Human Blood Basophils Display a Unique Phenotype Including Activation Linked Membrane Structures

By Christoph Stain, Hannes Stockinger, Michael Scharf, Ulrich Jäger, Heinz Gössinger, Klaus Lechner, and Peter Bettelheim

To evaluate the membrane marker profile of human basophils, a panel of well-established monoclonal antibodies (MoAbs, n = 60) was used for a combined toluidine/immunofluorescence staining procedure. Myeloid-associated MoAbs (particularly MoAbs against the LFA-1 family (CD11, CD18), MoAbs directed against lactosylceramide (CD17), anti-glycoprotein (gp) 150 MoAbs MCS 2 and MY 7 (CD13), anti-gp 67 MoAb MY 9, anti Fcγ-receptor (mol wt 40 kd) MoAb CIKM5, anti-CR 1 MoAb E 11, and the antiglycolipid MoAb VIM-2) were reactive with basophils, indicating a close relationship to other mature myeloid cells. Under normal conditions, basophils surprisingly express at least three activation-linked structures not detectable on mature neutrophils, i.e., the p45 structure defined by MoAbs OKT-10 and VIP-2b, the p24 structure identified by the CD9 MoAb BA-2, and the receptor for interleukin 2 (IL-2) recognized by three different MoAbs (anti-TAC, IL2RI, anti-IL-2). Moreover, under short-term culture conditions basophils both in mononuclear cell (MNC) suspension and as purified fractions display the HLA-DR and T4 antigens. The neutrophil/eosinophil structure 3-fucosyl-N-acetyllactosamine is expressed on basophils only after neuraminidase treatment. Basophils were not stained at all by CD16 MoAbs directed against the Fc γ-receptor (mol wt 50 to 70 kd) of neutrophils, by the MoAb 63D3 (CD12) recognizing the monocyte/granulocyte-associated p 200 antigen, and by the CD14 antibodies (VIM-13, Mo 2) defining the monocyte-specific structure p 55. Enriched basophils freshly obtained from chronic granulocytic leukemia (CGL) patients yielded identical results in FACS analyses. In summary, these data indicate that basophils generate a unique combination of surface determinants and possibly represent an activated cell population.

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MATERIALS AND METHODS

Cell Preparation

MNC were isolated by gradient centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) and were then subjected to staining with MoAbs. Neutrophils obtained from healthy controls (n = 3) were prepared by standard techniques as previously described. Eosinophils were isolated from two patients with hypereosinophilia and from two patients suffering from transient drug allergy. Purity of these cell fractions was >90%.

MoAbs

The MoAbs used in this study and referred to as belonging to a cluster of differentiation (CD) or for being reactive with a well-defined structure were: Leu 1 (CD5), Leu 2a (CD8), Leu 3a (CD4), Leu 4 (CD3), Leu 7 [human natural killer (NK) cells], Leu 11a (CD16), and anti-HLA-DR (Becton Dickinson, Sunnyvale, CA); MY 7 (CD13), MY 9 (anti-p67), Mo 2 (CD14), T4 (CD4), T8 (CD8), T11 (CD2), B1 (CD20), B2 (CD21), B4 (CD19), and IL 2 R1 (CD25) (Coulter Immunology, Hialeah, FL); BA-1 (CD24), and BA-2 (CD9) (Hybritech, San Diego); OKT-1 (CD5), OKT-9 (antitransferrin receptor), OKT-10 (p45), OKM-1 (CD11), and OKB-2 (CD24) (Ortho Pharmaceuticals, Raritan, NJ); and 63D3 (CD12) (BRL; Gaithersburg, MD); anti-II 2 (Eurogenetics, Aalst, Belgium); BMA-022 (HLA-DR) and BMA-0111 (PanT-ERF receptor) (Behringwerke AG, Marburg, FRG).

The MoAbs VIP-1 (antitransferrin receptor), VIP-2b (p45), VID-1 (anti-HLA-DR), VIL-A1 (CD10), VIM-12 (CD11), VIM-13 (CD14), VIM-D5 (CD15), VIM-2 (not clustered), VIM-C6 (CD15), VIB-C5 (CD24), VIT-3b (CD3), VIT-4 (CD4), VIT-6 (CD1), VIT-8 (CD8), VIT-12 (CD6), VIFcR2 (CD25), and VIE-G4 (anti-glycoporphin A) were produced at the Institute of Immunology, University of Vienna. The MoAb NA1/34 (CD1) was kindly provided by Dr A. J. McMichael. WT-1 (CD7) antibody was sent by Dr W. I. M. Tax, and MCS-2 (CD13) antibody was donated by Dr J. Minowada. VEP-13 (CD16) was supplied by Dr Rumpold (Vienna). Anti-TAC (CD25) was kindly

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provided by Dr T. A. Waldmann. CLBery3 was sent by Tetteroo. MoAbs L4F3, IG10 (CD15), KIM5 (CD11), T5A7 and (G)025 (CD17), TSF/18.11 and MMH 23 (CD18, LFA-1, b-chain; McMichael, personal communication, 1984, 1986). CIKM5 (40 kd Fcγ-receptor) was selected from the myeloid panel of the Second International Workshop on Human Leukocyte Differentiation Antigens. MoAb E II (anti-CR I receptor) was taken from the myeloid panel of the Third International Workshop on Human Leukocyte Differentiation Antigens.

Neuraminidase Treatment of Cells

Superantigen from the IgE-producing human myeloma cell line U-266 and a mouse anti-human IgE MoAb (E124 2-8, Immunotech, Marseille, France) was used for detection of membrane-bound IgE (positive control). Monoclonal antibodies of either IgG 1, IgG 2a, IgG 2b, and IgM subclass with irrelevant specificity were used as negative controls.

Immunologic Characterization With MoAbs

As previously described,13 the binding of the various antibodies to isolated MNCs was assessed by indirect immunofluorescence with fluoresceinated goat F(ab')2 anti-mouse IgG and IgM antibodies. Fluorescence of MNCs was evaluated by means of a Leitz Ortholux microscope (Leitz, Wetzlar, FRG). Purified basophils of CGL patients and enriched neutrophils of one healthy donor were analyzed with a FACS 440 (Becton Dickinson).

Combined Immunologic/Toluidine Staining Procedure

Blood samples from 15 volunteers were drawn for testing the entire panel three times. After the indirect immunofluorescence staining procedure, cells (6 x 10^5 for each MoAb) were fixed in glutaraldehyde at room temperature (RT) for 1 minute (0.025% glutaraldehyde in fixation buffer: 0.1 mol/L Tris buffer, pH 7.8). After three washings with phosphate-buffered saline (PBS, pH 7.2) cells were incubated with toluidine blue (Sigma, St Louis) (0.0125% wt/vol in PBS) at RT for 15 minutes. Subsequently, cells were washed three times in PBS and subjected to analysis as follows: Toluidine-stained cells were first identified in brightfield and then examined for antibody binding under fluorescence light.

The antibodies we used either stained practically all cells examined or did not stain basophils at all. For FACS analyses, a reactivity of >20% was considered positive.

Neuraminidase Treatment of Cells

MNCs (4 x 10^5/mL) were incubated with V. cholerae neuraminidase 0.2 U/mL (Behringwerke AG) in a shaking water bath at 37°C for 30 minutes. The reaction was terminated by washing the mixture three times with RPMI 1640 medium supplemented with 10% fetal calf serum (FCS).

Enrichment of Basophils

To compare the phenotype of normal basophils with that of CGL patients (two patients in stable phase, one patient in accelerated phase), the combined immunofluorescence technique and an analysis of enriched basophils was used. Enrichment of basophils was achieved by incubating MNC with a mixture of antibodies and subsequent rabbit complement lysis. After informed consent was obtained from each patient, heparinized peripheral blood of three CGL patients (5%, 11%, and 15% basophils, respectively) underwent MNC isolation as described above; 5 x 10^6 cells were incubated with 1 mL RPMI at 4°C for 45 minutes, the milliliter of RPMI containing 100 μg of MoAB VIM-D5. After being washed, cells were exposed to 5 mL rabbit complement (Behringwerke AG) at 37°C for 90 minutes. Monocyte depletion of washed cells was achieved with scrubbed nylon wool (37°C for 45'). Cells were then incubated with a mixture of antibodies consisting of 25 μg VIT-3, 25 μg VIB-C5, 25 μg Leu 1, 50 μg Leu 7, 25 μg VIE-G4, 25 μg anti-HLA-DR, CLBery3 (ascites: 1:500 diluted), 25 μg VIM-D5 at 4°C for 45 minutes, and subsequently incubated with 4 mL rabbit complement at 37°C for 90 minutes. Cells were again centrifuged on Ficoll-Hypaque and washed twice. Cytospin preparation of this cell fraction revealed >95% basophils (Fig 1).

Culture Studies

Activation of neutrophils and basophils by formyl-methionyl-leucyl-phenylalanine (fMLP). As reported previously,23 PMNs (1 x 10^6/mL) or MNCs (1 x 10^7/mL) were incubated at 37°C for 10 minutes, the medium (RPMI 1640, 5 g/L human albumin, pH 7.2) containing 1 μmol/L fMLP and cytochalasin B 10 μg/mL as well as dimethylsulfoxide (DMSO) (final concentration 0.01%) as a solvent. The reactivity of stimulated MNCs was evaluated by means of the combined immunofluorescence/toluidine staining procedure.

Activation of basophils from two healthy donors (in MNC suspension) with the Ca-ionophore A23187. Cells (1 x 10^5) were incubated with the Ca-ionophore A23187 (1 μg/mL, Sigma) at 37°C for 30 minutes. After this procedure degradation of basophils was evident.

Short-term cultures with biologic factors. Basophils were cultured either in MNC suspension (five healthy controls) or as purified fractions (CGL basophils from two patients) after lysis with a mixture of antibodies (>95% purity as determined by Wright staining of cytospin preparations) for 5 days in RPMI 1640 (Flow Laboratories, McLean, VA) plus 10% FCS, supplemented with either 20 U/mL human recombinant IL 2 (Boehringer Mannheim), Interferon Sciences, New Brunswick, NJ), supernatant of the HTB-9 cell line24 (9:1 vol/vol), or supernatant of the T cell hybridoma producing basophil-like promoting activity factor (BaPA, 4:1 vol/vol) kindly provided by Dr B. Stadler.16,17 Phenotyping (normal basophils in MNC fractions: combined toluidine immunofluorescence technique; purified CGL basophils: FACS analyses) of cultured cells was performed on days 0, 2, and 5.

3H-incorporation. Incorporation of 3H-thymidine was carried out as described previously.27 In brief, purified basophils from two CGL patients (10^7 cells/well) were incubated with factor-conditioned medium (γ-IFN, BaPA, HTB-9 supernatant, and IL 2) at 37°C for up to 5 days in a humidified atmosphere with 5% CO2. One
microcurie per well \( ^{1}H \)-thymidine (New England Nuclear) was added 12 hours before harvest. The incorporated radioactivity was collected on glass-fiber filters and counted in a liquid scintillation counter. Values are given as cpm \( \times 10^{-3} \pm \) SD of triplicate cultures.

Enrichment of neutrophils by MoAb + complement lysis. Heparinized blood drawn from a normal donor was first centrifuged on Ficoll-Hypaque. The sedimented cell pellet containing erythrocytes, neutrophils, and residual MNCs, subsequently underwent dextran sedimentation. Neutrophils (1 \( \times \) 10⁶) obtained by this method were incubated with a mixture of MoAbs comprising 25 \( \mu \)g anti-HLA-DR, 25 \( \mu \)g BMA0111, 25 \( \mu \)g B1, 25 \( \mu \)g VIM-13, and CLBery3 (ascites, 1:500 diluted) at 4°C for 45 minutes. Cells were then exposed to 1 mL rabbit complement at 37°C for 90 minutes. After two washings, cytospin examination revealed >95% neutrophils.

RESULTS

By means of a toluidine staining procedure in suspension, basophils are easily identified by their purple cytoplasmic granules. This procedure, combined with the simultaneous application of the immunofluorescence technique allowed determination of the basophil marker profile (Fig 2A and B).

A panel of 60 MoAbs was used to delineate the phenotype of basophils from that of neutrophils and eosinophils. Thirty MoAbs reacted with at least one type of mature granulocytes and showed a homogeneous staining for positive subsets in all samples tested. Table 1 shows that 20 MoAbs stained basophils. MoAbs giving positive results and identifying common granulocytic membrane structures were, in particular, anti-CRI MoAbs (220 kd), anti-Fcγ-receptor (40 kd) MoAbs, anti-C3bi-receptor antibodies (OKM-1, VIM-12, and Ki M5/CD 11), anti-LFA-1-β chain MoAbs (CD18), anti-gp 150 MoAbs (CD13), and MoAb VIM-2, which recognizes a glycolipid structure. Of all MoAbs tested in this study, antilactosylceramide MoAbs (CD17) showed the strongest reactivity with basophils (Fig 2A and B). MoAbs detecting the p 67 membrane determinant bound to basophils moderately and, in our hands, gave a very weak reactivity with neutrophils as well.

In contrast to eosinophils and/or neutrophils, the structure 3-fucosyl-N-acetyllactosamine (identified by MoAbs clustered as CD15) and the 50- to 70-kd Fcγ-receptor (recognized by CD16 MoAbs) were not detectable on the surface membrane of circulating basophils. The CD6, CD4, and CD8 anti-monocyte-associated MoAbs tested also failed to react with basophils.

We furthermore tested nonmyeloid-associated MoAbs of clusters CD1-10, 19 through 21, 24, 25, MoAbs directed against the T10 antigen and an antitransferrin receptor MoAb. A constant and reproducible finding was the reactivity of various activation-linked markers with basophils. All three anti-IL 2 receptor MoAbs (CD25) tested, both anti-T10 MoAbs, and the anti-p24 MoAb BA-2 (CD9) gave positive results. HLA-DR antigens and the transferrin receptor, also activation-linked structures, were not detectable on freshly obtained basophils. MoAbs directed against lymphocyte-associated structures (CD 1 through 8, 10, 19 through 21, 24, anti-human NK cell MoAb Leu7) failed to stain basophils at all. They included anti-common acute lymphocytic leukemia (cALLA) MoAb VIL-A1 (CD10) and CD24 MoAbs known to bind to neutrophils.

Neuraminidase Treatment of MNCs

Seven MoAbs (VIM-D5, BA-2, T5A7, VEP-13, VID-1, VIP-1, and anti-TAC) were tested after neuraminidase
### Table 1. Reactivity Pattern of MoAbs Binding to Mature Granulocytes

<table>
<thead>
<tr>
<th>MoAbs Reactive With Peripheral Blood Granulocytes*</th>
<th>WHO-Cluster/ Reactive Structure</th>
<th>Basophils</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKM-1 VIM-12 KIM 5</td>
<td>CD11/CR3</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MCS2 MY 7</td>
<td>CD13/p150</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TSI/18.11 MHM 23</td>
<td>CD18/LFA1β-chain</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E11</td>
<td>p220/CR1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C1KM5</td>
<td>40-kd Fc γ-receptor</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VIM-2</td>
<td>NK</td>
<td>+</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>TSA7† G1O35†</td>
<td>CD17/lactosylcer</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>MY 9 LAF3</td>
<td>p67</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>VIM-D5 VIM-C6 1G10</td>
<td>CD15/3-FAL</td>
<td>–</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>VIB-C5 BA-1 OKB-2</td>
<td>CD24</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VIL-A1</td>
<td>CD10/cALLA</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>63D3</td>
<td>CD12</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>VEP-13 Leu 11a</td>
<td>CD16/50- to 70-kd-Fcy-receptor</td>
<td>–</td>
<td>+ + +</td>
<td>–</td>
</tr>
<tr>
<td>OKT-10 VEP-2a</td>
<td>p45/T10</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-TAC IL 2R1</td>
<td>CD25/IL 2 receptor</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BA-2</td>
<td>CD9/p24</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NK, not known.

*Each antigranulocytic MoAb showed a homogeneous staining for positive subsets in all samples tested.

†Reactive with eosinophils in hypereosinophilic syndrome.

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treatment of cells. When pretreated with neuraminidase basophils, which are normally VIM-D5 (anti-3-FAL) negative, reacted strongly with this very MoAb. All other MoAbs tested displayed the same reactivity pattern with basophils both before and after neuraminidase treatment.

**Purification and Phenotypic Analysis of CGL Basophils**

After MoAb plus complement lysis, purity of basophils was >95% (Fig 1). Purified cells underwent indirect immunofluorescence staining with MoAbs of major significance and were evaluated by FACS analyses (Fig 3A and B). Identical results were obtained with all three CGL patients on day 0. These FACS data therefore confirmed the results obtained with normal basophils by use of the combined toluidine staining procedure.

**Phenotypic Analysis of Purified Neutrophils**

The phenotype of neutrophils has already been well defined. To examine whether the MoAb + complement lysis may cause an alteration of surface structures, purified neutrophils were stained with a panel of MoAbs and measured on a FACS. The phenotype of neutrophils appeared to remain unchanged by this procedure and was identical to the neutrophil reactivity pattern obtained by indirect immunofluorescence microscopy (data not given).

**Culture Studies**

**fMLP effect on basophils and neutrophils.** The binding of MoAbs (VIM-D5/CD15, T5A7/CD17, BA-2/CD9, V1D-1/HLA-DR, VIP-1/transferrin receptor, and anti-TAC/IL 2 receptor) was tested before and after fMLP activation of basophils and neutrophils (two healthy controls). Neutrophils displayed a distinct increase of the fluorescence intensity with MoAb VIM-D5, whereas T5A7 bound neutrophils to a lower extent after this procedure. With regard to basophils, no change of fluorescence intensity was evident with any MoAb tested.

Effect of Ca-ionophore A 23187 on basophils. When basophils, in MNC preparations, were exposed to the ionophore A23187, the reactivity of the MoAbs studied remained unchanged (same MoAbs were tested as are listed in the fMLP experiment).

**CGL basophils cultured in factor-conditioned media.** Purity of 5-day-cultured basophils still was >95% in all samples tested (as determined by morphological criteria). Trypan blue staining revealed a viability of at least 98% of cells. FACS analyses of basophils obtained from two CGL patients were performed on days 0, 2, and 5. The reactivity of anti-TAC, already detectable on unstimulated cells, was increased on days 2 and 5 and was not dependent on the addition of biologic factors. An example of this reactivity pattern is given in Fig 3C. The HLA-DR and T4 antigens, expressed neither on basophils obtained from healthy donors nor on CGL basophils on day 0, became detectable on days 2 and 5. All three surface structures in question were also identified on cells in the control medium. Table 2 shows a comparison of the anti-TAC, anti–HLA-DR, and T4 reactivities of basophils cultured in the control medium (as determined by FACS analyses on days 0, 2, and 5). The expression of HLA-DR was stronger on BaPA and γ-IFN–stimulated basophils than on those in the other factor-conditioned media and the control medium, respectively (Fig 3D). Moreover, a moderate reactivity of the three anti-T4 antibodies (VIT-4, T4, Leu 3a) was noted in all culture samples, which is shown in Fig 3E. The reactivity of all other MoAbs tested remained unchanged (data not shown).

Normal basophils cultured in factor-conditioned media. Phenotypic analyses (combined toluidine/imunologic staining procedure) of normal basophils from all but one donor (in MNC preparations) cultured for 5 days in...
factor-conditioned media gave identical results (HLA-DR+, T4+) as compared with purified CGL-basophils.

3H-Incorporation studies. Table 3 shows the proliferation kinetics as measured by 3H-thymidine uptake on days 0, 2, and 5. It is evident that the 3H-thymidine incorporation was higher on day 2 than on day 5. A distinct increase of the 3H-thymidine incorporation was noted in the HTB-9-conditioned medium. As compared with the control medium, all other samples revealed no significantly elevated 3H-uptake over a period of 5 days.

DISCUSSION

In this study, toluidine-stained cells were phenotyped with a panel of MoAbs to evaluate the antigenic membrane profile of peripheral blood basophils. The results clearly indicate that basophils express a unique pattern of membrane determinants.

The most conspicuous finding was the detection of three activation-linked structures on the surface membrane of basophils. The presence of the structures in question, ie, the IL 2 receptor, the T10 antigen, and the structure p 24, is consistent with the common idea of basophils representing an activated cell type and/or a population responding immediately to certain stimuli.

So far, reactivity of MoAbs directed against the IL 2 receptor was observed with activated T and B lymphocytes as well as monocytes stimulated by γ-IFN. With the exception of the recently established eosinophilic cell line EoL and a subset of myeloid blast cells, no granulocytic cells

Table 2. Expression of Surface Determinants on CGL Basophils Cultured in Control Medium as Measured by FACS Analyses

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Antibody Designation</th>
<th>Days 0</th>
<th>Days 2</th>
<th>Days 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-TAC</td>
<td>74</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Anti-HLA-DR</td>
<td>8</td>
<td>21</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>3</td>
<td>18</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Anti-TAC</td>
<td>59</td>
<td>67</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Anti-HLA-DR</td>
<td>4</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>2</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. 3H-Thymidine Incorporation of CGL Basophils on Days 0, 2, and 5

<table>
<thead>
<tr>
<th>Medium</th>
<th>Patient No.</th>
<th>Days 0</th>
<th>Days 2</th>
<th>Days 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>0.6 ± 0.05</td>
<td>5.2 ± 0.3</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>medium</td>
<td>2</td>
<td>0.5 ± 0.2</td>
<td>4.8 ± 0.9</td>
<td>0.7 ± 0.06</td>
</tr>
<tr>
<td>HTB-9</td>
<td>1</td>
<td>0.9 ± 0.05</td>
<td>13.6 ± 0.8</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.7 ± 0.07</td>
<td>8.2 ± 0.5</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>γ-IF</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.6 ± 0.05</td>
<td>4.3 ± 0.8</td>
<td>0.8 ± 0.02</td>
</tr>
<tr>
<td>IL-2</td>
<td>1</td>
<td>0.4 ± 0.02</td>
<td>4.7 ± 0.2</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.5 ± 0.06</td>
<td>5.4 ± 0.2</td>
<td>0.7 ± 0.02</td>
</tr>
<tr>
<td>BaPA</td>
<td>1</td>
<td>0.6 ± 0.03</td>
<td>8.2 ± 0.8</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.6 ± 0.1</td>
<td>3.7 ± 0.3</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

NT, not tested. Values are given in cpm × 10^{-3} ± SD.
have yet been reported to be reactive with anti-IL 2 receptor antibodies.

Basophils also reacted with MoAb BA-2 (CD9), recognizing the p24 structure. This antibody, which in our hands fails to react with neutrophils, was initially reported to identify a lymphohematopoietic cell surface structure. Recently, the structure p24 has also been detected on activated T cells,39 and from affinity-purified antigen preparations it is now evident that the p24 molecule is responsible for a protein kinase and for growth-promoting properties.36

Moreover, basophils were stained by MoAbs detecting the T10 antigen, which represents a new and quite unexpected observation. Whereas this structure was reported to be expressed on immature precursor cells as well as on activated lymphocytes, maturing myeloid cells have so far been considered unreactive with anti-T10 antibodies.37,38

Based on the assumption that basophils represent cells in an activated state or that these cells rapidly respond to stimuli, we also studied the basophil phenotype after in vitro culture. To this end, normal basophils in MNC suspension and CGL basophils as purified fractions were exposed to FMLP/cytochalasin B and Ca-ionophore and cultured for 5 days in medium containing either hrIL 2, hr-γ-IFN, supernatant of the HTB-9 cell line (which is reported to enhance blast colony formation),38 or BaPA synthesized by a T cell hybridoma.25,26 As compared with freshly obtained normal basophils and CGL basophils, the phenotype of cells in short-term cultures differed in three respects: First, after 2 days of culture, the reactivity of anti-TAC was significantly increased in all samples tested and remained unchanged until day 5. Second, the expression of HLA-DR was detectable on days 2 and 5. Third, the T4 antigen became demonstrable with three different MoAbs directed against this structure. At this point, we cannot definitely say whether the expression of HLA-DR and T4 on basophils is a phenomenon restricted to culture conditions or whether this may also occur in vivo. A differentiation to a “late” HLA-DR-positive state may be conceivable with regard to the other activation-linked structures. Whether the expression of differentiation structures or the susceptibility of receptors corresponds with mediator release requires further investigation.

The expression of various activation-linked structures and the T4 antigen on basophils again raises the question of a possible relationship of T lymphocytes and metachromatically stained cells.39 The detection of the surface structures in question suggests that the functional properties and/or the differentiation of basophils are influenced by T cells or T cell modulating factors. Despite the occurrence of the T4 antigen on cultured basophils, the existence of a common precursor cell, as proposed by Burnet,40 seems unlikely with regard to the numerous myeloid-associated determinants. The detection of the T4 antigen on cultured basophils does not represent a novel finding concerning myelomonocytic cells, nor does the presence of this antigen provide evidence for a common precursor since monocytes and γ-IFN-stimulated HL-60 cells were reported to display the T4 antigen as well.41,42

The myeloid determinants we detected on basophils were in particular the CR3 receptor recognized by CD11 MoAbs, the structure p150 (CD13 MoAbs), the β chain of LFA-1 (CD18 MoAbs), lactosylceramide (CD17 MoAbs), the CR1 receptor (MoAb E11), the 40-kd Fcγ-receptor (MoAb CIK5M), and the glycolipid structure identified by MoAb VIM-2, present on all granulocytic cells beyond the stage of promyelocytes. Moreover, basophils clearly displayed the structure p67 (MoAbs MY9, L4F3), which had so far been known to be generated only by myeloid progenitors and monocytic cells.43

The p67 determinant was reported to be lost gradually with ongoing granulocyte differentiation.43 Because staining of basophils was evident in our study, the decrease of antibody binding seems to be restricted to neutrophil and eosinophil differentiation. Basophils we obtained from healthy adults therefore showed a different reactivity with MY9, as did in vitro-cultured fetal basophilic cells which were reported to be MY9 negative.44

Certain well-defined neutrophil-associated structures were missing: The structure 3-fucosyl-N-acetyllactosamine identified by CD15 MoAbs (VIM-D5, 1G10, VIM-C6) was not detectable. After neuraminidase treatment, however, 3-FAL is expressed on basophils, which represents a phenomenon we already observed with myeloid and lymphatic blast cells.18 An observation of apparent functional importance was that basophils lack the 50- to 70-kd Fcγ-receptor (recognized by MoAbs VEP-13, Leuha, ViFcR2). This receptor is of major importance for the phagocytic capacity of neutrophils. Antibodies against this structure completely block the binding and ingestion of IgG-coated sheep erythrocytes.45 Because basophils are known to bind aggregated human IgG,46 minor differences of the receptor’s molecular structure on the different types of granulocytes could be an explanation for the nonreactivity of the CD16 MoAbs with basophils. An alternative possibility which cannot yet be excluded either is that the 40-kd Fcγ-receptor (recognized by MoAb CIK5M) is the sole IgG binding site of basophils.

CR 1 and the LFA-1 structures, which are further receptors contributing to the functional process of phagocytosis mainly by providing attachment to target particles, were identified by corresponding MoAbs on all types of mature granulocytes. These data confirm previous findings of the CR 1 receptor on basophils, obtained by use of complement-coated particles,47 and are consistent with the role assigned to basophils in the immune response.

So far, basophils have been explored mostly by morphological,48,49 histochemical,50,51 and functional52 analyses. These studies were laborious and, with a few exceptions, could not be performed simultaneously. The knowledge of the basophil phenotype and the technique used in this study offers a new possibility for the simultaneous determination of differentiation and function and provides the opportunity to influence this cell type specifically through its different surface structures.

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