Identification of Antigen-Capturing Cells as Basophils

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Identification of Antigen-Capturing Cells as Basophils

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Binding of intact Ag is a hallmark of Ag-specific B cells. Apart from B cells, a small number of non-B cells can bind Ag with comparable efficacy as B cells and are found in the peripheral blood, spleen, and bone marrow of mice. This population has been observed for a long time and recently named “Ag-capturing cells.” Their identity remained enigmatic. In this study, we show that these cells are basophilic granulocytes. Their ability to capture Ags is dependent on surface IgE receptors and on Ag-specific plasma IgE molecules appearing after immunization. Several surface markers including surface bound IgE, IL-3R, CD11b, CD16/32, and the chemokine receptor CCR2 were used to clearly identify these cells. Cross-linkage of surface IgE results in the release of large amounts of IL-4 and IL-6. The data identify basophils as Ag-capturing cells and support the concept of basophils as important regulators of humoral immune responses. The Journal of Immunology, 2005, 174: 735–741.

Searching for CCR2-positive cells in the mouse to get more insight into the phenotype of CCR2-deficient mice or mice treated with a blocking CCR2 Ab (4), we found CCR2 expression on a cell population that did not meet any criteria for T cells, B cells, NK cells, monocytes, or neutrophils. These cells were located within the lymphocyte gate with a somewhat higher side scatter than B and T cells and most characteristically expressed high levels of surface IgGs but completely lacked any markers of B cells. We decided to isolate these cells and found that they release very high levels of IL-4. This finally led to the discovery that these cells are basophils and prompted us to reanalyze the Ag-capturing cells described by Bell and Gray (3).

Murine basophils are notoriously hard to identify as summarized in a recent article by Dvorak (5) with the title “murine basophils: rare and rarely recognized cells.” Basophils complete their differentiation in the bone marrow and appear as mature basophils in the peripheral blood. Under inflammatory conditions, they can be recruited to sites of inflammation (6). Human basophils show high expression of CCR3 and CCR2 but weak expression of CCR1 and CCR5 (7–9). Upon stimulation by cross-linking of surface IgE or IgG and by some parasite products, basophils release histamine, leukotriene C4, and a variety of cytokines including IL-4 and IL-13 (6, 10, 11). After recruitment they play an important role in allergic, late phase reactions and the host response against parasites (12, 13). Basophils however also have major impacts on B and T cell function. They can induce class switch to IgE independently of T cells (14) and induce a shift toward Th2 differentiation (15, 16). Our finding that basophils are the only cell type in the peripheral blood, bone marrow, and spleen that can capture Ags in significant amounts underlines the importance of this cell type in innate and adaptive immune responses.

Materials and Methods

Immunizations

C57BL/6 mice were injected i.p. with 80 μg soluble allophycocyanin (Prozyme) in 200 μl of PBS with 107 heat-killed Bordetella pertussis. Three weeks after the first immunization, a boost dose of 80 μg soluble allophycocyanin with B. pertussis was given. Mice were analyzed between 10 and 30 days after the boost. Governmental approval was given for the animal experiments.

Cell preparations

Blood was drawn from the retro-orbital plexus and anticoagulated with 1 mM EDTA. Plasma was obtained by centrifugation of blood for 10 min at
Flow cytometry

To detect allophycocyanin-binding cells, cell preparations were washed three times in ice-cold PBS and stained with 10 μg/ml allophycocyanin (Prozyme) dissolved in PBS. The following Abs were purchased from BD Pharmingen: CD45/LECA-FITC (30-F11), CD11b-FITC (M1/70), CD19-FITC (1D3), CD11c-PE (HL3), CD16/32-PE (2.4G2), CD19-PE (1D3), HLA IA/IE-PE (MS/1/14.15.2), CD4-Cy5 (RM4-5), CD8-Cy5 (53–6.7), Gr-1-APC (RB6–8C5), c-kit-PE (2B8) CD123-biotin (5B11), CD138-biotin (8C12), CD54-biotin (3E2), CD123-biotin (SB11), CD138-biotin (281-2), B220-biotin (RA3-6B2), DX-5-biotin, anti-IgE-FITC (R35-72), anti-IgG1-FITC (R35-72), anti-IgG2a-biotin (R19-15), anti-IgG2b-biotin (R12-3), anti-IgG3-biotin (R40-82), anti-IgM-biotin (II/41), and purified mouse IgE isotype standard (C48-2). Anti-IgG1-biotin was purchased from Pharmingen: CD45(LCA)-FITC (30-F11), CD11b-FITC (M1/70), CD19-FITC (1D3), CD21/CD19-PE, and Gr1-APC for 30 min on ice. Basophils were identified by staining with CD45-FITC, blocking step with 5% rat serum was included before staining with primary Abs. RBC were lysed with FACS-lysing solution (BD Pharmingen) and analyzed on a FACS Calibur (BD Biosciences).

Isolation of basophils from peripheral blood and spleen

Basophils were prepared from unimmunized mice. Erythrocytes were lysed by addition of 0.2% sodium chloride for 10 s with subsequent correction of osmolarity with an identical volume of 1.6% sodium chloride. To isolate basophils from the peripheral blood, the cells were stained with CD45-FITC, CD16/32-PE, and Gr1-APC for 30 min on ice. Basophils were identified by staining on the lymphocyte gate, by medium expression of CD45, by high expression of CD16/32, and by absence of Gr-1 and were sorted on a cell sorter (MoFlo; DakoCytomation). The purity of the basophils was routinely ≥98% by subsequent FACS analysis. For isolation of basophils from the spleen, CD19+ B cells and CD11b− monocytes and neutrophils were first depleted with microbeads against CD19 and CD11b using LD columns (Miltenyi Biotec) and the remaining cells were stained with PE-labeled rabbit anti-mouse IgG (R0439; DakoCytomation), followed by blockade with 5% mouse serum and staining with CD45-FITC and Gr1-allophycocyanin. Basophils were identified by staining on the lymphocyte population, by medium expression of CD45, by high expression of surface IgG (R0439), and by the absence of Gr-1. The purity of the basophils was routinely ≥98%. Using eight mice, ~150,000 basophils were recovered from the peripheral blood and 350,000 from the spleens. Staining with anti-IgE, CD123 (IL-3R), CCR2, HLA IA/IE, CD19, B220, CD35, CD21, and CD138 Abs showed that the isolated cells were basophils.

Depletion of IgE from murine plasma

Sixty microliters of streptavidin-Sepharose (Amersham Biosciences) was loaded with 60 μg biotinylated anti-IgE Abs (clone R35-118) or biotinylated anti-IgG2a (clone R19-15) as control and washed five times to remove unbound Abs. Plasma from allophycocyanin-immunized mice (200 μl) was incubated twice for 1 h at room temperature with 30 μl of anti-IgE- or anti-IgG2a-coated Sepharose. The Sepharose was removed by centrifugation and complete depletion of IgE was shown by ELISA as described below.

Cell culture

Basophils (40,000/well) were cultured in round-bottom plates for 3 days in RPMI 1640 with 10% FCS and 1% penicillin/streptomycin. Where indicated, cells were stimulated with a polyclonal rabbit anti-mouse IgG (Dianova, Hamburg, Germany).

ELISA

IL-4 and IL-6 were measured in the cell culture supernatant with a commercially available kit (OptEIA; BD Pharmingen).

For measurement of total IgE in plasma, ELISA plates were coated with anti-IgE mAb (clone R35-72; 10 μg/ml) and bound IgE was detected with a biotinylated anti-IgE mAb (clone R35-118) followed by streptavidin-HRP (DakoCytomation) according to the manufacturer’s recommendations.

To test cross-reactivity of isotype-specific mAbs against murine IgG1, IgG2a, and IgG2b with purified murine IgE (BD Pharmingen), IgE was coated overnight at a concentration of 10 μg/ml followed by incubation with the biotinylated isotype-specific mAbs and streptavidin-HRP. No cross-reactivity of IgG isotype-specific Abs was detectable, while an Ab against IgE showed good reactivity with plate-bound IgE (data not shown).

**Results**

Detection and characterization of Ag-capturing cells

C57BL/6 mice were immunized twice with allophycocyanin using killed *B. pertussis* as adjuvant. Bone marrow, peripheral blood leukocytes, and splenocytes were stained with allophycocyanin and Abs against CD19 or B220. As described by Bell and Gray (3), two populations of allophycocyanin-binding cells were detected in allophycocyanin-immunized mice. In addition to allophycocyanin-positive B cells, a second population of CD19-negative/B220-negative allophycocyanin-binding cells was detectable at a frequency of 0.58% of total leukocytes in the peripheral blood, 0.77% in the bone marrow (Fig. 1a), and 0.21% in the spleen (data not shown).

**FIGURE 1.** Detection and characterization of allophycocyanin-binding non-B cells. *a.* Peripheral blood cells (*left column*) and bone marrow cells (*right column*) from C57BL/6 mice immunized twice with allophycocyanin (*upper row*) or control mice (*lower row*) were stained with CD19-PE and allophycocyanin. Apart from the small fraction of allophycocyanin-positive CD19− B cells, a clear population of allophycocyanin-positive CD19-negative cells is detectable in immunized mice. These allophycocyanin-positive non-B cells are more frequent in the bone marrow than in the peripheral blood. *b.* Light scatter properties of total peripheral blood cells (*left panel*) and allophycocyanin-positive non-B cells (*right panel*). Allophycocyanin-positive non-B cells are located within the lymphocyte region. *c.* The population of allophycocyanin-positive non-B cells was isolated by magnetic beads and FACS sort and stimulated in vitro by cross-linkage of surface Igs for 3 days. Cytokines were measured in the culture supernatant by ELISA. Cross-linkage of surface Igs resulted in a strong release of IL-4 and IL-6.

500 x g. Single-cell suspensions of spleen were prepared in ice-cold RPMI 1640 medium with 10% FCS and filtered to remove cell debris (Miltenyi Biotec). Bone marrow was obtained from the femur bones by flushing out the cells with ice-cold RPMI 1640 medium with 10% FCS.
In the bone marrow, allophycocyanin-positive B cells were detected at a very low frequency, while allophycocyanin-positive non-B cells were much more abundant. The allophycocyanin-positive B cells show a wide range of allophycocyanin staining intensity, as expected for an oligoclonal B cell population with variable affinity and avidity for the Ag. In contrast, the allophycocyanin staining on the non-B cell compartment is very homogenous and of identical signal strength in bone marrow, spleen, and peripheral blood. By backgating we could localize the allophycocyanin-positive non-B cells within the lymphocyte gate with somewhat higher side scatter properties than T or B cells (Fig. 1b). Costaining with several surface markers revealed that the allophycocyanin-positive non-B cells express low amounts of CD11b, but are negative for the additional B cell or plasma cell markers HLA-II, IgM, CD138, and CD21/35 (data not shown). Staining with a variety of other surface markers for T cells, NK cells, and monocytes did not result in identification of this cell population. However, a combination of staining for CD45, Gr-1, CD16/32, and surface Ig could unambiguously identify this cell population. They showed reduced expression of the common leukocyte Ag CD45, comparable to neutrophils, but are negative for the neutrophil marker Gr-1. In addition, they are highly positive for CD16/32 and express large amounts of surface IgG, as detected with a polyclonal Ab against IgG (Fig. 2b and data not shown). Combination of these surface markers enabled us to isolate the cell population in sufficient quantities for in vitro analysis from the peripheral blood and the spleen of mice with a purity of $>98\%$. The most striking finding was the release of large amounts of IL-4 and also some IL-6 after stimulation by cross-linkage of surface Ig with a polyclonal anti-mouse IgG Ab (Fig. 1c). LPS (50 $\mu$g/ml) was used as additional stimulus but did not result in a significant release of IL-4. These functional properties were very much reminiscent of the previously described IL-4-secreting non-B non-T cells (19) and raised the possibility that the cell population might consist of basophilic granulocytes that are known to release IL-4 after stimulation (20, 21).

Indeed, staining with Abs against IgE and the IL-3R (CD123) showed that all allophycocyanin-positive non-B cells were highly positive for both molecules (Fig. 3a) and that all IgE highly positive cells strongly bind allophycocyanin (Fig. 3b). Strong binding of IgE and homogeneous expression of the IL-3R is a hallmark for basophilic granulocytes that typically localize within the lymphocyte gate by light scatter properties (8, 9). It also became evident that allophycocyanin-positive cells were either CD19-positive B cells or IgE/CD123-positive basophils. The additional IgE-negative population of Ag-binding cells described by McHeyzer-Williams et al. (2) was not detectable in our mice.

We can exclude that eosinophils or mast cells contribute to the allophycocyanin-positive non-B cells. Eosinophils express only marginal levels of IL-3R (100-fold less than basophils) and are not located in the lymphocyte gate, but in the neutrophil gate (22). In addition, human eosinophils do not express the chemokine receptor CCR2 (23), while basophils do and respond to CCR2 ligands (7, 8). Staining of murine basophils for CCR2 and CCR5 reveals a high expression of CCR2 (Fig. 3c), while CCR5 was not detectable (data not shown). Expression of CCR2 was confirmed by down-modulation of the CCR2 receptor after incubation of the cells with murine macrophage chemotactic protein 1 (Il-1) for 30 min at 37°C. Mast cells are not found in the peripheral blood and can therefore be excluded as Ag-capturing cells at least in the blood (6). In addition, we stained the cells with an Ab against c-kit that

![Figure 2](image-url)  
**FIGURE 2.** “Capturing” of Igs by basophils. *a,* Binding of IgG and IgE subclasses by various cells types in the peripheral blood of mice was detected by FACS analysis. In comparison to other cells, basophils show the highest surface levels of IgG subclasses and IgE. *b,* Reduced expression of CD45 (common leukocyte Ag) and high expression of the low-affinity IgG receptors CD16/CD32 on basophils in the peripheral blood. Only the Gr-1-negative leukocytes are depicted in the dot plot.

![Figure 3](image-url)  
**FIGURE 3.** Identification of APC$^+$ non B cells as basophilic granulocytes. *a,* Allophycocyanin-positive non-B cells in the peripheral blood of immunized mice were costained for the surface markers CD123 (IL-3R) or surface IgE in flow cytometry. All allophycocyanin-positive non-B cells homogeneously stained positive for IL-3R and surface IgE. *b,* Basophilic granulocytes identified by high surface expression of IgE stained homogeneously positive for allophycocyanin in immunized mice (*upper row*), while no allophycocyanin binding is detectable in control mice. This indicates that all basophils and not only a subpopulation of basophils can bind allophycocyanin. *c,* Detection of the chemokin receptor CCR2 on basophils. Basophils identified by medium expression of CD16, high expression of CD16/32, and absence of Gr-1 were stained with the CCR2 Ab MC-21 (thick line) or an isotype control Ab (gray area). Preincubation of the cells with murine macrophage chemotactic protein 1 (0.5 $\mu$g/ml) for 30 min at 37°C markedly reduced surface staining of CCR2 (thin line).
showed a very weak expression on Ag-capturing cells in all three compartments, the peripheral blood, spleen, and bone marrow. c-kit-positive cells in the bone marrow with 40-fold higher expression of c-kit than basophils did not capture allophycocyanin, indicating that the Ag-capturing cells are no mast cells. Dendritic cells could also be excluded by the absence of CD11c and HLA II on Ag-capturing cells. A summary of the analyzed surface markers is given in Table I.

We also performed cytospin on the isolated basophils and stained the cells with May-Grünwald-Giemsa. The cells are slightly granular with basophilic granules and have indented nuclei with lobulation seen in some cases (data not shown). This is consistent with the morphological description of murine basophils that characteristically have few granules and a nucleus with heavily condensed chromatin in two lobes (5).

Ag capture is dependent on IgE receptors on basophils and Ag-specific IgE

We analyzed the binding of various Ig subtypes on basophils in comparison to other cell types (Fig. 2a). Using Abs against murine IgG1, IgG2a, IgG2b, and IgE, we found that basophils in the peripheral blood of BALB/c mice have severalfold more Igs bound on their surface than monocytes, neutrophils, and even B cells (Fig. 2a). These differences were most pronounced for IgE that was by far the most predominant Ig on basophils and was hardly detectable on other cell types. However, also various IgG subtypes were detectable at higher levels on basophils than on other cell types. This binding of IgG subtypes might result from a higher expression of Ig FcRs on basophils. This was indeed the case for the low- and medium-affinity FcRs for IgGs, FcγRIII, and FcγRII (CD16/32) (Fig. 2b) that would at least mediate the binding of IgG containing immune complexes. Using recently described mAbs against CD64 (18), we were not able to demonstrate CD64 (FcγRI) expression on basophils in the peripheral blood and found only a marginal expression of CD64 on basophils in the bone marrow (data not shown). However, we could demonstrate that the binding of allophycocyanin-specific Igs on basophils is very stable. When basophils from allophycocyanin-immunized mice were washed several times and incubated for 48 h in medium, no significant reduction in their ability to bind allophycocyanin was observed (data not shown). These data suggest that the capture of Igs by basophils occurs with rather high affinity or avidity.

The concept of Ab capturing by basophils was tested in vitro. Bone marrow cells from unimmunized C57BL/6 mice were incubated for 1 h at 37°C with plasma from control and allophycocyanin-immunized mice and washed three times before allophycocyanin was added. Incubation with plasma (75% of total volume) from allophycocyanin-immunized mice enabled the basophils to bind large amounts of allophycocyanin (Fig. 4, upper panel), whereas other cell types showed little allophycocyanin binding (Fig. 4, lower panel). Incubation with plasma from control mice did not allow allophycocyanin binding to basophils.

We then wanted to analyze which Ig FcRs mediate the binding of allophycocyanin-specific Igs and which Ig isotypes are responsible for the allophycocyanin-binding capacity of basophils. We therefore preincubated bone marrow cells from naive C57BL/6 mice for 1 h at 37°C with a high concentration of an IgE isotype control Ab (50 μg/ml) or a combination of IgG1, IgG2a, IgG2b, and IgG3 isotype control Abs (50 μg/ml each) to block unoccupied high-affinity IgE or IgG receptors, respectively. Without washing, cells were further incubated for 1 h at 37°C with plasma (75% of total volume) from allophycocyanin-immunized mice or naive control mice. Preincubation with IgE Abs reduced the ability of basophils to capture allophycocyanin-specific Igs by 90% (Fig. 5, a and b), whereas preincubation with the combination of IgG molecules had no effect (Fig. 5c). Preincubation with lower concentrations of IgE control Abs (5 μg/ml) also markedly reduced the ability of basophils to capture allophycocyanin-specific Igs (80% inhibition). To determine whether the isotype of the allophycocyanin-specific Igs that bind to basophils is indeed IgE, we completely depleted the IgE molecules from the plasma of allophycocyanin-immunized mice with anti-IgE-coated Sepharose. IgE-depleted plasma lost again >90% of its ability to mediate allophycocyanin binding to basophils in comparison to undepleted plasma (Fig. 6). Sepharose coated with a control Ab did not reduce the ability of plasma to mediate allophycocyanin binding (data not shown). These experiments show that IgE binding sites are required for the capture of allophycocyanin-specific Igs from mouse plasma and that allophycocyanin-specific IgE molecules are responsible for the ability of basophils to capture allophycocyanin.

The observation that basophils are able to capture allophycocyanin-specific IgE molecules during a 1-h exposure with plasma from allophycocyanin-immunized mice suggests that in unimmunized mice the IgE binding sites on basophils are not completely occupied and/or that the exposure of basophils with IgE induces an up-regulation of FcεRI and prevents the removal of unoccupied FcεRI from the cell surface (24–26). As expected, the amount of IgE molecules bound to basophils from unimmunized mice increased after exposure to IgE for 1 h at 37°C. The mean channel

Table I. Surface characteristics of murine basophils from peripheral blood and spleen as determined by flow cytometry

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FIGURE 4. Ag capture by basophils in vitro. Bone marrow cells from unimmunized control mice were incubated for 1 h at 37°C with plasma (75% of volume) from unimmunized control mice (gray area) or allophycocyanin-immunized mice (white area) and allophycocyanin binding was measured by flow cytometry. Basophils (upper panel) were identified by high expression of IgE and absence of CD19. All cells except basophils are shown in the lower panel. After incubation with plasma from allophycocyanin-immunized mice, only basophils show a strong binding of allophycocyanin, while incubation with control plasma does not allow allophycocyanin binding.
fluorescence for IgE bound to these basophils was 447 after exposure to IgE-depleted plasma, 824 after exposure with an IgE isotype control Ab (50 μg/ml), 862 after incubation with plasma from allophycocyanin-immunized mice but only 582 after incubation with plasma from unimmunized control mice. The increased binding of IgE by basophils after incubation with plasma from immunized mice compared with control mice is consistent with ELISA data measuring the total amount of IgE in the plasma. Immunized mice have ~8-fold higher IgE levels in their plasma than unimmunized mice (4.6 vs 0.55 μg/ml). It is reasonable to assume that a fraction of these additional IgE molecules will have specificity for allophycocyanin and mediate the ability of basophils to capture allophycocyanin-specific Igs.

Since IgE induced up-regulation of surface FcεRI is supposed to occur only at 37°C, we also analyzed whether the capture of allophycocyanin-specific IgE molecules from the plasma of immunized mice is higher at 37°C than on ice. Incubation of basophils from unimmunized mice with plasma from allophycocyanin-immunized mice on ice resulted only in a low binding of allophycocyanin, whereas allophycocyanin binding was much higher when the incubation with plasma was performed at 37°C (Fig. 7a). The same differences were observed when potentially unoccupied IgE receptors on the basophils were first saturated by preincubation with IgE isotype control Abs (75 μg/ml) and after thorough washing the subsequent incubation with plasma was performed on ice or at 37°C (Fig. 7b).

Discussion
After immunization of mice with Ags (e.g., fluorescent proteins like allophycocyanin) a very small fraction of leukocytes is able to bind intact Ags on the cell surface. Although low binding of intact Ag is detectable on a larger fraction of leukocytes (e.g., monocytes and neutrophils), strong binding is only observed on two distinct cell populations. One cell population consists of the small subpopulation of Ag-specific CD19-positive B cells. Ag binding on these B cells is inhomogeneous and somewhat higher than on the second cell population that was described in several manuscripts without being identified. In a recent report, Bell and Gray (3) demonstrated that these cells passively capture Igs on their surface and cannot be considered as a new population of CD19 and B220-negative memory B cells as previously suggested (2). We were now able to demonstrate that the Ag-capturing cells are basophilic granulocytes that capture Ag-specific Igs on their cell surface and thereby bind the Ag. We further show that the binding of Ag is dependent on surface IgE receptors and Ag-specific IgE in the plasma of immunized mice.

Several parameters were used to identify these cells as basophils. By light scatter properties in flow cytometry they are located in the lymphocyte gate with a somewhat higher side scatter than the majority of B or T cells. The Ag-capturing cells are negative for B cells markers (CD19, B220, CD21/35, IgM, HLA II) and the plasma cell marker CD138 (Syndecan-1). They show a high expression of surface IgE and the IL-3R CD123, and release large quantities of IL-4 after cross-linkage of surface IgE. In the peripheral blood, bone marrow, and spleen, only one cell population with high expression of IgE and CD123 was detectable. This population was identical to the allophycocyanin binding non-B cells, which means that all basophils identified by a high surface expression of IgE and CD123 were allophycocyanin-positive and that all allophycocyanin-positive non-B cells homogeneously express high levels of surface IgE and CD123. We also performed costaining with CD11c and HLA-II to exclude dendritic cells as Ag-capturing cells, as some dendritic cell subpopulations have been described to express the IL-3R (27–29). In addition, we excluded mast cells as Ag-binding cells. Allophycocyanin-positive
cells in all three compartments (blood, spleen, bone marrow) expressed only marginal levels of c-kit (mean channel fluorescence, ~25) and cells in the bone marrow that were clearly c-kit-positive (mean channel fluorescence, ~1000) did not capture allophycocyanin. Further evidence that the allophycocyanin- binding non-B cells are basophilic granulocytes comes from light microscopy that showed slightly granular cells with basophilic granules and indented nuclei with lobulation. We also analyzed the expression of the chemokine receptors and found a high expression of CCR2 and an absence of CCR5, a feature that has also been described for human basophils.

We then wanted to know why only basophils and not other FcR-positive leukocyte subsets (like monocytes or neutrophils) are able to capture Ag-specific IgGs and bind intact Ag. We therefore analyzed the amount of surface-bound IgGs on basophils compared with other cell types. Using isotype-specific Abs against IgM, IgG1, IgG2a, IgG2b, and IgE, we found that IgM is absent on basophils, while the IgG subtypes and especially IgE is detectable in large amounts on the surface of basophils. The amount of IgE captured by basophils by far outweighs the amount of IgG on basophils. Binding of IgE was only marginal on other cell types. The virtual absence of the high-affinity IgG Fc receptors FcγRI (CD64) and the ability of basophils to still capture allophycocyanin after several washing steps and even culture for 2 days in medium suggested that high-affinity receptors for IgE and allophycocyanin-specific IgE molecules might be responsible for the Ag-binding capacity of basophils. To analyze which Ig receptors and which Ig isotypes are responsible for the allophycocyanin binding, we allowed basophils from unimmunized mice to capture IgGs from the plasma of allophycocyanin-immunized mice by in vitro incubation for 1 h at 37°C. Incubation with plasma from allophycocyanin-immunized mice in contrast to plasma from naive mice allowed basophils to bind large amounts of allophycocyanin, while other cell types showed little allophycocyanin binding. The capture of allophycocyanin-specific IgGs on basophils could almost completely be prevented by preincubation of basophils with an excess of IgE IgGs. In addition, depletion of IgE from the plasma of allophycocyanin-immunized mice markedly reduced the capture of allophycocyanin-specific IgGs by basophils from naive mice. These data show that receptors for IgE on basophils and allophycocyanin-specific IgE molecules in the plasma are required for Ag binding on basophils. Our results are consistent with a previous report by Bell and Gray (3) that have shown the absence of Ag-capturing cells in mice deficient in the common FcR γ-chain. These mice are unable to express IgG receptors FcγRI and FcγRIII as well as the high-affinity receptor for IgE. In conjunction with our data, one now can conclude that basophils capture Ags by binding Ag-specific IgE molecules on high-affinity IgE receptors. The high-affinity IgG Fe receptor CD64 that was recently shown to be expressed on murine mast cell lines (P815, 10P2) and on APCs (dendritic cells, macrophages) has no major contribution to Ag binding on basophils.

How could basophils obtained from naive mice with IgE molecules already bound to their IgE receptors capture additional allophycocyanin-specific IgE molecules from the plasma of allophycocyanin-immunized mice? First, the IgE receptors on basophils from naive mice might not be completely occupied by the low concentrations of IgE (0.55 μg/ml) detected in the plasma of these mice. Second, the incubation of basophils with plasma from allophycocyanin-immunized mice containing much higher IgE concentrations (4.6 μg/ml) might result in an up-regulation of high-affinity IgE receptors and a decreased loss of surface FcεRI (24–26). It was shown that IgE molecules (5 μg/ml) induce an ÿ 4-fold up-regulation of FcεRI expression within 3 h on murine mast cells (25). Up-regulation of high-affinity IgE receptors might explain the much higher binding of allophycocyanin, when basophils were incubated with plasma from allophycocyanin-immunized mice at 37°C compared with an incubation on ice. In addition, up-regulation of IgE receptors also appears as the most likely explanation why the blockade of potentially unoccupied IgE receptors with IgE control Abs did not prevent the subsequent capture of allophycocyanin-specific IgE Abs at 37°C. Since basophils were extensively washed after incubation with plasma, an interaction of allophycocyanin-specific IgE molecules with low-affinity receptors for IgE appears unlikely. The up-regulation of FcεRI by IgE molecules would enable basophils to bind newly added IgE Abs and always present a representative sample of plasma-derived IgE molecules on their surface despite the high-affinity interaction between IgE and FcεRI.

Basophils are important regulators of humoral immune responses. They influence B cell function by secretion of cytokines and also express CD40L that stimulates B cell proliferation (14, 30, 31). Via CD40L and the secretion of the cytokines IL-4, IL-6, and IL-13, basophils induce a Th2 deviation of the immune response and induce class switching to IgE and IgG4 in humans (14–16, 31, 32). The capture of IgGs by basophils might be important during both the recognition phase and memory responses. In both phases, the amount of Ag is greater than the amount of specific IgGs in the plasma and therefore free Ag is available for cross-linkage of surface IgGs on basophils. At later time points, an excess of specific Ab would prevent binding of Abs to basophils and thereby exclude basophils from participating in immune regulation. The high expression of the chemokine receptor CCR2 that is also critical for migration of APCs (monocytes/macrophages and dendritic cells) may help to localize basophils to sites of Ag recognition and enhance their influence on humoral immune responses, especially in spleen and bone marrow.
In summary, we have identified Ag-capturing non-B cells as basophilic granulocytes that bind intact Ags in an IgE and IgE receptor-dependent manner. By their ability to capture Ags and respond to cross-linkage of surface IgS, basophils may play an important role in supporting and accelerating humoral immune responses in the recognition phase and during a memory response.

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References