Immunology 1994 **81** 584–591

Human eosinophils as antigen-presenting cells: relative efficiency for superantigen- and antigen-induced CD4$^+$ T-cell proliferation

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SUMMARY

Human eosinophils become hypodense and express class II major histocompatibility (MHC) molecules when activated by granulocyte–macrophage colony-stimulating factor (GM-CSF) *in vitro* or *in vivo* in pathological conditions such as allergic disorders. In this study, we examined the capacity of class II MHC-expressing eosinophils to serve as antigen-presenting cells (APC) for resting and activated CD4$^+$ T cells. Eosinophils were isolated from healthy donors and incubated in conditioned medium (CM) containing GM-CSF for 2–4 days, after which 15–92% of the cells expressed class II MHC (HLA-DR). Preincubated eosinophils induced resting T cells to proliferate in response to the staphylococcal superantigens, *Staphylococcus* enterotoxins A, B and E. Furthermore, superantigen-induced T-cell proliferation correlated with the proportion of eosinophils expressing class II MHC molecules. When eosinophils and macrophages were compared for their ability to act as accessory cells for superantigen-induced T-cell proliferation, macrophages were more efficient than eosinophils. Eosinophils were not effective APC for microbial antigens (Ag), which required processing. Proliferative responses to purified protein derivative, tetanus toxoid, or *Brugia malayi* antigen were observed in only three of nine studies. The three positive studies included activated CD4$^+$ T cells, whereas no responses were observed with resting CD4$^+$ T cells. Macrophages and mononuclear cells were effective APC for these Ag for both resting and activated CD4$^+$ T cells. These data indicate that although class II MHC-expressing eosinophils can serve as APC, they are relatively inefficient for the activation of CD4$^+$ T cells by Ag, which require processing.

INTRODUCTION

Blood and tissue eosinophilia characterize the haematological responses in allergic disorders and infections with tissue-invasive metazoan parasites.[1,2] Because of their origin in the myelocytic cell series and biochemical similarities to neutrophils, many studies of the functional role of eosinophils have been directed at their activities in the effector limb of the immune response.[3–6] Eosinophils may contribute to the pathophysiology of allergic disorders by the release of potent lipid mediators of cell–cell adherence and smooth muscle contraction, such as platelet-activating factor and leukotriene C_4.[2,7] In addition, *in vitro* studies indicate that eosinophils kill multicellular helminths by a variety of mechanisms,[6,8] although their role as effectors of host defence against these organisms has been challenged by observations that depletion of eosinophils *in vivo* fails to alter worm burdens in mice infected with *Schistosoma mansoni* or *Trichinella spiralis*.[9,10]

Several biological features of human eosinophils suggest they may also perform functions in the afferent limb of the immune response. Eosinophils are long-lived cells following release from the bone marrow.[11] The majority of mature eosinophils are distributed throughout the skin and mucosae of the gastrointestinal and respiratory tracts. Notably, the prevailing cytokine milieu in inflammatory parasitic and allergic lesions, which includes granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3) and IL-5, induces blood eosinophils to differentiate and become activated.[12,13] Activated eosinophils demonstrate decreased buoyant density, enhanced toxicity for helminths, and elevated production of oxyradicals and leukotrienes.[14,15] Tissue eosinophils isolated from the airways or peritoneal fluid show the activated phenotype.[15,16] The discovery that eosinophil viability *in vitro* is prolonged from less than 48 hr to 14 days by culture in conditioned medium (CM) from fibroblasts or endothelium,[17] or with GM-CSF, IL-3 or IL-5,[18] has permitted more detailed investigations into their function as immunoregulatory cells. Peripheral blood eosinophils from normal donors incubated in this manner,[19] or tissue eosinophils obtained from patients,[15,16]

Received 13 August 1993; revised 1 November 1993; accepted 4 November 1993.

Abbreviations: APC, antigen-presenting cell; CM, conditioned medium; GM-CSF, granulocyte–macrophage colony-stimulating factor; MHC, major histocompatibility complex molecules; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells; PPD, purified protein derivative of *Mycobacterium tuberculosis*; SEA, SEB, SEE, *Staphylococcus aureus* enterotoxins A, B, and E; TcR, T-cell receptor; TT, tetanus toxoid.

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express on their surface class II major histocompatibility complex (MHC) and adhesion molecules involved in T-cell activation (e.g. ICAM-1).[15,20] In this regard, Hansel *et al.*[20] demonstrated that human eosinophils can serve as antigen-presenting cells (APC) for a CD4+ T-cell clone specific for the bee venom antigen (Ag) phospholipase A2. Del Pozo *et al.*[20] observed a similar phenomenon using murine eosinophils and T-cell clones specific for *Mesocestoides corti* Ag. It is not known if the putative Ag presenting function of eosinophils is an *in vitro* phenomenon limited to selected CD4+ T-cell clones. Furthermore, it has not been established whether interaction between eosinophils and CD4+ cells is restricted to allergens and helminthic Ag or if the level of class II MHC expression correlates with the ability of these cells to serve as APC. Of particular relevance to the immunological significance of these observations is the efficiency of the human eosinophil APC function relative to 'professional' APC such as the monocyte/macrophage.

In the current study, we describe the capacity of class II MHC-expressing human eosinophils to serve as APC for resting and activated CD4+ T cells, and examine their efficiency in these activities relative to monocyte/macrophages. The interaction of eosinophils and CD4+ T cells was evaluated in two ways. First, the proliferative response to *staphylococcus aureus* enterotoxins was assessed. These superantigens do not require processing by APC, stimulate a large proportion of T cells by cross-linking class II MHC and certain Vβ T-cell receptors (TcR), and have limited co-stimulatory requirements for T-cell activation.[22-25] Second, we examined responses to microbial Ag, including *Mycobacterium tuberculosis* purified protein derivative (PPD), tetanus toxoid (TT) and *Brugia malayi* Ag. These Ag require the APC to internalize and degrade protein to peptides for insertion in the groove of the class II MHC molecule, and co-stimulatory signals for CD4+ T-cell activation.[26-28]

MATERIALS AND METHODS

Antibodies, cytokines and antigens

OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8), OKM1 (anti-human monocyte/granulocyte CR3 complement receptor) and OKDR and OKIa1 (anti-HLA-DR) antibodies were purchased from Ortho Diagnostics (Raritan, NJ). The hybridomas for L243 (anti-HLA-DR), 9.3F10 (anti-HLA-DR) and W6/32 (anti-HLA-A,B,C framework determinant) monoclonal antibodies (mAb) were purchased from ATCC (Rockville, MD) and used to make ascites. 84H10 (anti-ICAM-1) and AICD-58 (anti-LFA-3) were purchased from AMAC (Westbrook, ME). TS1/18 (anti-LFA-1) was kindly provided by Dr R. Rothlein (Boehringer Ingelheim, Ridgefield, CT). BB1 (anti-B7) was kindly provided by Dr E. Clark, University of Washington, Seattle, WA. Human recombinant GM-CSF was purchased from Genzyme (Cambridge, MA).

PPD from *M. tuberculosis* was a gift from Lederle laboratories (Pearl River, NY). TT was purchased from the Massachusetts State Laboratory Institute (Jamaica Plain, MA). *Brugia malayi* Ag was a generous gift from Dr C. King at Case Western Reserve University and prepared as described elsewhere.[29] The *S. aureus* enterotoxins A, B and E (SEA, SEB and SEE, respectively) were purchased from Toxin Technology (Sarasota, FL).

Isolation and in vitro *culture of eosinophils*

Five asymptomatic donors, prescreened for consistency of eosinophil yield, were evaluated for class II MHC molecule expression on eosinophilsm after *in vitro* culture as described below. Isolation of eosinophils was performed according to Koenderman *et al.*[30] Peripheral blood was separated by density centrifugation into a mononuclear cell (PBMC) interface and a pellet containing neutrophils, eosinophils, and red cells. The pellet was resuspended in 3% dextran T-500 (Sigma, St Louis, MO) in phosphate-buffered saline (PBS; pH 7·4) and allowed to settle by sedimentation. Red cells were lysed with three volumes of H_2O and remaining cells rescued with 1 volume of 3·6% saline. Neutrophils and eosinophils ($50–75 \times 10^6$/cells/ml) were incubated with 1×10^{-8} M N-formyl-met-leu-phe (Sigma) for 10 min at 37° before washing and resuspension in enriched medium (RPMI-1640 supplemented with 10% v/v fetal calf serum, 2 mM L-glutamine, 1 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin, 0·1 mM non-essential amino acids and 1 mM sodium pyruvate). Cells were placed on a two-layer gradient of 71% and 80% isotonic Percoll (Pharmacia, Uppsala, Sweden), and spun at 1000 *g* for 18 min. Eosinophils were removed from the lower interface and washed twice in RPMI-1640 before resuspension in enriched medium. These preparations contained 90–99% eosinophils, with neutrophils representing the major contaminating cells. Mononuclear cell contamination (<0·4%) was evaluated by non-specific esterase staining, staining for CD3 by indirect immunofluorescence (FACS, see below), and microscopic inspection of $\geqslant$1000 cells per cytospin preparation stained with Wright's Giemsa.

To induce class II MHC expression, purified eosinophils ($0·5–1 \times 10^6$/ml) were suspended in medium containing 50% (v/v) (CM from bovine pulmonary artery endothelial cell monolayers (ATCC no. CCL 209), as described by Rothenberg *et al.*[17] In some experiments, eosinophils were incubated in medium supplemented with 100 U/ml GM-CSF. This cytokine induced the same amount of class II MHC expression as 50% CM. Eosinophils used in APC experiments were maintained in 50% CM or 100 U/ml GM-CSF for 1–7 days. Their viability was >90% as measured by trypan blue exclusion and propidium iodide staining. The preparations contained $\geqslant$99% eosinophils.

Preparation of resting and activated CD4+ T cells

To prepare resting T cells, PBMC were isolated, monocytes removed by adherence, and non-adherent cells passed over a nylon-wool column (Fenwall Laboratories, Grove Park, IL) which had been preincubated at 37° in 10% fetal calf serum. Lymphocytes were eluted from the nylon-wool column after 30 min at 37°, and placed on a discontinuous gradient of 40%, 43·5%, 47% and 54% Percoll. Cells at the 47–54% interface were collected, washed and depleted of residual class II MHC-expressing cells by treatment with OKIa1 and rabbit complement (Pel-Freez, Brown Deer, WI). Remaining high-density lymphocytes were >90% T cells by FACS analysis and dependent on the addition of APC for proliferative responses to recall Ag and staphylococcal superantigens.

To purify resting CD4+ T cells, PBMC were first depleted of monocytes by plastic adherence. Cells were then incubated with saturating amounts of mAb to CD8 (OKT8), CD11 (60.1), CD20 (Leu-11), CD16 (Leu-M3) and CD14, washed, and goat anti-mouse immunoglobulin-coated magnetic particles added. Subsequent exposure to a strong magnetic field allowed for

negative selection of CD4+ T cells with greater than 98% purity based on FACS analysis with OKT4 mAb staining, as described elsewhere.[31]

Ag-specific CD4+ T-cell lines to PPD and TT were initiated and maintained as described by Boom *et al.*,[32] and were considered to be activated CD4+ T-cell populations. CD4+ T-cell lines were used 7–10 days after the last stimulation with Ag and APC.

Monocyte–macrophage preparation

PBMC were added to plastic tissue culture dishes (Falcon 3003; Oxnard, CA) precoated with pooled human serum. Non-adherent cells were removed after incubation for 1 hr at 37°. Plastic-adherent cells (consisting of more than 90% monocytes by Wright's, peroxidase and non-specific esterase staining) were collected by scraping with a rubber policeman, washed and counted. Monocytes were cultured in RPMI supplemented with 10% fetal calf serum, L-glutamine, penicillin and streptomycin for 24–48 hr and irradiated (3500 rads) before use in T-cell proliferation assays.

T-cell proliferation assays

T cells were co-cultured for 72–96 hr with irradiated monocyte/macrophages, PBMC or non-irradiated eosinophils (as APC), and superantigen or Ag. Eosinophils were maintained in 50% CM or 150 U/ml GM-CSF during the 72–96 hr proliferation assay. Cells were pulsed with 1 μCi [^{3}H]thymidine ([^{3}H]TdR; ICN, Costa Mesa, CA) for the final 18 hr and incorporation measured by liquid scintillation counting. Results are expressed as mean c.p.m. of duplicate or triplicate samples. Duplicates varied less than 10% of the mean. Triplicate results are presented with the mean and SD. Cell proliferation was considered significant when the ratio of c.p.m. obtained in the presence of Ag or superantigen was $\geqslant$ three times the c.p.m. in the absence of Ag or superantigen. Experiments in which the APC function of eosinophils for superantigen was measured were conducted with resting T cells. For Ag which required processing, both resting and activated CD4+ T cells were used. Since the maximal contamination of eosinophil preparations with mononuclear cells was $<1\%$, control studies to confirm T-cell responsiveness were performed with monocytes/macrophages added to purified T cells at 1:100 ratios. No T-cell proliferative responses to Ag and superantigens were observed in the absence of added APC (eosinophils or monocyte/macrophages). Furthermore, incubation of monocytes/macrophages in 50% CM or 150 U/ml GM-CSF for 2–4 days prior to use in APC experiments did not enhance APC function of these cells.

Phenotypic analysis

Purified eosinophils were harvested and stained by indirect immunofluorescence. Cells were incubated with a saturating amount of a primary mAb for 30 min, followed by FITC-conjugated goat F(ab')$_2$ anti-mouse IgG (Cappell, Malvern, PA) for 30 min. After washing, cells were analysed on a Cytofluorograph IIs instrument (Becton Dickinson, Mountain View, CA). A gate was set to correspond to live eosinophils based on forward and 90° light scatter patterns, previously validated by cell sorting. The gate for negative control cells stained with isotype-matched second antibody was set to be less than 5% of the extreme tail of a normal distribution. The experimental samples were recorded as the percentage positive cells beyond this gate. Initially, mAb L243, 9.3F10, OKIa1, and OKDR (all anti-HLA-DR) were used to examine class II MHC molecules on eosinophils, and found to give identical results. L243 was used in the studies described in this report. W6/32 (anti-HLA-A, B, and C) was used as a positive control. Eosinophil preparations were 90–100% positive for staining with mAb W6/32.

Cytotoxicity assays

Freshly isolated eosinophils were incubated for 18 hr in 50% CM with or without 20 μg/ml PPD prior to use as effector cells. Autologous cells from a PPD-specific CD4+ T-cell line were labelled for 1 hr with 100 μCi ^{51}Cr (New England Nuclear, Boston, MA). Eosinophils were added to T cells (10^4) at ratios of 1:1, 5:1, 10:1, and 20:1 (triplicate samples for each). After 4 hr incubation, 50 μl culture supernatants was harvested and ^{51}Cr release measured with a gamma counter. Spontaneous release (10–20% of the maximum) was measured in wells containing T cells and medium alone. Maximal release was determined by treating targets with 2% SDS. Percentage specific release was calculated using the following equation: $100 \times$ [(mean c.p.m. experimental release) − (mean c.p.m. spontaneous release)]/[(mean c.p.m. maximum release) − (mean c.p.m. spontaneous release)]. Ten per cent or greater specific release was considered significant cytotoxicity.

RESULTS

Variability and levels of eosinophil MHC class II

Freshly isolated eosinophils expressed no to low levels of class II MHC ($<15\%$). Incubation of eosinophils in CM resulted in a time-dependent up-regulation of class II MHC. Increased proportions of eosinophils had detectable class II MHC after 1 day (5–88%), with peak levels of 17–92% by 2–4 days, as shown for five donors in Fig. 1. These results confirm previous observations of Lucey *et al.*[19]

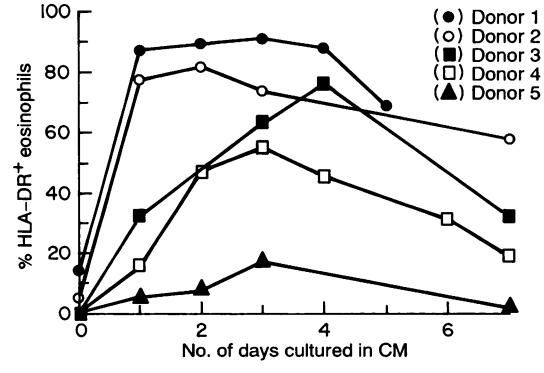

Figure 1. HLA-DR expression by *in vitro* activated eosinophils. Peripheral blood eosinophils were obtained from five healthy asymptomatic donors. Eosinophils were purified and cultured in 50% CM, as indicated in the Materials and Methods, for 0–7 days before measuring HLA-DR expression (mAb L243) by FACS analysis. Background staining with the IgG2a isotypic control was $<5\%$ and subtracted from the results. Cell viability was assessed by trypan blue exclusion and propidium iodide staining and was $>90\%$.

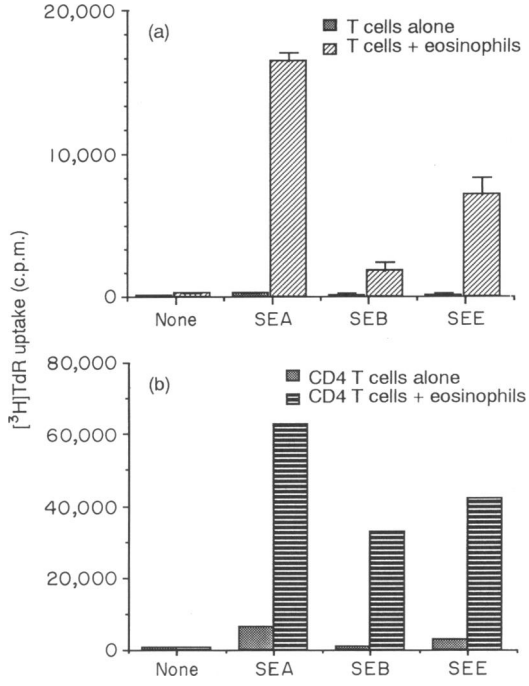

Figure 2. (a) Response of unselected resting T cells to staphylococcal superantigens presented by HLA-DR-bearing eosinophils. 7×10^4 autologous purified resting T cells were cultured alone, or with 7×10^4 HLA-DR-bearing eosinophils and 100 ng/ml of the indicated staphylococcal superantigen for 72 hr. Data are shown as the mean of triplicate cultures $\pm$SD. [^{3}H]TdR uptake by eosinophils alone with superantigen was 243 c.p.m. (b) Response of purified resting CD4$^+$ T cells to superantigens presented by HLA-DR-expressing eosinophils. Human peripheral blood T cells were purified by immunomagnetic negative selection resulting in a 98% pure population of CD4$^+$ T cells. 1×10^5 CD4$^+$ T cells were used alone or with 1×10^5 allogeneic cultured eosinophils and with 100 ng/ml of the indicated staphylococcal superantigen. The data are shown as the mean of duplicate cultures with the actual results varying less than 10% from the mean. [^{3}H]TdR uptake of eosinophils alone with superantigen was 232 c.p.m.

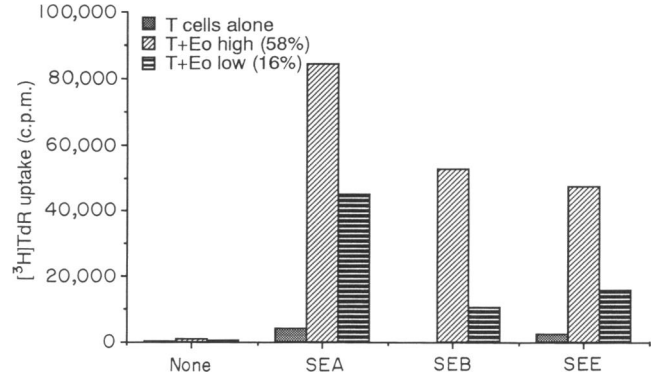

Figure 3. Correlation between the level of T-cell proliferation to superantigens with the amount of HLA-DR expression by eosinophils. 1×10^5 purified, resting T cells from a donor with HLA-DR haplotype (DR 3,4) were incubated with 8×10^4 eosinophils from two HLA-DR mismatched allogeneic donors. Donor 2 (Fig. 1) had HLA-DR haplotype DR w15, and the class II MHC expression on eosinophils was 58% in this experiment (Eo high). Donor 4 had the HLA-DR haplotype DR 7,w8 and low class II MHC expression at 16% (Eo low). Staphylococcal superantigens were added at 100 ng/ml. The data are presented as the mean of duplicate cultures. The actual results varied less than 15% from the mean. [^{3}H]TdR uptake by eosinophils alone with SEA and SEE was 727 and 344 c.p.m., respectively.

The magnitude of up-regulation of class II MHC on eosinophils was consistent for eosinophils from the same donor (< 20% variability at day 3 for cells from five individuals). The proportions of eosinophils expressing class II MHC for donor 4 described in Fig. 1 were 45·6%, 38·9%, 51·6%, 52·6% and 36·5% (mean $\pm$SD 45·1 $\pm$ 7·3%) after 48 hr in five experiments. After 72 hr in CM, class II MHC was detectable on 56·1%, 55·7% and 53·8% of eosinophils from the same donor in three experiments. Similar kinetics and peak levels of class II MHC were observed when eosinophils were incubated with GM-CSF rather than CM. Based on these results, eosinophils expressing consistently high levels of MHC class II ($\geq$24%) were used for APC experiments.

Eosinophils as APC for superantigen-induced proliferation of T cells

To assess the functional role of class II MHC expressed on the surface of eosinophils, we first evaluated the ability of these cells to serve as APC for the *S. aureus* superantigens SEA, SEB, and SEE. Freshly isolated autologous resting T cells were incubated with CM-maintained eosinophils (73·2% positive for HLA-DR), at a ratio of 1:1, and superantigen. When SEA, SEB or SEE (100 ng/ml optimal; 1–500 ng/ml range) were added, no proliferative responses were observed. In contrast, inclusion of class II MHC-expressing eosinophils with superantigen resulted in significant T-cell proliferation (stimulation indices for SEA, SEB, and SEE were 67·7, 10·2, and 46·4; Fig. 2a). T cells incubated with CM-activated eosinophils in the absence of superantigen did not proliferate ([^{3}H]TdR uptake 188 c.p.m.). Four experiments with similar results were performed.

To determine that eosinophils could stimulate resting CD4$^+$ T cells, the same experimental protocol was followed using freshly isolated, negatively selected CD4$^+$ cells (98% purity). As shown in Fig. 2b, the pattern of response was similar to that described for unselected T cells (stimulation indices for SEA, SEB and SEE of 15·2, 104 and 52, respectively).

Relationship between level of eosinophil class II MHC expression and superantigen-induced T-cell stimulation

The efficiency of Apc function for T-cell proliferation correlates with the amount of class II MHC expression.[33] Since superantigen-induced proliferation is class II MHC-dependent but not HLA-haplotype restricted,[22] we were able to evaluate superantigen-induced proliferation of resting T cells in the presence of eosinophils with different levels of class II MHC expression. Eosinophils from two donors (DR 7, w8, and DR w15/w15) with class II MHC expression of 58% and 16%, were cultured with resting T cells from an unrelated donor (DR 3,4). Eosinophils with higher class II MHC expression induced greater T-cell proliferation than their counterparts expressing less class II MHC (Fig. 3). Similar results were obtained using eosinophils obtained from two other donors.

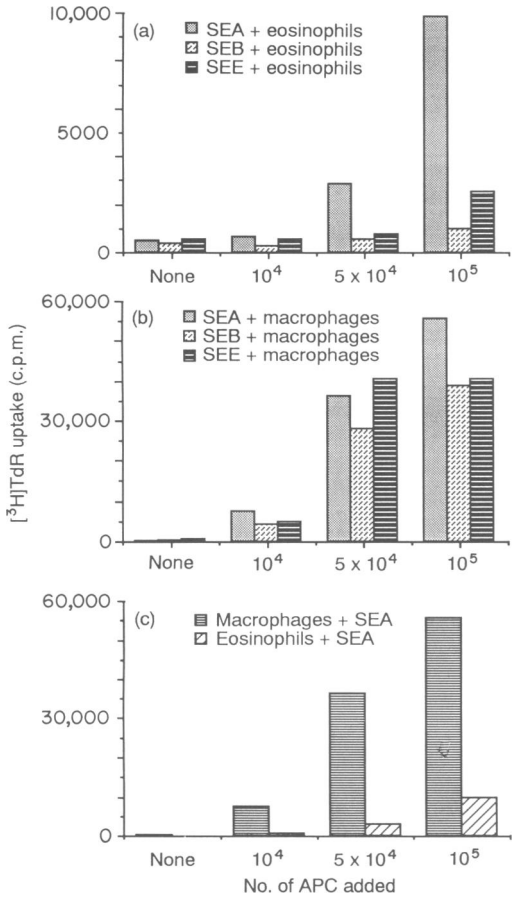

Figure 4. Efficiency of HLA-DR-expressing eosinophils as APC compared to monocyte/macrophages for superantigen. 1×10^5 autologous, resting T cells were incubated with SEA, SEB or SEE at 100 ng/ml and APC (eosinophils or macrophages) for 96 hr. Varying numbers of eosinophils expressing 87·1% HLA-DR (a), or monocyte/macrophages expressing 83·8% HLA-DR (b) were cultured with T cells and superantigens. In (c), a direct comparison of eosinophils and macrophages for SEA is shown. The results are expressed as the mean [^{3}H]TdR uptake. T cells alone with SEA had 483 c.p.m., 1×10^3 macrophages added to 10^5 resting T cells and SEA had 578 c.p.m., and eosinophils with SEA had 343 c.p.m.

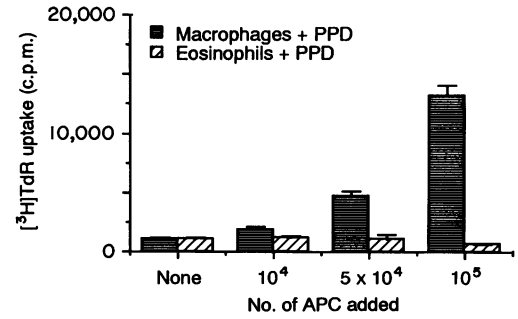

Figure 5. Relative efficiency of HLA-DR-expressing eosinophils and monocyte/macrophages as APC for PPD-specific CD4$^+$ T cells. 1×10^5 T cells from a PPD-responsive CD4$^+$ T-cell line was incubated with the same eosinophils and monocyte/macrophages used in the experiment described in Fig. 4 with or without PPD (10 μg/ml). The results are expressed as the mean [^{3}H]TdR uptake $\pm$ SD of triplicate cultures. T cells alone with PPD had 1087 c.p.m., monocyte/macrophages alone had 846 c.p.m., and eosinophils alone had 703 c.p.m.

Relative efficiencies of eosinophils and monocyte/macrophages in superantigen-induced T-cell proliferation

To examine the efficiencies of eosinophils and monocyte/macrophages as APC for resting T cells, increasing numbers of autologous eosinophils or monocyte/macrophages were added to a fixed number of T cells in the presence of superantigen. In the experiment described in Fig. 4, eosinophils and monocyte/macrophage preparations which were, respectively, 87·1% and 83·8% HLA-DR positive were co-incubated with 100 ng/ml SEA, SEB or SEE. When 10^4 eosinophils were used as APC (Fig. 4a), proliferative responses to SEA, SEB or SEE were not observed. SEA-induced T-cell proliferation occurred when the number of eosinophils was increased to 5×10^4. T-cell proliferation to all three superantigens developed when 10^5 eosinophils were included. When monocyte/macrophages were APC, proliferative responses were observed with as few as 10^4 cells (e.g. mean [^{3}H]TdR uptake $\geqslant$5000 c.p.m. for SEA, SEB or SEE versus $<$950 c.p.m. in the absence of monocytes/macrophages) (Fig. 4b). These values increased to $>$29,000–55,000 c.p.m. as monocyte/macrophages were increased to 5×10^4 or 10^5/well. In all cases, the magnitude of proliferation associated with eosinophil APC function was less than that with monocyte/macrophages. Figure 4c emphasizes the inefficiency of eosinophils relative to monocyte/macrophages as APC for SEA, which of all the superantigens tested consistently resulted in the greatest degree of T-cell stimulation when eosinophils were APC.

Supernatants from these cell cultures taken after 72 hr incubation were evaluated for interferon-γ (IFN-γ) production by ELISA. Only the highest number of eosinophils (10^5) in the presence of SEA and SEE resulted in IFN-γ production (1371 and 956 ng/ml for SEA and SEE, respectively). In contrast, 10^4 monocyte/macrophages induced T cells to produce IFN-γ (597 and 888 ng/ml for SEA and SEE).

Capacity of eosinophils to present Ag which require processing

We next evaluated the ability of eosinophils to present processed microbial antigens. Ag which require processing have different and more stringent co-stimulatory requirements, such as B7-CD28, for T-cell activation and proliferation.[28,31,34,35] In Fig. 5, eosinophils and monocyte/macrophages were compared for the presentation of PPD to a PPD-specific CD4$^+$ T-cell line. Both APC populations from this donor were able to present superantigen to resting CD4$^+$ T cells, as shown in Fig. 4. PPD-specific proliferative responses were not detected when eosinophils were used as APC. In contrast, monocyte/macrophages induced strong Ag-specific proliferation (stimulation indices of 8 and 22 with 5×10^4 and 10^5 monocyte/macrophages, respectively).

The inefficiency of class II MHC-bearing eosinophils to function as APC for protein Ag (PPD, TT and *B. malayi* Ag) was examined further in nine additional experiments (Table 1). When resting CD4$^+$ T cells were used (experiments 1–3), no proliferative responses were observed with eosinophils as APC. In all three experiments, eosinophils were functional for superantigen-induced T-cell proliferation ([^{3}H]TdR uptake 6902–24,758 c.p.m.).

When activated antigen-specific CD4$^+$ T-cells lines were used (experiments 4–9), significant proliferative responses to PPD were observed in three of six experiments (stimulation

Table 1. Inefficiency of activated eosinophils as APC for processed microbial antigens for resting and activated CD4+ T cells

Exp.	CD4+ T cell	Class II MHC on eosinophils (%)	Ag	[3H]TdR uptake (c.p.m.) No Ag	+Ag	+SuperAg
1	Resting	73·8	*B. malayi*	245	409	9329
2	Resting	77·4	TT	581	542	24,748
3	Resting	ND*	PPD	256	385	6902
4	Activated	ND	PPD	317	*1557*	1783
5	Activated	38·9	PPD	267	*1235*	6143
6	Activated	24·4	PPD	537	*2659*	ND
7	Activated	32·5	PPD	62	41	2121
8	Activated	87·9	PPD	696	613	9833
9	Activated	ND	TT	103	307	3795

Eosinophils were cultured with resting CD4+ T cells (exps 1–3) or activated CD4+ T cells (antigen-specific CD4+ T-cell lines; exps 4–9) with and without the designated antigen. The concentrations were 10 μg/ml for PPD and *B. malayi* Ag, 1·0 μg/ml for TT, and 100 ng/ml for staphylococcal superantigen. Antigen and superantigen stimulated CD4+ T-cell proliferation in the presence of class II MHC-expressing eosinophils were measured in parallel experiments.

*ND, not done.

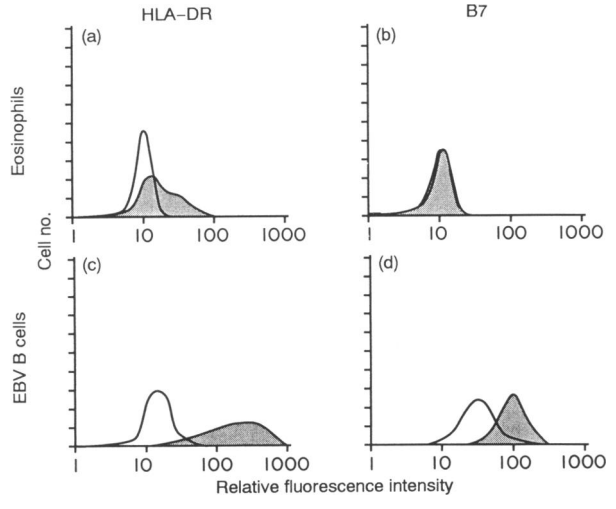

Figure 6. Surface expression of HLA-DR and B7 by eosinophils. Eosinophils from donor 5 in Fig. 1 and EBV-transformed B cells were stained with mAb to HLA-DR (a and c) and B7 (BB1) (b and d) and expression measured by indirect immunofluorescence and FACS analysis. Eosinophils were cultured in CM for 72 hr before analysis. Log fluorescence intensity (*x*-axis) as a function of relative cell number (*y*-axis) is shown for test antibody (stippled area) superimposed on fluorescence determined by Ig isotype controls (open area).

indices of 4·6, 4·9 and 4·9). These responses, however, were less than those observed when irradiated PBMC or monocyte/macrophages were used as APC (ranges of stimulation indices 4·1–108·9 and 17·6–34·1, respectively). Eosinophils were functional as APC for superantigen-induced T-cell proliferation in five of six experiments.

B7 expression by eosinophils

Because co-stimulatory molecules such as B7 on APC are important for T-cell activation,[31,34-36] B7 expression on activated eosinophils was examined. Eosinophils incubated in CM for 72 hr did not express B7, as detected by mAb BB1. Thirty-seven per cent of these eosinophils expressed class II MHC (Fig. 6). Epstein–Barr virus (EBV)-transformed B cells from the same donor expressed high levels of B7 (50·8%) as well as class II MHC (91·4%). This experiment was repeated twice with cells from different donors and similar results were obtained.

Activated eosinophils are not toxic for T cells

To exclude the possibility that poor T-cell proliferative responses were due to cytotoxicity mediated by eosinophils, unpulsed and PPD-pulsed eosinophils were co-incubated with ^{51}Cr-labelled autologous CD4+ T cells from a PPD-specific CD4+ T-cell line. Specific ^{51}Cr release was $< 5\%$ at up to 100:1 eosinophil to T-cell ratios (mean of three experiments). This value was not different from spontaneous release for T cells incubated alone. Further, the addition of eosinophils to resting T cells (1:1 ratio) did not affect the proliferative response to SEA or SEB when monocyte/macrophages were used as primary APC.

DISCUSSION

These studies were undertaken to determine the function and efficiency of class II MHC-expressing human eosinophils as APC for resting and activated CD4+ T cells. APC function of eosinophils was measured for non-processed bacterial superantigens (SEA, SEB and SEE) and for microbial Ag which require processing by APC (PPD, TT, *B. malayi* Ag). Our results were that human eosinophils expressed class II MHC after *in vitro* culture in the presence of GM-CSF containing medium, as reported by others.[19] The degree of class II MHC up-regulation varied among individuals, with a consistent pattern for each donor. Eosinophils expressing class II MHC were able to present bacterial superantigens to resting CD4+ T cells. Their ability to do so correlated with the amount of class II MHC

expressed and was heterogeneous with respect to various *S. aureus* enterotoxins (relative efficiency for SEA greater than for SEB or SEE). However, class II MHC-expressing eosinophils were inefficient as APC for the activation of resting and activated CD4+ T cells when microbial Ag requiring processing were used as T-cell stimuli. The inefficiency was highlighted when eosinophils were compared to monocytes/macrophages, which efficiently presented superantigens and processed microbial Ag to both resting and activated CD4+ T cells.

Two requirements must be fulfilled for APC to function for protein Ag.[28] First, the APC must process and present peptide fragments of protein Ag on class II MHC for recognition by the TcR of CD4+ cells.[37] Second, once ligand binding to the TcR occurs, the APC must provide co-stimulatory signals to induce CD4+ T-cell activation, cytokine production and proliferation.[26,28,34-36] The inefficiency of eosinophils as APC for resting CD4+ T cells therefore may be due to an inability to process peptide Ag efficiently, a deficiency in co-stimulatory function, or a combination of both.

The ability of superantigens to induce resting CD4+ T-cell proliferation in the presence of eosinophils suggests that the latter cells possess the necessary co-stimulatory signals for stimuli which do not require uptake and processing and which have high affinity for both MHC class II and TcR.[38] The observations that eosinophils express on their surface ICAM-1[15,20] and LFA-3 (S. D. Mawhorter *et al.* unpublished data) and have the capacity to produce IL-1 and IL-6[39,40] support their function as APC in this context. In contrast, a deficiency in the more stringent co-stimulatory requirements for processed Ag may contribute to the inability of the eosinophils to induce resting CD4+ T-cell proliferation and only minimal proliferation of activated Ag-specific CD4+ T-cell lines in response to processed microbial Ag.[36] In particular, B7 interaction with CD28 has been found to influence IL-2 production, suggesting that it is an important contributor to proliferative responses by T cells.[34,35] Our observation that class II MHC-bearing eosinophils do not express B7 support this explanation.

Alternatively, it is possible that eosinophils are inefficient in the intracellular processing of protein Ag, an event which is critical for stimulation of CD4+ T cells. Studies with murine and human eosinophils and CD4+ T-cell clones demonstrate that eosinophils are capable of processing Ag by a chloroquine-sensitive pathway.[20,21] However, CD4+ T-cell clones are highly differentiated cells and may have decreased or different co-stimulatory requirements and enhanced reactivity to nominal Ag relative to the resting T cells from which they were selected.

In contrast to our results, Weller *et al.*[41] recently reported that HLA-DR+ eosinophils supported Ag (TT)-driven proliferation of resting T cells. The manner in which APC function was examined may contribute significantly to differences in the results of this investigation and the current study. Whereas we evaluated APC function of living eosinophils co-incubated with Ag, these investigators used eosinophils which had been fixed with paraformaldehyde following an Ag pulse. Indeed, their data indicate that unfixed eosinophils fail to facilitate Ag-driven T-cell proliferation, which is consistent with our results. Inefficient APC function by viable eosinophils was not due to toxicity for T cells, as measured by the lack of increased ^{51}Cr-release from labelled T cells or inhibition of superantigen-mediated T-cell proliferation when eosinophils were added to cell cultures which included monocyte/macrophages as APC.

Finally, induction of a partial state of tolerance may account for the poor proliferative responses of CD4+ T cells to Ag presented by eosinophils. Presentation of Ag by accessory cells deficient in critical co-stimulators is a known mechanism of inducing a state of Ag-specific tolerance.[28] Further studies on the immunoregulatory function of activated class II MHC-expressing eosinophils, particularly the possibility that these cells may produce cytokines which down-regulate T-cell activation,[42] should advance our understanding of their role in modulating cellular immune responses to helminths and allergens.

ACKNOWLEDGMENTS

This work was supported by AI-27243 (W. H. Boom), AI-15351 (J. W. Kazura) and T32-AI-07024 (S. D. Mawharter). We thank Kerry J. Schimenti and James W. Jacobberger for help with the FACS analysis; Maria Mincek for preparing resting T cells; Christopher King for the purified CD4+ T cells and help with the IFN-γ ELISA; Jerrold J. Ellner, Eric Pearlman and Christopher King for many helpful discussions; Carl June for purified resting CD4+ T cells; and Pat Amato for secretarial assistance.

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