IL-3 Induces B7.2 (CD86) Expression and Costimulatory Activity in Human Eosinophils

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Eosinophils in tissues are often present in intimate contact with T cells in allergic and parasitic diseases. Resting eosinophils do not express MHC class II proteins or costimulatory B7 molecules and fail to induce proliferation of T cells to Ags. IL-5 and GM-CSF induce MHC class II and B7 expression on eosinophils and have been reported in some studies to induce eosinophils to present Ag to T cells. The cytokine IL-3, like IL-5 and GM-CSF, is a survival and activating factor for eosinophils and the IL-3 receptor shares with the IL-5 and GM-CSF receptors a common signal transducing β-chain. IL-3-treated eosinophils expressed HLA-DR and B7.2, but not B7.1 on their surface and supported T cell proliferation in response to the superantigen toxic shock syndrome toxin 1, as well as the proliferation of HLA-DR-restricted tetanus toxoid (TT) and influenza hemagglutinin-specific T cell clones to antigenic peptides. This was inhibited by anti-B7.2 mAb. In contrast, IL-3-treated eosinophils were unable to present native TT Ag to either resting or TT-specific cloned T cells. In parallel experiments, eosinophils treated with IL-5 or GM-CSF were also found to present superantigen and antigenic peptides, but not native Ag, to T cells. These results suggest that eosinophils are deficient in Ag processing and that this deficiency is not overcome by cytokines that signal via the β-chain. Nevertheless, our findings suggest that eosinophils activated by IL-3 may contribute to T cell activation in allergic and parasitic diseases by presenting superantigens and peptides to T cells. ***The Journal of Immunology,*** **2001, 167: 6097–6104.**

At least two signals are required for T cell activation and proliferation. The first signal is the recognition by the TCR of an antigenic peptide bound to MHC molecules on APCs (1). The second signal is the interaction between counterreceptors on the surface of APCs with costimulatory receptors on T cells (2). APCs fall into two categories: professional APCs and nonprofessional APCs. Professional APCs, which include dendritic cells and monocytes/macrophages, constitutively express surface MHC class II molecules, as well as costimulatory molecules and are able to process Ag and present it to resting T cells. Nonprofessional APCs may not express surface MHC class II molecules or costimulatory molecules in their resting state and include cells from both hemopoietic lineage (e.g., B cells) and nonhemopoietic lineage (e.g., endothelial cells). Activation of nonprofessional APCs by cytokines induces expression of MHC class II molecules and costimulatory molecules and can enhance their capacity to process Ag rendering them capable of Ag presentation.

The B7 family of molecules includes B7.1 (CD80) and B7.2 (CD86), which interact with CD28 and with its homolog CTLA-4, and B7RP-1 which interacts with the CD28 homolog inducible costimulator and the newly described member B7-H1 (3–5). Interaction between B7.1 and B7.2 on the surface of APCs and the costimulatory molecule CD28 on T cells plays a critical role in the T cell response to Ag (3). Thus, T cells from mice deficient in CD28 fail to proliferate in response to specific Ag (6). B7.1, and to a lesser degree B7.2, are expressed constitutively on the surface of professional APCs, although nonprofessional APCs do not express significant amounts of B7 molecules unless activated by cytokines (7–9), by cross-linking of surface receptors such as surface immunoglobulins on B cells (10), or by ligation of CD40 (11, 12).

Eosinophils play an important role in allergic inflammation and in the response to parasitic infection (13). Resting eosinophils do not express detectable MHC class II molecules or B7 molecules on their surface and are unable to present Ag to T cells (14, 15). Following their activation with the cytokines GM-CSF, IL-4, IFN-γ, and IL-5, eosinophils express MHC class II molecules (14–17). Furthermore, in vivo activated eosinophils, isolated from blood on the basis of their low density or isolated from the bronchoalveolar lavage (BAL)[3] fluid of patients with chronic lung eosinophilia, also express MHC class II molecules (16, 18). There is little information on the expression of B7 molecules on eosinophils. B7.2 only or both B7.1 and B7.2, were reported to be expressed on activated eosinophils in vivo (19, 20). GM-CSF was shown to induce B7.1 and B7.2 expression on eosinophils from IL-5 transgenic mice and Abs to these B7 molecules inhibited Ag presentation by these eosinophils (21). GM-CSF- and IL-5-treated eosinophils have been reported to present Ag to activated T cells, although much less efficiently than macrophages (14, 17, 22), and were either unable to present Ag to resting T cells, or supported very weakly the proliferation of resting T cells to native Ag (15, 17, 21, 22). More recently, in vivo activated eosinophils isolated from the BAL from the inflamed airways of mice sensitized to and challenged by inhalation with OVA, were shown to be competent in presenting OVA to T cells (20). Because tissue eosinophils come in intimate contact with Ag, particularly with allergens and

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Received for publication November 2, 2000. Accepted for publication September 19, 2001.

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[1] This work was supported by U.S. Public Health Service Grants AI-31541, AI-31136, AI-07512, and by Baxter, Aventis-Behring, and Olsten Corporations.

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[3] Abbreviations used in this paper: BAL, bronchoalveolar lavage; TSST-1, toxic shock syndrome toxin 1; βc, β-chain; TT, tetanus toxoid; HA, hemagglutinin.

0022-1767/01/$02.00

helminthic Ags, these findings suggest that activated eosinophils may play a role in Ag presentation to T cells.

IL-3 is a hemopoietic growth factor that supports the proliferation of early multilineage progenitors at early stages of development and is a differentiation factor for human basophils and murine mast cells (23, 24). IL-3 signals via the IL-3R which shares a common signal transducing β-chain (βc) with IL-5 and GM-CSF. Like IL-5 and GM-CSF, IL-3 is a survival factor for eosinophils and causes eosinophils to become hypodense (25). However, unlike IL-5, IL-3 is not a growth factor for eosinophils, and unlike GM-CSF it is not a differentiation factor for granulocytes and monocytes/macrophages. This suggests that IL-3 may activate signaling pathways that are not identical with those activated by IL-5 and GM-CSF.

Because IL-3 is produced by activated T cells, mast cells, and eosinophils from tissues in allergic inflammation (26–30) we wished to examine the effect of IL-3 on the differentiation of eosinophils into APCs. In this study, we demonstrate that IL-3 induces human eosinophils to express HLA-DR and B7.2, but not B7.1, on their surface. IL-3-treated eosinophils were able to support the proliferation of autologous resting purified peripheral blood T cells to the superantigen toxic shock syndrome toxin 1 (TSST-1), but not to the Ag tetanus toxoid (TT) which requires processing. However, they supported the proliferation of TT-specific and hemagglutinin (HA)-specific T cell clones to Ag-derived peptides. Presentation of superantigen- and Ag-derived peptides by IL-3-treated eosinophils was specifically inhibited by anti-B7.2 mAb, suggesting the involvement of the B7-CD28 pathway. In parallel experiments, IL-5 and GM-CSF also enabled eosinophils to present TT peptide Ags, but not native TT Ag, to T cells, suggesting that eosinophils are deficient in Ag processing and that this deficiency cannot be overcome by cytokines that signal signaling via the βc.

Materials and Methods

Reagents and Abs

Anti-CD16 mAb was generated from the 3G8 hybridoma kindly provided by Dr. T. Springer (Harvard Medical School, Boston, MA). Anti-CD80 and anti-CD86 mAbs were purchased from BD PharMingen (San Diego, CA). Anti-HLA-DR mAb was purchased from BioSource International (Camarillo, CA) and Biomag goat anti-mouse IgG from Perseptive Diagnostics (Framingham, MA). The anti-Mac1 mAb OKM1 was purchased from Ortho Diagnostics (Raritan, NJ). PE-conjugated mAbs to B7.1 and B7.2, FITC-conjugated anti-HLA-DR mAb and anti-CD28 mAb were from BD PharMingen. Recombinant human IL-2 and IL-5 were from R&D Systems (Minneapolis, MN). Recombinant human IL-3 and GM-CSF were obtained from Genetics Institute (Cambridge, MA); TSST-1 was obtained from Toxin Technology (Sarasota, FL). Mouse IgG1 and histopaque 1083 were purchased from Sigma-Aldrich (St. Louis, MO). HA was a kind gift from Dr. J. Skehel (National Insitute of Medical Research, Mill Hill, U.K.), Peptides derived from the amino acid sequence of HA-spanning residues 307–319 and from the amino acid sequence of TT-spanning residues 1273–1284, were synthesized commercially.

Eosinophils purification

Eosinophils were purified as described previously (31) with slight modifications. Briefly, 60–120 ml of heparinized blood was obtained from normal volunteers. The blood was diluted 1/1 with PBS, and granulocyte preparations were obtained by density centrifugation on Ficoll with a density of 1.083. RBC were lysed twice with cold distilled water for 30 s. Granulocyte pellets were incubated with anti-CD16 mAb on ice for 30 min. The cells were washed to remove excess Ab and incubated with Biomag goat anti-mouse Ig at a ratio of 50 beads per target cell at 4°C for 45 min. Eosinophils were negatively selected by applying a magnetic field and collecting the medium. Cells were washed and May-Grunwald staining was done to verify purity. Preparations had uniformly >98% purity and contained no detectable $CD14^+$ cells by FACS analysis (data not shown). Furthermore, RT-PCR analysis of eosinophil populations revealed no detectable mRNA for the monocyte surface marker CD14 (data not shown). Eosinophils were suspended in RPMI 1640 with 10% FCS and 1% penicillin/streptomycin (complete medium) and were cultured with rIL-3 and in some experiments with rIL-5 and rGM-CSF in 24-well plates at a concentration of 10^6/ml. In some cases, 1–1.5 $\times$ 10^5 eosinophils were cultured directly in 96-well microtiter plates.

Immunostaining

Cells (0.5–1.0 $\times$ 10^6) were washed and suspended in 100 μl of staining buffer containing 2% FCS and 0.01% sodium azide. Cells were incubated for 10 min with mouse IgG to block FcγR, then PE-conjugated mAbs to B7.1 or B7.2, or with FITC-conjugated mAb to MHC class II were added. The appropriate PE- and FITC-conjugated isotype controls were used as controls. After 30 min of incubation in the dark at 4°C, the cells were washed twice with staining buffer, suspended in PBS, and analyzed immediately by FACS.

Preparation of purified T cells and monocytes

Autologous resting T cells were prepared by E-rosetting and double complement lysis. Briefly, 60–120 $\times$ 10^6 PBMCs were incubated overnight with AET-treated SRBC. PBMCs were then resuspended and centrifuged on Ficoll. The T cell pellet was lysed with ice-cold distilled water for 30 s and washed three times with Hanks before adherence to a petri dish for 1 h. Nonadherent cells were placed in a new petri dish overnight then subjected to two cycles of complement lysis by incubation with anti-HLA-DR and OKM1 mAbs on ice for 45 min followed by the addition of rabbit complement at 37°C for 30 min. After the second lysis, the cells were washed three times with Hanks and suspended in complete medium at 10^6/ml. Purified T cell populations contained >99% $CD3^+$ cells and completely failed to proliferate to PHA at concentrations that were optimal for the proliferation of PBMCs. Monocytes were prepared by adherence to plastic as previously described and were irradiated with 2500 rad before use.

Proliferation to TSST-1 and TT Ag

Autologous T cells (1 $\times$ 10^5) were added to 1 $\times$ 10^5 eosinophils which have been cultured for 48 h in 96-well flat-bottom plates with 20 ng/ml rIL-3. TSST-1 (1 μg/ml) or TT (10 μg/ml) were added and the cells were cultured for 4 days for TSST-1 or 6 days for TT, then were pulsed with 1 μCi (37 kBq) of [*methyl*-^{3}H]thymidine for 12 h and harvested onto filters, and radioactivity was measured in a scintillation counter.

Proliferation of TT- and HA-specific T cell clones

The HA-reactive clone HA1.7 recognizes amino acid residues 307–319 in association with HLA-DR1 (32). The TT-reactive clone F6 recognizes amino acid residues 1273–1284 in association with HLA-DR3 (Ref. 33 and our unpublished observations). T cell clones (2 $\times$ 10^4 cells/well) were cultured with an equal number of irradiated (2500 rad) monocytes or IL-3-treated eosinophils derived from donors matched for HLA-DR Ags in the presence or absence of Ag or peptide. Proliferation was assessed after 3 days by [^{3}H]thymidine incorporation.

Results

IL-3 induces B7.2 expression by peripheral human blood eosinophils

Populations of highly pure eosinophils (>98%) freshly isolated from the blood of normal subjects expressed no detectable B7.1, B7.2 or HLA-DR molecules on their surface as assessed by FACS analysis (Fig. 1). Following culture with 20 ng/ml IL-3, surface B7.2 expression became consistently detectable at 48 h, and further increased at 72 h (Fig. 1). The mean $\pm$ SD percentage of $B7.2^+$ cells was 15 $\pm$ 3% at 48 h and 23 $\pm$ 8% at 72 h (n = 7). At both of these time points, the viability of eosinophils as assessed by trypan blue exceeded 90%. In contrast to its induction of B7.2, IL-3 failed to induce expression of B7.1 on eosinophils (<1% $B7.1^+$ cells at all time points). As previously reported (14), IL-3 induced HLA-DR expression on eosinophils by 24 h, with vigorous expression at 48 and 72 h with 60 $\pm$ 13% and 70 $\pm$ 13% $HLA\text{-}DR^+$ cells respectively (n = 7).

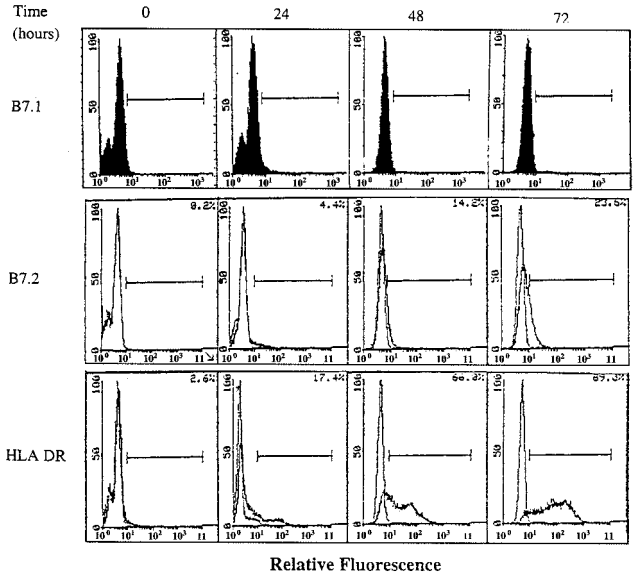

FIGURE 1. Surface expression of HLA-DR and B7.2 on peripheral blood eosinophils by direct immunofluorescence. Purified human blood eosinophils (>98% pure) were cultured in medium containing IL-3 (20 ng/ml) for 72 h. At 0, 24, 48 and 72 h, the cells were analyzed by FACS for expression of B7.1, B7.2 and HLA-DR. The experiment shown is representative of seven experiments performed on eosinophils from seven different donors.

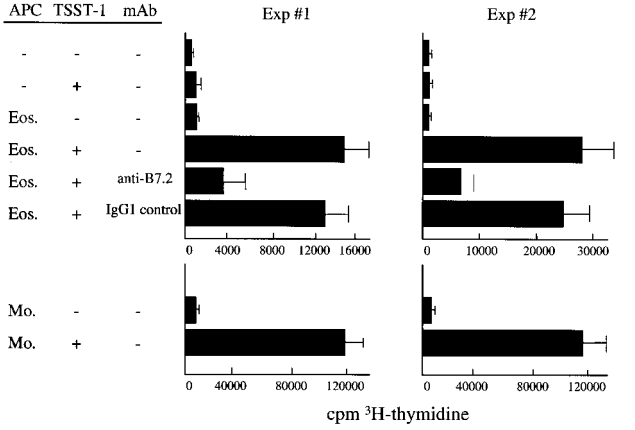

FIGURE 2. IL-3-treated eosinophils support T cell proliferation to the superantigen TSST-1 in a B7.2 dependent manner. Highly purified 1×10^5 eosinophils were cultured in 96-well microtiter plates for 48 h in medium containing IL-3 (20 ng/ml). Subsequently, an equal number of autologous purified resting T cells was added with or without TSST-1 (1 μg/ml) and the cultures were assessed for proliferation 4 days later. Parallel cultures contained equal numbers of T cells with or without addition of 2.5×10^4 irradiated (2500 rad) autologous. In blocking experiments, cultures received 10 μg/ml anti-B7.2 or mouse IgG1 before the addition of T cells. All cultures were run in triplicates. The bars represent the mean and SDs of triplicate cultures.

IL-3-stimulated eosinophils support T cell proliferation to superantigens and deliver costimulatory signals to T cells via B7.2

Superantigens directly bind to MHC class II molecules and are recognized by the TCR in a Vβ-restricted fashion with no requirement for processing (34–37). T cell proliferation to superantigens requires, in addition to cross-linking of the TCR, the costimulatory molecule CD28 (38, 39). Because IL-3-stimulated eosinophils express both MHC class II molecules and the CD28 ligand B7.2, their capacity to induce T cell proliferation to superantigens was examined. Highly purified T cells, depleted of monocytes and of MHC class II-positive cells by treatment twice with OKM1 and anti-MHC class II mAb plus complement, did not proliferate to TSST-1 (Fig. 2). Addition of autologous irradiated monocytes resulted in vigorous T cell proliferation to TSST-1. Eosinophils failed to support the proliferation of T cells to TSST-1. In contrast, IL-3-treated eosinophils supported the proliferation of autologous T cells to TSST-1, albeit to a lesser extent than autologous monocytes (Fig. 2).

To examine the role of B7.2 in the ability of IL-3-stimulated eosinophils to support T cell proliferation to superantigen, the effect of blocking B7.2-CD28 interactions on eosinophil presentation of TSST-1 to T cells was assessed. Addition of mAb to B7.2, but not of mouse IgG1 isotype control, significantly inhibited T cell proliferation to TSST-1 presented by IL-3-treated eosinophils (Fig. 2). The mean ± SD percent inhibition was 60 ± 17% (n = 3) Thus, IL-3-treated eosinophils express functional B7.2 molecules important in the induction of T cell proliferation to TSST-1 by IL-3-treated eosinophils.

IL-3-stimulated eosinophils fail to support the proliferation of purified autologous T cells to TT Ag

T cells recognize antigenic peptides, which are processed by APCs and presented in the context of MHC molecules. To determine whether IL-3-stimulated eosinophils are competent in Ag processing, their capacity to present TT Ag to highly purified autologous T cells was examined. These highly purified T cell populations contained >99% CD3$^+$ cells and completely failed to proliferate to PHA at concentrations that were optimal for the proliferation of PBMCs (data not shown). Highly purified T cells did not proliferate to TT Ag (Table I). Addition of 10% irradiated autologous monocytes supported vigorous T cell proliferation to TT. In contrast, in seven experiments from seven different donors, neither untreated eosinophils (data not shown), nor IL-3-treated eosinophils (Table I), were capable of supporting the proliferation of autologous T cells to TT. The failure of IL-3-treated eosinophils to support T cell proliferation to TT Ag was not specific to TT, but was also observed with diphtheria Ag (data not shown).

Table I. *IL-3-treated eosinophils fail to support T cell proliferation to TT Ag*[a]

Costimulator	TT	cpm [^{3}H]Thymidine Expt. 1	Expt. 2
Medium	−	2,817 ± 424	1,215 ± 364
Medium	+	1,931 ± 189	1,480 ± 155
Monocytes	−	1,441 ± 498	2,132 ± 340
Monocytes	+	82,089 ± 7,750	58,620 ± 3,349
Eos	−	2,401 ± 459	2,005 ± 217
Eos	+	2,713 ± 524	1,920 ± 320
Monos + Eos	−	1,887 ± 256	2,420 ± 621
Monos + Eos	+	78,554 ± 6,120	55,080 ± 3,285
Eos + anti-CD28	−	2,337 ± 578	2,411 ± 428
Eos + anti-CD28	+	2,202 ± 1,557	2,705 ± 213
Eos + IL-2	−	608 ± 33	2,098 ± 167
Eos + IL-2	+	934 ± 52	2,561 ± 322
IL-2	−	604 ± 55	2,564 ± 67
IL-2	+	642 ± 96	2,145 ± 218

[a] Highly purified T cells were incubated for 6 days with autologous IL-3-activated eosinophils, autologous monocytes irradiated with 2500 rad, or both. Cultures were stimulated with 10 μg/ml TT and harvested 6 days later. Anti-CD28 mAb was added at 10 μg/ml. The results represent the mean ± SD of triplicate cultures. Similar results were obtained in five other experiments. Eos, eosinophils; Monos, monocytes.

Several alternative possibilities that may account for the failure of IL-3-treated eosinophils to present Ag to T cells were examined and ruled out. First, do eosinophils release factors that inhibit T cell proliferation to Ag? This was clearly not the case, because addition of IL-3-treated eosinophils to cultures containing T cells and monocytes did not inhibit T cell proliferation to TT (Table I). Next, the possibility that interactions between B7.2 on eosinophils and CD28 on T cells may not be sustained throughout the 6-day culture period because of poor eosinophil survival and/or down-regulation of B7.2 expression was considered. However, addition of anti-CD28 mAb did not reverse the inability of IL-3-treated eosinophils to support T cell proliferation to TT (Table I). Finally, we considered the possibility that IL-3-treated eosinophils may not support sufficient IL-2 production by the T cells to result in detectable proliferation. However, addition of recombinant IL-2 did not reverse the inability of IL-3-treated eosinophils to support T cell proliferation to TT (Table I). Taken together, these results suggest that IL-3-treated eosinophils are not competent in Ag processing.

IL-3-stimulated eosinophils support the proliferation of T cell clones to antigenic peptides

Eosinophils accumulate at sites of inflammation where antigenic peptides may be processed and released by other cells. Because IL-3-treated eosinophils express both HLA-DR and B7.2 they may be able to present antigenic peptides to T cells. To examine this possibility, an HLA-DR3-restricted TT peptide 1273–1284-specific T cell clone, F6, and an HLA-DR1-restricted HA peptide

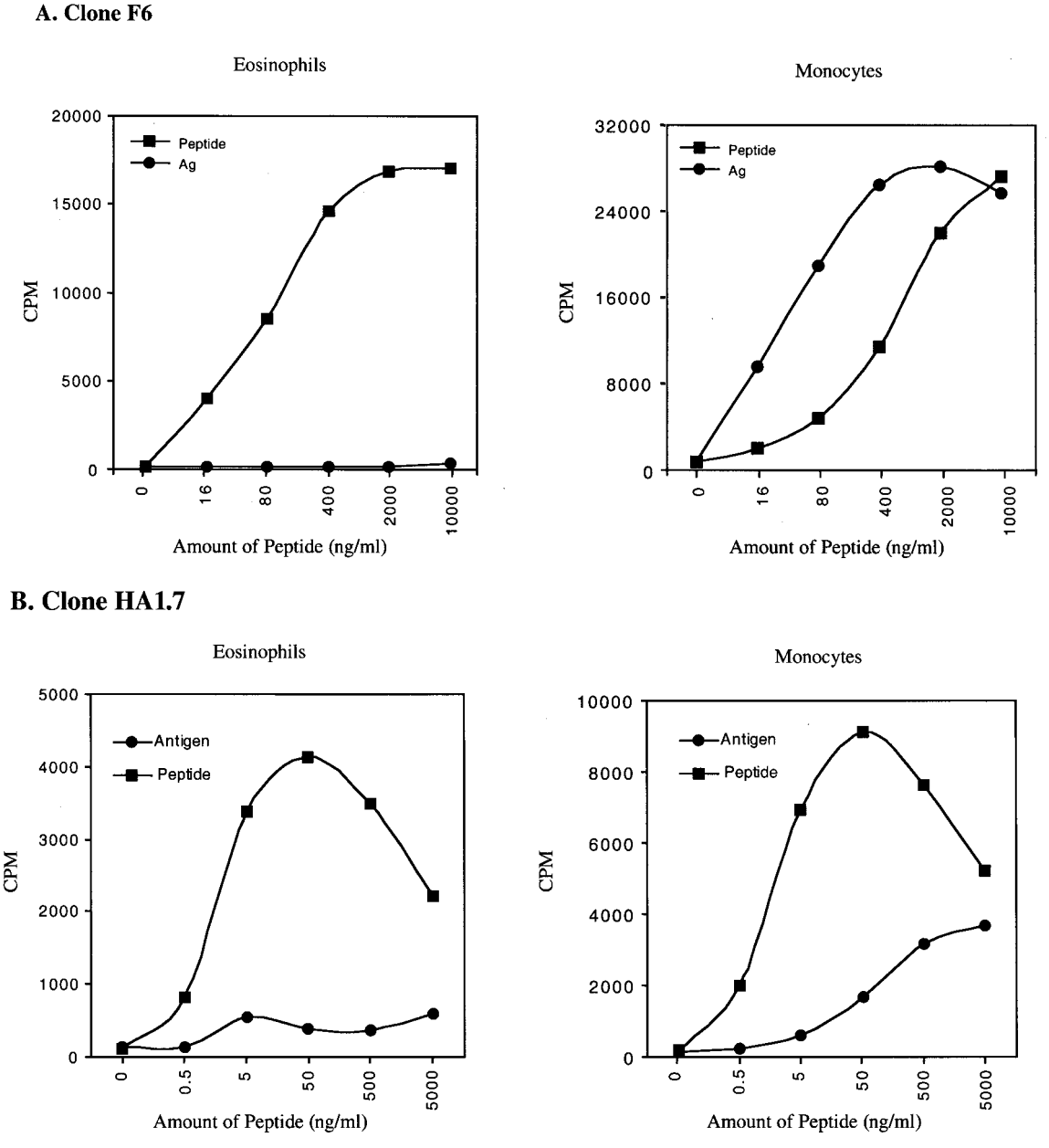

FIGURE 3. IL-3-treated eosinophils present Ag-derived peptides to T cell clones. The TT-specific clone F6 (*A*) and the influenza HA-specific clone HA1.7 (*B*) were cultured with equal numbers of eosinophils or irradiated monocytes derived from HLA-DR matched donors (HLA-DR1 for HA.7 and HLA-DR3 for F6). Cultures were stimulated with either Ag or Ag-derived peptides and assessed for proliferation 4 days later. Similar results were obtained in two other experiments.

307–319-specific T cell clone, HA1.7 were used. Proliferation of these clones to intact TT or HA requires Ag processing; fixation of monocytes with paraformaldehyde abolishes their capacity to support the proliferation of the clones to native Ag. In contrast, recognition of the peptides by the clones is not dependent on Ag processing because the clones proliferate to the appropriate peptides in the presence of monocytes fixed with paraformaldehyde (Ref. 32 and data not shown).

The clones were cultured in the presence of irradiated monocytes or IL-3-treated eosinophils derived from donors who expressed the appropriate restricting HLA-DR Ag and stimulated for 72 h with native Ag or peptide. Both clones proliferated to native Ag as well as to Ag-derived peptides in the presence of monocytes bearing the appropriate HLA-DR Ags (Fig. 3). As in the case of freshly isolated T cells, T cell clones failed to proliferate to native Ag in the presence of IL-3-treated eosinophils. However, IL-3-treated eosinophils were competent in supporting the proliferation of the clones to the appropriate peptides, albeit to a slightly lesser extent than monocytes derived from the same donors. These results strongly support the notion that eosinophils are defective in Ag processing, but can effectively load processed antigenic peptides onto their surface MHC class II molecules and present them to the T cells.

Finally, mAb to B7.2, but not mouse IgG1 isotype control, significantly inhibited the proliferation of clone F6 to TT peptide 1273–1284 presented by monocytes as well as by IL-3-treated eosinophils (Fig. 4). Mean inhibition was 56 + 15% (n = 3). Thus, B7.2 plays an important costimulatory role in the presentation of antigenic peptides by activated eosinophils.

Effect of IL-3 concentration on accessory function of eosinophils

IL-3 may be released at sites of inflammation from a number of potential sources that include T cells, mast cells, and others (29, 40). The concentrations of IL-3 achieved in tissues are not known, with the exception of nasal fluids where concentrations of up to 20 pg/mg tissue were measured (19). The dose effect of IL-3 on the accessory function of eosinophils was examined. Treatment of eosinophils with concentrations of IL-3 as low as 20 pg/ml enabled them to present superantigen to autologous T cells and TT peptide to clone F6 (Fig. 5). These results that IL-3 induces accessory function in eosinophils at concentrations that may be achieved in vivo.

IL-5- and GM-CSF-stimulated eosinophils support the proliferation of T cell clones to antigenic peptides, but not to native Ag

There is conflicting data regarding the capacity of IL-5 and GM-CSF to enable eosinophils to present native Ag to T cells (15, 17, 21, 22). We examined in our system in parallel the effect of IL-3, IL-5, and GM-CSF on the Ag presenting capacity of eosinophils. Treatment with IL-5 or GM-CSF rendered eosinophils capable of presenting superantigen to resting T cells and TT peptides to T cell clones to a degree comparable to that observed with eosinophils treated with IL-3 (Fig. 6, *A* and *B*, *left panels*). In contrast, eosinophils treated with IL-5 or GM-CSF, like IL-3-treated eosinophils, remained incapable of presenting native TT Ag to either resting T cells or T cell clones (Fig. 6, *A* and *B*, *right panels*). These results suggest that eosinophils have a defect in Ag processing, which is not overcome by cytokines that signal via the βc.

Discussion

The results of the present study show that IL-3 induces MHC class II and B7.2 expression on eosinophils and renders them capable of supporting T cell proliferation to superantigen and Ag-derived peptides. However, neither IL-3 nor the cytokines IL-5 and GM-CSF that signal via the βc were able to present native Ag to T cells.

Stimulation of freshly isolated eosinophils with IL-3 induced the expression of B7.2, but not expression of B7.1 (Fig. 1). Similarly, IL-10 has been shown to differentially regulate B7.1 and B7.2 expression on dendritic cells (41). These observations suggest that expression of B7.1 and B7.2 is differentially controlled. Eosinophils activated in vivo can also express B7.2, but not B7.1 (19), suggesting that the differential expression of these two costimulatory molecules on eosinophils is physiologically relevant. It has been suggested that B7.1/CD80 acts preferentially as a costimulator for the generation of Th1 cells while B7.2 costimulates and induces Th2 cells (42–44). Thus, it is possible that eosinophils activated by IL-3 derived from T cells or other cells at sites of allergic inflammation participate in further skewing of the Th response toward Th2.

IL-3-treated eosinophils, but not untreated eosinophils, were able to present the superantigen TSST-1 to T cells (Fig. 2). It is unlikely that this was due to contaminating monocytes. Eosinophil preparations were uniformly >98% pure as demonstrated by May-Grunwald staining and contained no detectable $CD14^+$ cells by FACS analysis and no detectable CD14 mRNA by RT-PCR (data not shown). Furthermore, superantigen presentation by APCs to T cells requires binding of superantigen to MHC class II molecules (35) and the delivery of costimulatory signals to the T cell which include B7-CD28 interactions (38, 39, 45). Presentation of TSST-1 by IL-3-treated eosinophils is consistent with the induction by IL-3 of HLA-DR and B7.2 expression on these cells. A role for B7.2 in superantigen presentation by IL-3-treated eosinophils was revealed by the observation that mAb to B7.2 significantly inhibited, by ~60%, the capacity of eosinophils to present TSST-1 to T cells.

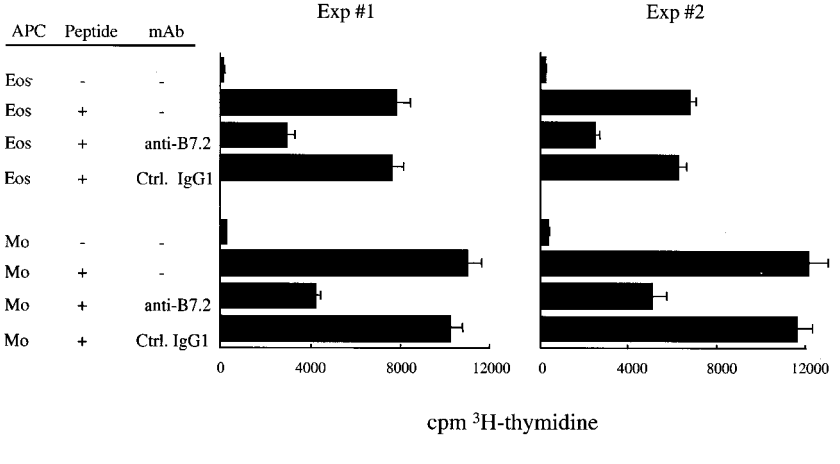

FIGURE 4. Peptide presentation by IL-3 activated eosinophils is dependent on B7.2. IL-3 activated eosinophils and monocytes from an HLA-$DR3^+$ donor were used to present TT peptide 1273–1284 to clone F6 in the presence or absence of 10 μg/ml anti-B7.2 or mouse IgG1. The bars represent the mean and SDs of triplicate cultures.

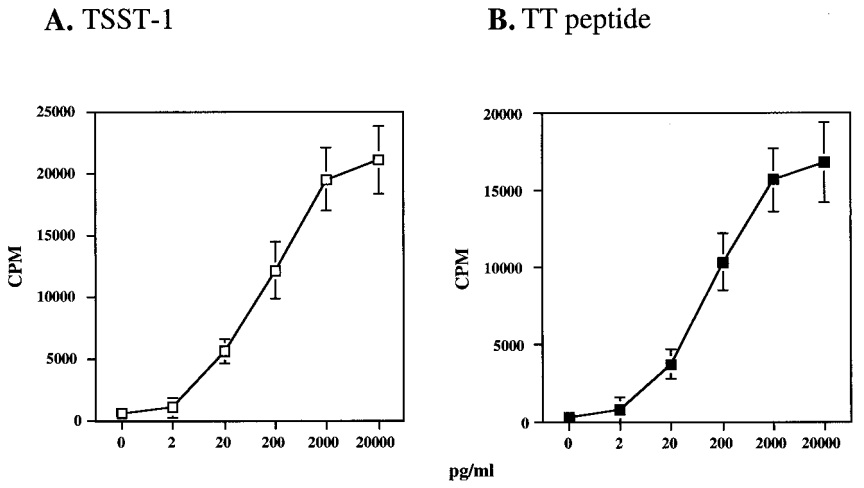

FIGURE 5. Dose-response curve of IL-3 induction of accessory function in eosinophils. Eosinophils activated with various concentrations of IL-3 were used to support the proliferation of autologous T cells to superantigen TSST-1 (*A*) and of HLA-DR3 matched clone F6 to TT peptide 1273–1284 (*B*). The bars represent the mean and SDs of triplicate cultures.

This suggests that B7.2 plays an important role in the costimulatory activity of IL-3-treated eosinophils. It is also consistent with the previously demonstrated role of B7 molecules in superantigen presentation by GM-CSF-treated eosinophils (21). The fact that the inhibition was not complete suggests the presence of other costimulatory molecules. These may include ICAM-1, which is induced on eosinophils by IL-3 (46) and plays a role in superantigen presentation (47).

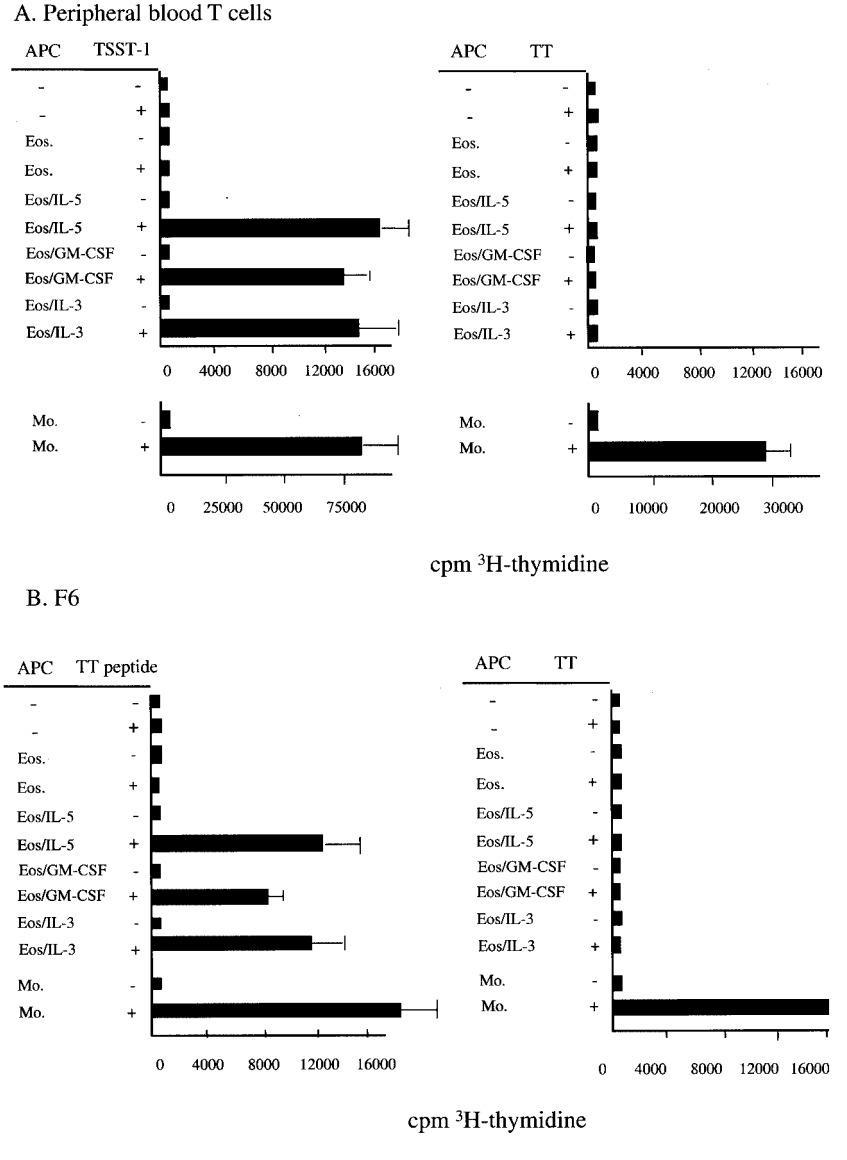

FIGURE 6. IL-5 and GM-CSF activated eosinophils present TT peptides, but not native TT Ag to T cells. *A*, IL-5, GM-CSF and IL-3 activated eosinophils and monocytes were used to present TSST-1 superantigen and TT Ag to autologous T cells. *B*, IL-5, GM-CSF and IL-3 activated eosinophils and monocytes from an HLA-DR3^{+} donor were used to present TT peptide 1273–1284 and native TT Ag to clone F6. The bars represent the mean and SDs of triplicate cultures.

Although IL-3-treated eosinophils expressed both HLA-DR and B7.2, they remained unable to present native TT Ag to T cells (Table I). Because the fraction of T cells that recognize TT Ag is much smaller than the fraction of T cells that recognize TSST-1, it is possible that IL-3-treated eosinophils provide less than the optimal costimulatory signals required for IL-2 synthesis and T cell proliferation to TT. However, neither ligation of CD28 on T cells nor addition of IL-2 overcame the failure of IL-3-treated eosinophils in supporting T cell proliferation to TT. This suggests that IL-3-treated eosinophils are not competent in processing Ag. This was directly demonstrated using T cell clones that recognize well defined antigenic peptides. IL-3-treated eosinophils supported the proliferation of TT as well as HA-specific T cell clones to the appropriate peptides, but not to native TT or HA Ags. Furthermore, B7.2 on IL-3-treated eosinophils played an important role in delivering the costimulatory signal to T cell clones, because mAb to B7.2 significantly inhibited the proliferation of TT-specific T cells to TT-derived peptides presented by IL-3-treated eosinophils.

We were unable to detect presentation of native TT Ag by IL-5- or GM-CSF-treated eosinophils, to purified T cells although the same eosinophils presented superantigen successfully to the same T cells, and more importantly, presented TT peptide to T cell clones (Fig. 6). This suggests that eosinophils are deficient in their capacity to process Ag and present it to T cells. This deficiency could result from rapid degradation of endocytosed Ag in the granules of these cells. Some previous studies, but not others, have shown that GM-CSF- and IL-5-treated eosinophils can present native Ag to T cells (15, 17, 21, 22). In these studies, the magnitude of T cell proliferation to Ag in the presence of eosinophils is quite modest compared with that in the presence of monocytes as APC. The reason for the discrepant results may be related to the degree of T cell purity achieved. In our experiments we used T cells that were rigorously depleted of APCs by the strict criterion of failure to proliferate to doses of PHA that cause optimal proliferation of PBMCs.

Recently, eosinophils recovered from BAL fluid of OVA-sensitized mice following Ag challenge with OVA were found to express MHC class II and B7 molecules and to support the proliferation of OVA sensitized T cells (20). This may be explained in part by the presence on these activated eosinophils of already processed Ag, as suggested by their ability to induce the proliferation of OVA sensitized T cells in the absence of added OVA. This processed Ag may have been derived from BAL fluid macrophages, Nevertheless, addition of native OVA to the cultures enhanced T cell proliferation suggesting that the in vivo activated eosinophils were also capable of Ag processing. It is possible that stimuli other than cytokines that signal via the βc may confer Ag processing capacity on eosinophils.

The concentrations of IL-3 measured in nasal polyps are in the range of 20 pg/mg (30). At this concentration IL-3 caused a measurable, albeit modest, induction of accessory function in eosinophils (Fig. 5). The effective concentrations of IL-3 at sites of contact between activated T cells and eosinophils in allergic and parasitic inflammation are unknown and may well exceed the concentrations measured in nasal polyps. Thus, it is likely that accessory cell function could be induced in eosinophils by concentrations of IL-3 that may be achieved in vivo. The skin of patients with atopic dermatitis, the parenchyma of the lung of patients with asthma and the sites of invasion of a number of parasites are infiltrated with T cells and eosinophils (48–50). Ag-derived peptides can be generated at these sites. Furthermore, the skin of patients with atopic dermatitis is frequently colonized with bacteria that produce superantigens (51). Our findings suggest that eosinophil activated by IL-3 may contribute to T cell activation in allergic and parasitic diseases.

Acknowledgements

We thank Leslie Woods for help in the preparation of eosinophils and Dr. Peter Weller for reading and discussing the manuscript.

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