40% glycerol, 2% sodium dodecyl sulfate [SDS] pH 6.8) for 2 minutes. For reducing conditions, 5% 2-mercaptoethanol was added to the gel buffer and the samples were boiled for 5 minutes. Immunosorbents were prepared by incubating 25 μL hybridoma ascites with 100 μL protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) at room temperature for 1 hour. Samples were analyzed by SDS in a discontinuous buffer system on 12% polyacrylamide slab gels. Dried gels were fluorographed on Kodak XS-5 films (Kodak, Rochester, NY) at −70°C.

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Hybridization was performed overnight at 42°C with a 32P nick-translated pIL-2R2 cDNA probe (kindly provided by Dr Warner Greene, Bethesda, MD) in 50% formamide, 5X SSPE, 5X Denhardt's solution, and 100 μg/mL transfer RNA (tRNA) from brewer's yeast (Boehringer Mannheim GmbH, Penzberg, FRG). Afterward the blots were washed three times with 0.1X SSPE and 0.1% SDS at 42°C for 30 minutes. Bound radioactivity was detected by exposing the blots to X-S films (Kodak) at -70°C.

Histamine Release Assay

To evaluate the possible role of the IL-2 binding site on CGL basophils, we examined the effect of rhIL-2 on histamine release from basophils obtained from a CGL donor, after informed consent was given. Basophils were enriched (17.5% basophils) by dextran sedimentation as described.17 Cells, 5 x 10^6, were exposed to various concentrations (ie, 10, 100, and 1,000 U/mL) of rhIL-2 in the wells of 96 multiwell microtiter plates (Costar, Cambridge, MA). Cells were also exposed to MoAb E124-2-8 (anti-IgE, 1 μg/mL) as positive control. After incubation at 37°C for 45 minutes, cells were centrifuged to recover cell-free supernatant. Histamine was measured by a commercial radioimmunoassay (RIA).17 Total histamine content of cell suspensions was quantified after cell lysis in distilled water and freeze thawing. Histamine release was expressed as percentage of total histamine.

RESULTS

Purification and Culture of CGL Basophils

Basophils from three CGL donors could be enriched to near homogeneity, ie, to 92%, 97%, and 99% purity, respectively, with most of the remaining cells representing immature myeloid cells as determined by Giemsa staining. Almost all of the purified CGL basophils (more than 90%) were viable cells as determined by trypan blue exclusion as well as by viability staining using flow cytometry and ethidium bromide. The loss of a significant subpopulation of basophils could be excluded by measuring the histamine content before and after exposure to MoAbs + complement versus control medium, which gave identical results (data not given). A significant contamination (ie, more than 1%) with T cells, monocytes, eosinophils, B cells, or neutrophils could be excluded by staining with CD3 MoAb VIT3, VIM13 (CD14), and MoAb VIB-C5 (CD24). On the other hand, 94% ± 4% of the purified cells reacted with MoAb BA-2, which was shown to stain human basophils.8

No loss of cell viability could be observed with purified basophils during a culture period of 5 days. The total number of CGL basophils also essentially remained unchanged during a 5-day incubation period.

Reactivity of CGL Blood Basophils With CD25 MoAbs

Fluorescence activated cell sorter (FACS) profiles of two purified CGL basophil preparations stained with anti-Tac MoAb are shown in Fig 1. Approximately one fourth of the cells (23% ± 6%) were found to react with anti-Tac MoAb. Virtually identical results were obtained with all other CD25 MoAbs tested (CD25-3G10, 25% ± 9%; CD25-4E3, 28% ± 9%; CD25-9G8, 22% ± 8%). Interestingly, a two- to threefold increase in binding of CD25 MoAbs to purified basophils was observed during a 2- to 5-day cultivation period as determined by calculation of anti-Tac staining intensity on the basis of the mean fluorescence intensity (Fig 2). No significant influence of rhIL-3 (10, 100, and 1,000 U/mL tested), rhIL-2 (10, 100, and 1,000 U/mL tested), or rhIFNγ (100 and 1,000 U/mL tested) on anti-Tac binding to basophils was observed.

Molecular Weight of Tac-Reactive Peptides

To further characterize the Tac-reactive membrane component on human basophils, freshly isolated as well as purified and cultured (48-hour culture) CGL basophils were...
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**Fig 2.** Enhancement of anti-Tac binding by culturing purified basophils. Basophils were cultured in RPMI 1640 medium containing 10% FCS, and stained with anti-Tac at time intervals as indicated (numbers represent days of culture). Determination of percentage positive cells (%) and arbitrary units (AU) was performed with linear amplification on a FACS 440. One AU corresponds to approximately 1,000 FITC molecules per cell. Staining of enriched CGL basophils with an isotype IgG2a control MoAb gave the same negative results on all days examined.

labeled with $^{125}$I, and the Tac-reactive structure precipitated from their lysates. Polyacrylamide gel electrophoresis (PAGE) showed a single band of about 60 Kd (Fig 3A, lanes 2 and 3), similar in size to the Tac-reactive component on PHA-activated T blasts run in parallel (Fig 3B, lane 1).

**Binding of rhIL-2 to Purified CGL Basophils, Number of Binding Sites, and Binding Constants**

To demonstrate binding of rhIL-2 to purified CGL basophils, these cells were incubated with increasing concentrations of radiolabeled rhIL-2. The number and affinity of IL-2 binding sites expressed on cultured human basophils were quantified by Scatchard plot analysis using the LIGAND program for data analysis and curve fitting. Results are summarized in Table 2. Eight experiments were performed. CGL basophils were found to express 12,000 ± 4,700 low affinity IL-2 binding sites, with a kd of 66 nmol/L. No high affinity IL-2 binding sites were calculated by the LIGAND program.

Cells of the T-cell leukemia cell line HUT 102, studied in parallel, were found to express 4,400 ± 1,100 high affinity and 315,000 ± 62,000 low affinity IL-2 binding sites, with kd values of 46 pmol/L and 36 nmol/L, respectively.

**CGL Basophils Express Tac Messenger RNA (mRNA)**

Total cellular RNA was isolated from cultured (24 hours) purified CGL basophils and hybridized with a cDNA probe coding for the Tac peptide. Two mRNA bands of 3.5 kilobases (kb) and 1.5 kb were seen (Fig 4A). RNA equivalent in size was found with T cells activated for 8 hours with PMA (Fig 4C). No detectable amount of Tac peptide message was found in RNA isolated from resting T cells (Fig 4B).

**Basophils Actively Synthesize the Tac Peptide**

To address the question if basophils actively synthesize the Tac peptide, we performed metabolic labeling studies on highly enriched CGL basophils. Under nonreducing conditions the immunoprecipitation pattern of anti-Tac MoAb showed proteins with molecular weights of 52, 95, and 105 Kd (Fig 5, lane 2). Under reducing conditions (Fig 5, lane 3), one major band of 58 Kd and two minor bands of 95 and 105 Kd were observed. Thus, CGL basophils actively synthesize the Tac peptide.

**Histamine Release From CGL Basophils**

To examine a possible effect of IL-2 on function of CGL basophils, we measured the amount of histamine released in response to rhIL-2. In this study we found that CGL basophils fail to release histamine in response to stimulation with various concentrations (ie, 10, 100, and 1,000 U/mL) of rhIL-2 (control medium: 1.35% release; rhIL-2, 100 U/mL: 1.88% release; and optimal dose of anti-IgE: 41.27% release of the total amount of histamine).
DISCUSSION

Recent data suggest that basophils express receptors for distinct lymphokines. In previous studies we demonstrated that basophils express receptors for IL-3. In this study we provide evidence that blood basophils express, in addition, receptors for IL-2. In particular, our experiments show that highly enriched CGL basophils: (1) bind CD25 MoAbs (as has been reported for normal human bone basophils); (2) contain specific messages for the Tac peptide and express this peptide on their surface membrane; and (3) bind radiodiiodinated rhIL-2 specifically.

On the surface membrane of human lymphocytes, three classes of IL-2 receptors have been identified, each differing in their affinity for IL-2. The high affinity IL-2 receptor apparently represents a multichain complex consisting of at least two subunits, with molecular weights of 55 to 60 and 70 to 75 Kd, respectively. Both chains alone display IL-2 binding capacity; however, the affinity is low (55 to 60 Kd) or intermediate (70 to 75 Kd). The 55- to 60-Kd subunit is identical to the Tac peptide and has also been termed α-chain or β-chain of the IL-2 receptor. This domain was initially thought to be restricted in its expression to activated T cells. Later it became evident that activated B cells, Langerhans cells, as well as myeloid cells (ie, activated eosinophils, activated monocytes, and some myeloid leukemia cells) also express the CD25 antigen. Our results show that CGL basophils synthesize and express the Tac peptide (CD25 molecule). In particular, we could demonstrate specific Tac protein messenger RNA in purified CGL basophils, and CD25 MoAbs precipitated a surface membrane molecule from CGL basophils corresponding to the Tac peptide. To directly demonstrate active synthesis of the Tac peptide in purified basophils, we performed immunoprecipitation studies with metabolically labeled cells. Using this method, anti-Tac MoAb was found to precipitate a protein (molecular weight of 52 Kd under nonreducing, and 58 Kd under reducing conditions) from CGL basophils, corresponding to the Tac peptide detected on human T blasts. The higher molecular weight bands (95 to 105 Kd) presumably represent dimeric forms of the Tac peptide, as these bands almost completely disappeared after reduction with 2-mercaptoethanol.

By means of a radioreceptor assay, we were able to demonstrate that basophils bind recombinant human IL-2 specifically. In particular, CGL basophils were found to...
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express about 12,000 low affinity IL-2 binding sites with a kd of about 66 nmol/L. No significant numbers of high affinity IL-2 binding sites on CGL basophils were calculated by the LIGAND program. These data correspond in approximation to the numbers and binding constants of IL-2 receptors detected on the surface of other myeloid cells, such as monocytes and myeloid precursor cells. The possibility that basophils, in addition to the Tac peptide, also express other chains of the IL-2 receptor complex, and that human basophils may also express high affinity IL-2 binding sites under certain conditions, is currently under investigation.

Thus far it remains unknown why CGL basophils display higher amounts of IL-2 surface membrane receptors after in vitro culture. One possibility could be a change in the kinetics of IL-2 receptor production during isolation or cultivation of CGL basophils. Alternatively, CGL basophils after isolation are less capable of removing IL-2 receptor molecules from their surface and/or to degrade those receptors.

Expression of IL-2 receptors on human monocytes can be modulated by rhIFN gamma, a compound that has been shown to induce monocyte activation. In our study, the reactivity of anti-Tac with CGL basophils increased in culture. However, none of the cytokines tested (ie, IL-2, IL-3, and IFNy) showed any effect on anti-Tac reactivity with CGL basophils.

A number of surface membrane structures expressed on basophils are involved in basophil function. Most significantly, basophils express IgE receptors that mediate release of inflammatory compounds. More recently, lymphocyte activation products, such as IL-3, have been shown to modulate basophil histamine release. Basophils also express activation linked surface membrane structures such as the CD9 and CD38 antigens. The constitutive expression of such activation-linked antigens, including the Tac peptide, probably indicates that basophils represent an intrinsically activated cell type. Thus far, we were unable to detect cellular effects of IL-2 binding to human basophils. In particular, IL-2 failed to induce histamine release from normal or CGL basophils. IL-2 also failed to modulate the response of basophils to histamine releasing stimuli, as has recently been reported for rhIL-3 and rhGM-CSF. In addition, IL-2 showed no effect on basophil growth, proliferation, or basophil differentiation in bone marrow suspension cultures. Thus, a physiologic role of IL-2 binding sites in basophil function remains to be elucidated.

REFERENCES

20. Auffray C, Rougeon F: Purification of mouse immunoglobul-
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