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Basophils Support the Survival of Plasma Cells in Mice

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We have previously shown that basophils support humoral memory immune responses by increasing B cell proliferation and Ig production as well as inducing a Th2 and B helper phenotype in T cells. Based on the high frequency of basophils in spleen and bone marrow, in this study we investigated whether basophils also support plasma cell survival and Ig production. In the absence of basophils, plasma cells of naive or immunized mice rapidly undergo apoptosis in vitro and produce only low amounts of Iggs. In contrast, in the presence of basophils and even more in the presence of activated basophils, the survival of plasma cells is markedly increased and continuous production of Iggs enabled. This effect is partially dependent on IL-4 and IL-6 released from basophils. Similar results were obtained when total bone marrow cells or bone marrow cells depleted of basophils were cultured in the presence or absence of substances activating basophils. When basophils were depleted in vivo 6 mo after immunization with an Ag, specific Ig production in subsequent bone marrow cultures was significantly reduced. In addition, depletion of basophils for 18 d in naive mice significantly reduced the number of plasma cells in the spleen. These data indicate that basophils are important for survival of plasma cells in vitro and in vivo. The Journal of Immunology, 2010, 185: 7180–7185.

Until recently, basophils were considered mainly as effector cells of an innate immune response linked to allergy and parasite infection. Only in the past few years were they recognized as important regulators of adaptive immunity. We have shown that during memory immune responses, basophils are able to bind significant amounts of intact Ags on their surfaces (1). Binding of Ags on basophils is achieved by Ag-specific IgE produced during the primary immune response and captured via high-affinity IgE receptors on the surfaces of basophils. After re-exposure to Ags, basophils become activated by cross-linkage of surface Fc receptors for IgE (FceRI) and IgG (FcyRIII) and constitute the main source of IL-4 and IL-6 in the spleen and bone marrow (2). Basophils markedly support the development of a humoral memory immune response and accelerate and enhance Ab production after re-exposure to Ags (2). Basophils may directly support B cells or enhance B cell responses by inducing a Th2 and B helper phenotype in CD4+ T cells (2–5). It has also been shown that basophils express MHC class II and under certain experimental conditions present Ag to CD4+ T cells (2–5). Basophils cooperate with dendritic cells in inducing a Th2 response (9) and have been shown to contribute to the development of lupus nephritis (10).

Apart from cross-linkage of surface Fc receptors, a large number of stimuli are known to activate basophils. These include cytokines (e.g., IL-1, IL-3, IL-5, IL-18, IL-33) (11–15), growth factors (e.g., IL-1, IL-3, IL-5, IL-18, IL-33) (11–15), and complement factors (C3a, C5a) (16, 24). FcyRIII, FcγRII, and the IL-3R CD123 contain the signal-transducing FcγRI and are important for activation of basophils in vivo (2, 25–28).

Activated basophils have been shown to release a large number of factors including cytokines (IL-4, IL-6, IL-13, thymic stromal lymphopoietin), histamine, leukotrienes, and platelet activating factor (1, 2, 5, 12, 28–31).

Long-lived plasma cells residing mainly in the bone marrow are an important component of the immunological memory (32). Plasma cell inherent factors and environmental signals are thought to enable longevity of plasma cells but are poorly defined to date (33). It was shown that plasma cells are located next to CXCL-12– and VCAM-1–expressing mesenchymal stromal cells and that CXCR4 expression on plasma cells is required for their survival in vivo (34). Also, BAFF and a proliferation inducing ligand (APRIL) were shown to support plasma cell survival in vivo (35, 36).

Whereas several factors (e.g., IL-5, IL-6, TNF-α, CXCL-12, anti-CD44, BAFF, APRIL, VLA-4 ligands) were found to support survival of plasma cells for up to 3 d, a combination of factors (e.g., IL-6 and anti-CD44) was required to mediate survival of plasma cells for up to 5 d in culture (37, 38). The combination of IL-6 with the B cell maturation Ag ligands BAFF and APRIL enabled reasonable survival of plasma cells for up to 10 d (36).

Based on the presence of basophils in bone marrow and spleen and the multiple pathways for activation of basophils and their potency in supporting B cell responses, we analyzed whether basophils support the survival of short- and long-lived plasma cells. In the absence of basophils, isolated plasma cells rapidly died, whereas addition of basophils enabled excellent survival of plasma cells and Ig production for up to 17 d.

Basophils being present in total bone marrow cells also supported plasma cell survival as shown with basophil-depleted bone marrow and by in vivo depletion of basophils in mice at 6 mo after immunization. Depletion of basophils in naive mice for 18 d significantly reduced the number of plasma cells in the spleen. These data indicate that basophils contribute to survival of plasma cells in mice.
Materials and Methods

Mice

Female C57BL/6 mice were purchased from Elevage Janvier (Le Genest Saint Isle, France) at an age of 10 to 12 wk. Mice were immunized twice by i.p. injection of 100 μg APCy (cross-linked APCy; Prozyme, Hayward, CA) or PE (cross-linked PE; Prozyme) with 2 × 10^7 heat-inactivated Bordetella pertussis (Deutsche Sammlung von Mikroorganismen und Zellkulturen DSMZ, Braunschweig, Germany) as adjuvant. If not otherwise indicated, plasma cells and basophils were isolated at 2 to 4 wk after the second immunization. All animal experiments were performed according to institutional guidelines and German federal laws on animal protection.

In vivo depletion of basophils

Mice were injected twice daily for 3 d with 5 μg anti-FcεRIα (MAR-1; eBioscience, San Diego, CA) or an isotype control Ab (hamster IgG) if not specified otherwise. Injections were repeated twice in weekly intervals for low-term depletion of basophils.

Isolation, depletion, and culture of cells

Bone marrow cells were collected from the femur and tibia bones. For depletion of basophils, cells were stained with FITC-labeled anti-IgE (R35-72) and removed with magnetic beads directed against FITC (Miltenyi, Bergisch Gladbach, Germany) using LD columns (Miltenyi). Sham depletion was performed also with LD columns after incubating the cells with PBS and anti-FITC beads.

Total bone marrow cells or bone marrow cells depleted of basophils were cultured in 96-well flat-bottom plates (0.5 × 10^5 cells/well in a total volume of 200 μl medium) with or without murine IL-3 (10 ng/ml; Peprotech, Rocky Hill, NJ). The culture medium consisted of RPMI 1640 with 10% heat-inactivated FCS, penicillin/streptomycin, nonessential amino acids, 1 mM Na-pyruvate, 100 μM HEPES, and 0.1 μM 2-mercaptoethanol.

Basophils were enriched from the bone marrow using magnetic beads directed against DX5 and MS columns (Miltenyi). Approximately 10% of the DX5^+ cells stained positive for surface IgE and could be identified as basophils. To obtain DX5^+ cells without basophils, bone marrow cells were first depleted of basophils as described earlier and then positively selected with magnetic beads against DX5 as described above. Enriched basophils or DX5^+ cells without basophils were added to the culture as indicated.

Plasma cells were isolated using the CD138^+ plasma cell isolation kit (Miltenyi). Isolated plasma cells (20,000–50,000 cells/well) were cultured in a total volume of 200 μl medium. Culture of isolated plasma cells and/or basophils was performed in 96-well round-bottom plates. Where indicated, APCy (0.1 μg/ml) and blocking Abs (20 μg/ml) against IL-4 (clone 30404.11) and IL-6 (clone MP520F3) were added.

Extracellular flow cytometry

The following Abs were used for flow cytometry: anti-CD16/32 (Fc-block 2.4G2), FITC-anti-CD45 (LCA; 30-F11), APCy-anti-CD45 (LCA; 30-F11), PE-anti-CD138 (281-2), PE-anti-CD19 (1D3; eBioscience), FITC-anti-IgE (R35-72), PE-anti-CD49b (DX5), APCy-anti-CD49b (DX5; Miltenyi), PE-anti-CD19 (1D3; eBioscience).

Unfixed cells were preincubated for 15 min on ice with Fc block (5 μg/ml) and then for 45 min with combinations of directly labeled Abs. After three washing steps, RBCs were lysed with FACS-lysing solution (BD Biosciences), and samples were analyzed on a FACSCalibur (BD Biosciences, Heidelberg, Germany) with CellQuest software (BD Biosciences).

Intracellular flow cytometry of cultured cells

Cells from the triplicate samples were pooled and first stained with PE-anti-CD138 and then treated with Fix-Perm and Perm-Wash solutions (BD Biosciences) according to the manufacturer’s instructions. Cells were then incubated with APCy (0.05–0.5 μg/ml) for 30 min for intracellular staining of anti-APCy or anti-PE Abs and determination of Ag-specific plasma cell survival. The number of live cells per well was quantified for each time point by addition of propidium iodide (10 μg/ml) and counting beads (Caltag/Invitrogen, Carlsbad, CA).

ELISA

Cell culture supernatant was used for determination of total IgG1, total IgG2a, APCy- or PE-specific IgG1 and IgG2a, and cytokines. ELISA plates were coated with 10 μg/ml APCy or PE in PBS overnight, washed, and blocked with 2% BSA (Sigma, Munich, Germany) in PBS. Cell culture supernatant was added in the indicated dilutions. Biotinylated Abs against IgG1 (A85-1; BD Biosciences) and IgG2a (R19-15; BD Biosciences) and streptavidin-linked HRP (DakoCytomation, Hamburg, Germany) followed by ABTS (Roche, Penzberg, Germany) were used to reveal bound Igs. Between all steps, the plates were washed with 0.05% Tween 20 in PBS. IL-4 and IL-6 were measured with commercially available ELISA kits (OptEIA; BD Biosciences).

Statistics

Error bars indicate the SEM in all figures. Cell culture experiments were performed in triplicate and reproduced at least three times with one representative experiment being depicted. The p values for significance were calculated with ANOVA and two-tailed Student t test.

Results

Basophils support the survival of isolated plasma cells

C57BL/6 mice were immunized twice with APCy. At 2 wk after the second immunization, CD138^+ plasma cells and DX5^+ basophils were isolated from the bone marrow using magnetic beads as described in Materials and Methods. Basophils were enriched via DX5 to avoid activation of basophils. Plasma cells (20,000/well) were cultured alone or in the presence of 10,000 DX5^+ cells consisting of ~1,000 basophils. As further control, DX5^+ cells (10,000/well) were cultured alone. At days 6, 10, 14, and 17 of culture, the amount of APCy-specific IgG1 and IgG2a as well as total IgG1 and IgG2a was measured in the supernatant by ELISA. The number of plasma cells producing Abs against APCy (APCy-positive plasma cells) or against other Ags (APCy-negative plasma cells) was quantified by FACS analysis with counting beads (Fig. 1).

Culture of plasma cells alone resulted in the release of only low amounts of total and APCy-specific Igs with a marginal increase from day 6 to day 17 (Fig. 1A). The numbers of total and APCy-specific plasma cells markedly decreased being almost undetectable on days 10–17 (Fig. 1B). In contrast, coculture of plasma cells with basophils resulted in a high and continuously increasing release of total and APCy-specific Igs and an excellent survival of both total and APCy-specific plasma cells (survival >60% at day 6 and 40% at day 17). DX5^+ cells alone did not contain plasma cells and did not produce any Igs (Fig. 1).

In the fraction of DX5^+ cells isolated from the bone marrow, only ~10% were basophils, as determined by staining for IgE and absence of c-Kit (data not shown). To verify that basophils and not other DX5^+ cells (e.g., NK cells) support survival of plasma cells, we first completely depleted IgE^+ basophils with magnetic beads and in a second step isolated DX5^+ cells. As control we used total DX5^+ cells, where the first step was performed in the absence of the anti-IgE Ab. Total DX5^+ cells or DX5^+ cells depleted of basophils were cocultured with isolated plasma cells for 7 or 14 d (Fig. 2). Compared with a culture of plasma cells alone, the coculture of plasma cells with basophil-depleted DX5^+ cells only minimally enhanced the release of total IgG1/IgG2a and APCy-specific IgG1/IgG2a. Likewise, only very few total and APCy-specific plasma cells were detectable on day 7 with a further drop until day 14 in both groups. In contrast, coculture of total DX5^+ cells with plasma cells strongly increased the number of plasma cells and markedly enhanced the release of Igs, indicating that basophils and not other DX5^+ cells enable plasma cell survival (Fig. 2).

We then asked the question whether activation of basophils further enhances their ability to support plasma cell survival and Ig production. For this purpose, we used mice immunized twice with APCy and PE. Plasma cells from these mice were cultured alone or with basophils (DX5^+ cells) from the same mice for 7 d. PBS or APCy (100 ng/ml) was added to the culture, and the release of total and PE-specific Igs was measured. Addition of APCy does not interfere with detection of PE-specific Igs and results in a strong activation of basophils with release of large amounts of IL-6 and IL-4 (data not shown). Unstimulated basophils (PBS group) mark-
edly enhanced the release of total and PE-specific Igs and the survival of plasma cells as shown before (Fig. 3). Stimulation of basophils with APCy further enhanced (≈2-fold) the release of Igs and the survival of plasma cells (Fig. 3). As described earlier in this article, activated basophils are known to release a variety of factors (e.g., IL-4 and IL-6) that may potentially support plasma cell survival (e.g., IL-6). To investigate the role of IL-4 and IL-6, we cocultured plasma cells and basophils from APCy and PE double immunized mice in the presence or absence of blocking Abs against IL-4 and IL-6 (Fig. 4). As shown before, unstimulated basophils and even more stimulated basophils enhanced the release of total and PE-specific Igs in the culture supernatant. Addition of Abs against IL-4 and IL-6 significantly interfered with the supportive effects of basophils and significantly reduced the release of Igs. However, blockade of IL-4 and IL-6 did not completely abolish the supportive effect of basophils, indicating that apart from IL-4 and IL-6, other basophil-derived factors support plasma cell survival (Fig. 4). Blockade of IL-4 and IL-6 in a culture of plasma cells with nonactivated basophils also slightly reduced the number of live plasma cells/well and decreased the levels of APCy-specific IgG1 and IgG2a (Supplemental Fig. 1), suggesting that the release of small amounts of IL-4 and/or IL-6 by nonactivated basophils contributes in promoting the survival and function of plasma cells. To investigate further whether membrane-bound factors or soluble factors from basophils support the survival of plasma cells, we performed experiments where we incubated plasma cells with activated basophils or just with the supernatant derived from the same number of activated basophils (Supplemental Fig. 2). The supernatant of activated basophils is as efficient in supporting the survival of plasma cells and their production of total and APCy-specific Igs as the addition of preactivated basophils themselves. These data suggest basophils support the survival of plasma cells in culture mainly by release of soluble factors.

To analyze whether basophils present at physiological numbers in the bone marrow also contribute to plasma cell survival, we cultured total bone marrow cells or bone marrow cells depleted of basophils (500,000 cells/well). Cells were obtained from mice at 2 wk after the second immunization with APCy. Culture of total bone marrow cells for 7 d resulted in 2- to 3-fold higher APCy-specific and total IgG1 and a better survival of plasma cells than that of culture of bone marrow cells without basophils (Fig. 5). If unstimulated basophils (10,000/well) were added back to the later culture, Ig production and plasma cell survival was restored. There was only a moderate increase in Igs and plasma cell survival when basophils were added to the culture of total bone marrow cells. When activated basophils were added instead of unstimulated basophils, there was a pronounced increase in the level of IL-6 measured in the culture supernatant on day 7. However, there was only a modest increase in APCy-specific IgG2a (Supplemental Fig. 3) and no increase in total or APCy-specific IgG1. These data indicate that a strong increase in IL-6 levels does not translate into a better plasma cell survival in total bone marrow cells. To analyze whether low amounts of IL-4 and IL-6 released from nonactivated basophils present in total bone marrow have an effect on plasma cells, we cultured total bone marrow cells or bone marrow cells depleted of basophils in the presence or absence of anti–IL-4 and anti–IL-6 Abs (Supplemental Fig. 4). Depletion of basophils markedly reduced the number of plasma cells and the levels of
total and APCy-specific IgG1. However, blockade of IL-4 and IL-6 had only a minor effect on survival and function of plasma cells.

We also investigated whether basophils support plasma cells in naive mice (Fig. 6). Due to the low frequency of CD138+ plasma cells in the bone marrow of these mice, we were unable to isolate sufficient numbers of plasma cells. We therefore cultured total bone marrow cells or bone marrow cells depleted of basophils in vitro and measured the amount of IgG1 from day 5 to 9. In total bone marrow, the IgG1 level was markedly lower in basophil-depleted bone marrow and did not increase during culture, indicating that only few viable plasma cells are present from day 5 to 9. To study the effect of basophils on plasma cell numbers in vivo, basophils were depleted in naive mice by repeated injection of MAR-1 Ab ($n = 9$). Control mice were treated with the same amount of an isotype-control Ab ($n = 9$) (Fig. 6B). After 18 d, mice were sacrificed and the absolute number of basophils and plasma cells determined in the spleen and bone marrow (one tibia bone/mouse). The number of basophils was markedly reduced both in the spleen and bone marrow, whereas the number of plasma cells was significantly reduced in the spleen but not in the bone marrow (Fig. 6B). Depletion of basophils with MAR-1 did not affect spleen size, as the number of leukocytes per spleen was not significantly different in both groups ($p = 0.51$) (data not shown).

To also analyze the effect of basophils on long-lived Ag-specific plasma cells in the bone marrow, we immunized mice two times with APCy and at 6 mo after the second immunization depleted basophils in vivo by injection of the Ab MAR-1 for 3 d, as described previously (Fig. 7). Control mice were treated with the same amount of an isotype-control Ab. Five days after the last injection, bone marrow was obtained, cultured for 14 d in plain medium, and the level of total and APCy-specific IgG1 measured in the supernatant. Treatment of mice with MAR-1 resulted in a complete disappearance of basophils from the bone marrow and significantly reduced the production of total and APCy-specific IgG1 in the subsequent culture of bone marrow cells (Fig. 7).

**Discussion**

Using two readouts, we show that basophils markedly support the survival of short- and long-lived plasma cells in bone marrow cul-
cells or recently immigrated plasma cells. Basophils also supported the survival of plasma cells from naive mice and the survival of APCy-specific plasma cells from mice at 6 mo after immunization, indicating that basophils also act on long-lived plasma cells. Culture of total bone marrow cells or bone marrow cells where basophils were selectively depleted demonstrated that physiological numbers of basophils are sufficient to enhance plasma cell survival.

We have shown previously that activation of basophils (e.g., by Ag-induced cross-linkage of surface Fc receptors or by cytokines, e.g., IL-3) and their subsequent release of IL-4 and IL-6 is a precondition for their ability to support activation of B cells and to induce Th2 cells during memory immune responses. In contrast, the positive effect of basophils on plasma cells in the bone marrow seems to be largely independent of such activation, as unstimulated basophils supported survival of isolated plasma cells, and specific depletion of basophils reduced plasma cell survival in the bone marrow. Artificial activation of basophils (e.g., with Ag or IL-3) markedly increased IL-6 levels in culture but had only a minor incremental effect on plasma cell survival and Ig production. In addition, blockade of IL-4 and IL-6 with Abs was unable to abolish the positive effects of basophils on plasma cells and had only a minimal effect on survival and function of plasma cells in total bone marrow cultures. This is in accordance with IL-6-deficient mice that do not have a plasma cell defect (39). We think that basophil-derived IL-6 is only one of several factors provided by basophils. The spectrum of basophil-derived factors, as mentioned earlier in this article, is large, and other substances released from basophils may also support plasma cells. The supernatant of activated basophils provided the same help to plasma cells as activated basophils themselves, indicating that mainly soluble factors and not membrane-bound factors from basophils support plasma cell survival.

It is difficult to determine in a strict sense whether activation of basophils is unnecessary for plasma cell support. A large variety of factors has been found to activate basophils, and we know from gene array studies that some of these factors result in a differential activation of basophils, inducing the release of different molecules and specific changes of membrane-associated molecules. Basophils may be continuously activated to a low degree in the bone marrow and spleen (e.g., by factors released from leukocytes or stromal cells) and thereby be part of a network of cells building the plasma cell niche.

The culture of isolated plasma cells or total bone marrow cells does not fully reflect the environment of plasma cells in spleen or bone marrow, where plasma cells have been found to be located next to CXCL-12- and VCAM-1-expressing stromal cells. Culture of single-cell suspensions is unlikely to result in self-organization of plasma cell niches comparable with those present in intact tissue. We therefore depleted basophils for prolonged periods of time (18 d) in naive mice and determined the absolute numbers of basophils and plasma cells in the spleen and bone marrow. Whereas basophils were clearly reduced in both organs, the number of plasma cells was significantly reduced only in the spleen. Reduced numbers of plasma cells in the spleen were also described in Lyn−/− mice at 6 d after deletion of basophils, whereas there was no difference in wild-type animals (10). We have observed a significant reduction of plasma cells also in the spleens of wild-type mice at 18 d after deletion of basophils. Depletion of basophils seems to affect plasma cell survival rather quickly (6 d) in Lyn−/− mice, which have increased numbers of basophils, but appears to take more time in wild-type animals (18 d). In a second type of experiment, we depleted basophils in immunized mice by injection of MAR-1 and measured in subsequent bone marrow cultures the production of total and Ag-specific IgG1.

![FIGURE 6. Basophils support plasma cell survival in naive mice. A. Total BM cells or BM cells without basophils (IgE− BM) from naive mice (0.5 × 10^6 cells/well) were cultured for 5 and 9 d with or without IL-3 (10 ng/ml), and the level of total IgG1 and IL-6 was measured in the supernatant by ELISA. Activation of basophils with IL-3 induced a strong release of IL-6 and significantly enhanced their ability to support basophils. Depletion of basophils significantly reduced production of IgG1. One of three representative experiments is shown. B, Basophils were depleted in naive mice by repeated injection of MAR-1 Ab (n = 9). Control mice were treated with the same dose of isotype control Ab (n = 9). After 18 d, mice were sacrificed and the absolute number of basophils and plasma cells determined in the spleen and bone marrow (one tibia bone/mouse). The number of basophils was markedly reduced both in the spleen and bone marrow. The number of plasma cells was significantly reduced in the spleen but not in the bone marrow. *p < 0.05; **p < 0.01. BM, bone marrow cells; IgE− BM, BM cells without basophils.](http://www.jimmunol.org/)

![FIGURE 7. In vivo depletion of basophils reduces plasma cell survival in the spleen and in subsequent bone marrow cultures. At 6 mo after the second immunization with APCy, basophils were depleted in vivo by injection of MAR-1 Ab (n = 5) or remained undepleted (injection of an isotype control Ab, n = 5). At 5 d after the last Ab injection, bone marrow cells were cultured for 14 d. The levels of total and APCy-specific IgG1 measured in the supernatant were significantly reduced in the MAR-1–treated mice. *p < 0.05.](http://www.jimmunol.org/)
MAR-1 treatment reduced the production of Abs in bone marrow cultures, indicating that in vivo depletion of basophils also affects plasma cell function in culture.

Taken together, we have shown that basophils enhance survival and Ig production of plasma cells in vitro and support plasma cell survival in the spleen.

Disclosures

The authors have no financial conflicts of interest.

References


