Farhan, Ruhaifah K. and Vickers, Mark A. and Ghaemmaghami, Amir M. and Hall, Andrew M. and Barker, Robert N. and Walsh, Garry M. (2016) Effective antigen presentation to helper T cells by human eosinophils. Immunology . ISSN 1365-2567

Effective antigen presentation to helper T cells by human Eosinophils

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This study was funded by grants from The Embassy of the State of Kuwait

Abstract

Although eosinophils are inflammatory cells, there is increasing attention on their immunomodulatory roles. For example, murine eosinophils can present antigen to CD4+ T helper (Th) cells, but it remains unclear whether human eosinophils also have this ability. This study determined whether human eosinophils present a range of antigens, including allergens, to activate Th cells, and characterized their expression of MHC class II and co-stimulatory molecules required for effective presentation.
Human peripheral blood eosinophils purified from non-allergic donors were pulsed with the antigens house dust mite extract (HDM), Timothy Grass extract (TG) or Mycobacterium tuberculosis purified protein derivative (PPD), before co-culture with autologous CD4+ Th cells. Proliferative and cytokine responses were measured, with eosinophil expression of HLA-DR/DP/DQ and the co-stimulatory molecules CD40, CD80 and CD86 determined by flow cytometry. Eosinophils pulsed with HDM, TG or PPD drove Th cell proliferation, with the response strength dependent on antigen concentration. The cytokine responses varied with donor and antigen, and were not biased towards any particular Th subset, often including combinations of pro- and anti-inflammatory cytokines. Eosinophils up-regulated surface expression of HLA-DR/DP/DQ, CD80, CD86 and CD40 in culture, increases that were sustained over 5 days when incubated with antigens, including HDM, or the major allergens it contains, Der p I or Der p II. Human eosinophils can, therefore, act as effective antigen-presenting cells to stimulate varied Th cell responses against a panel of antigens including HDM, TG or PPD, an ability that may help to determine the development of allergic disease.

Key words:

Human, eosinophil, helper T cell, antigen presentation, cytokine, allergen, major histocompatibility complex class II, co-stimulatory molecule, house dust mite, Timothy grass

Abbreviations:

HDM - house dust mite extract; TG - Timothy grass extract; APC - antigen presenting cell; DC - dendritic cell; Th - helper T; MHC - major histocompatibility complex; HLA - human leukocyte antigen; mAb – monoclonal antibody; PPD - purified protein derivative; SEB - staphylococcal enterotoxin B

Introduction

Eosinophil involvement in inflammatory conditions affecting the skin, gastrointestinal tract and upper and lower airways is well-documented.[1, 2] Asthma is now recognised as a heterogeneous condition with a number of phenotypes, some of which are stratified according to cellular infiltrates such as neutrophilic or eosinophilic asthma.[3] Eosinophilic asthma is characterized by increased blood or sputum eosinophils, [4] the numbers of which correlate with disease severity.[5] Infiltrating tissue eosinophils release their potent pro-inflammatory arsenal that includes such diverse elements as granule-derived basic proteins, lipid mediators, cytokines and chemokines.[2] These contribute to airway inflammation and lung tissue remodelling, including epithelial cell damage and loss, airway thickening, fibrosis and angiogenesis.[6] In addition to their role as degranulating effector cells, more recent findings emphasise the immunomodulatory properties of eosinophils,[7] and other important effector functions such as a potential role in maintaining host survival in life-threatening respiratory viral infections.[8]

One question that has attracted interest is whether eosinophils can modulate immune responses by acting as antigen presenting cells (APC) to stimulate $CD4^+$ helper T (Th) cell responses. It has been known for many years that *in vitro* culture of eosinophils with GM-CSF, typically added to prevent their apoptosis, can also induce expression of MHC Class II,[9] which could equip them for antigen presentation. MHC Class II expression by eosinophils has been observed in murine models of allergic airways inflammation[10] and intraperitoneal parasitic infection.[11] In man, tissue eosinophils have increased MHC Class II expression,[12] with upregulation observed in asthma,[13] chronic eosinophilic pneumonia[14] and eosinophilic esophagitis.[15]

Consistent with APC function, murine eosinophils home to lymphoid tissue and provide a second signal for T cell activation through the expression of key co-stimulatory molecules such as CD80 and CD86.[16, 17] Although it has been demonstrated that human eosinophils can express CD86 when taken from hypereosinophilic patients,[18] or stimulated with IL-3,[19] it is unclear how commonly, or in what circumstances, they display such co-stimulatory molecules. There are also reports that human eosinophils

can process and present antigen to activate specific T cells,[20] but, again, it remains to be established how widespread is such ability in different individuals and for different antigens. Despite the evidence that eosinophils have the potential to act as APC to drive Th cell responses and thereby propagate inflammation,[21, 22] other findings have suggested that this is limited to super-antigens and peptides, rather than proteins that require processing.[11, 16] The effects of Th activation are critically dependent on the subset(s) that respond, and the associated cytokines they produce, but it is not known whether eosinophil antigen presentation preferentially supports responses by any particular Th type.

To address the unanswered questions about their roles as APC, we performed a comprehensive examination of the ability of purified peripheral blood human eosinophils to present a variety of protein antigens to autologous $CD4^+$ Th cells. Helper responsiveness was tested to eosinophils pulsed with the allergens house dust mite extract (HDM), Timothy Grass extract (TG), Der p 1 Der p 2, or the microbial recall antigen *Mycobacterium tuberculosis* purified protein derivative (PPD), with eosinophil expression of MHC Class II molecules and the co-stimulatory molecules CD40, CD80 and CD86 characterized. It was also determined whether any Th cytokines elicited by eosinophil antigen presentation exhibited a bias towards particular effector or regulatory subsets. The focus here was on donors with no history of allergy, since we wished to establish the ability of eosinophils to contribute to the Th activation in the absence of any pre-existing strong pathogenic response.

Methods

Materials

CD-16 immunomagnetic beads, the Human $CD4^+$T Cell Isolation Kit II and magnetically activated separation columns were from Miltenyi Biotec (Surrey, UK). HDM (*Dermatophagoides pteronyssinus)* and TG extracts, (both certified LPS free and obtained from NIBSC, UK) were dialysed using slide-A- Lyzer dialysis cassettes (Thermo scientific, UK) for 24 hours and used at a final concentrations of 500—2500 IU/ml. PPD (Satens Serum Institute, Denmark) was added to cell cultures at a final concentration of 5 μg/ml. *Dermatophagoides pteronyssinus* allergens, Der p 1 or Der p 2 (Indoor Biotechnologies Ltd) were used at

final concentrations 10 μg/ml. All cells were cultured in RPMI 1640 (Labtech International Ltd, UK) supplemented with HEPES and 100 IU/ml penicillin, 100 μg/ml streptomycin, L- glutamine 5% (v/v) (Gibco, Life Technologies, Paisley, UK) and 5% (v/v) heat-inactivated autologous serum, with 10nM granulocyte-macrophage colony-stimulating factor (rhGM-CSF, R&D systems, Abingdon, UK). The latter was essential to prevent eosinophil apoptosis during co-culture. The following mAb were used in these studies: HLA-DR/DP/DQ (clone TL2.1), CD1a (clone H1149), CD40 (clone5 C3), CD80 (L307.4) and CD86 (clone 2331) were all from BD Pharmingen, Oxford, UK. Siglec-8 mAb (clone 7CP) was from Biolegend, London, UK. Cytokine levels were measured by Multiplex array for IFN-γ, IL-3, IL-6, IL-10, IL-17A and TNF-α (Luminex, Millipore, Watford, UK) and by ELISA for IL-9 and IL-13 (Biolegend).

Eosinophil and Th cell Isolation

Human eosinophils and $CD4^+$ Th cells were purified from individuals with no clinical history of allergy or eosinophilia ($\leq 0.5 \times 10^6$ eosinophils/ml), and who were not taking any medication for allergic disease. The inclusion of patients with hemochromatosis, who were being routinely bled to treat the disease, allowed relatively large numbers of eosinophils for some experiments to be collected from whole units of blood in the face of normal eosinophil counts. All subjects gave informed consent and the study was approved by the North of Scotland Research Ethics Service (ref 09/S0801/16). Eosinophils were purified from samples of peripheral blood using our standard technique[22] using dextran sedimentation and centrifugation on Percoll gradients followed by CD16-dependent negative immunomagnetic selection. To obtain $CD4^+$ Th cells, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation[23] and non-target cells depleted by negative selection following the manufacturer's instructions.

Using these methods, eosinophils and Th cells with respective purities of at least 98% were obtained, with greater than 98% viability as assessed by trypan blue exclusion.

Cell cultures

Eosinophils ($5x10^5$/ml) were incubated in culture medium for up to 5 days in the presence of rhGM-CSF (10 nM) to inhibit their spontaneous apoptosis as previously described,[24] with or without the addition of

antigens. When co-cultured with autologous $CD4^+$ Th cells, eosinophils were first pulsed with antigens by overnight incubation, then washed and added at $5x10^5$/ml in medium containing GM-CSF to the Th cells ($1x10^6$/ml) for 5 days; these conditions were found to give optimal Th responses in pilot experiments (data not shown). Cultures of autologous PBMC ($1.25x10^6$/ml), with or without antigen, provided controls for comparison of Th responsiveness.

Th cell responses

Proliferative Th cell responses were determined by incorporation of ^{3}H-thymidine in triplicate 100µl volumes withdrawn from cultures 5 days after stimulation as previously described, with results presented as CPM.[25] Cytokine levels in cultures were measured by bead array for IFN-γ, IL-3, IL-6, IL-10, IL-17A and TNF-α and by ELISA for IL-9 and IL-13, according to the manufacturers' instructions. Cytokine responses >2x background in unstimulated wells were considered significant.[23] To test the dependency of responses on MHC class II,[26] antigen-pulsed eosinophils were incubated with blocking antibody before co-culture with autologous $CD4^+$ Th cells.

Immunostaining and flow cytometry

Cell surface expression markers were examined using flow cytometry for HLA-DR,-DP,-DQ, and co-stimulatory molecules, CD40, CD80, and CD86 using established protocols.[22] Briefly, eosinophils and $CD4^+$ Th cells were removed from co-culture, washed and saturating quantities of primary antibodies or specific isotype controls were added to the cells and incubated for 40 min at 4^0C in the dark, washed and fixed. Human dendritic cells (DC) were identified by staining cells with CD1a while eosinophils were identified by staining with a specific marker, siglec-8.[27] Multiple panels of conjugated antibodies were used to identify the subpopulation of immune cells and the corresponding specific cell surface markers. Ten thousand events were collected on flow cytometer, LSRII (BD Biosciences) using FACS Diva software (BD Biosciences). Analysis was performed using FlowJo software (Treestar, Inc., Ashland, Oreg. USA).

Statistics

Data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA), and since D'Agostino-Person Omnibus and Shapiro-Wilk normality tests were failed, non-parametric methods were used (Wilcoxon-Signed-Rank-test or Mann-Whitney U Test as appropriate). Bonferoni correction was applied to correct for multiple comparisons. Data were considered to be statically significant if $p<0.05$, and are expressed as either median and interquartile range (IQR), or individual data points if $n<4$.

Results

Eosinophils pulsed with antigen induce proliferative Th responses

We first examined the ability of human eosinophils pulsed with a range of concentrations of the allergens HDM or TG, or with our standard concentration of the control microbial recall antigen PPD, to induce proliferation by autologous $CD4^+$ Th cells after 5 days of co-culture. In a series of 9 experiments, significant ($p<0.001$) increases in proliferation were observed when eosinophils had been pulsed by pre-incubation with the allergens HDM (Figure 1A) or TG (Figure 1B) at final concentrations of 1000, 1500 or 2500 IU/ml, compared with medium alone. The strongest responses were induced by HDM or TG at 2500 IU, and this concentration of the allergens was therefore used in all subsequent experiments. Eosinophils pulsed with the control antigen PPD also elicited significant proliferation at the standard concentration of 5 μg/ml. To confirm that responses to each stimulus required the presence of both eosinophils and Th cells, proliferation was compared in cultures containing each cell type alone or together, with or without antigen pulsing (Figure 2). Proliferative responses were significant only when both antigen-pulsed eosinophils and Th cells were added, and, strikingly, these responses were similar in size to those seen when unfractionated PBMC were stimulated with the respective antigen. The ability of Th cells to respond to antigen-pulsed eosinophils was MHC class II dependent, since incubating HDM-pulsed eosinophils with blocking mAb specific for HLA-DR/DP/DQ[(26)] significantly ($P<0.001$) reduced T cell proliferation by 77% (Supplemental Figure 1).

FIG 1

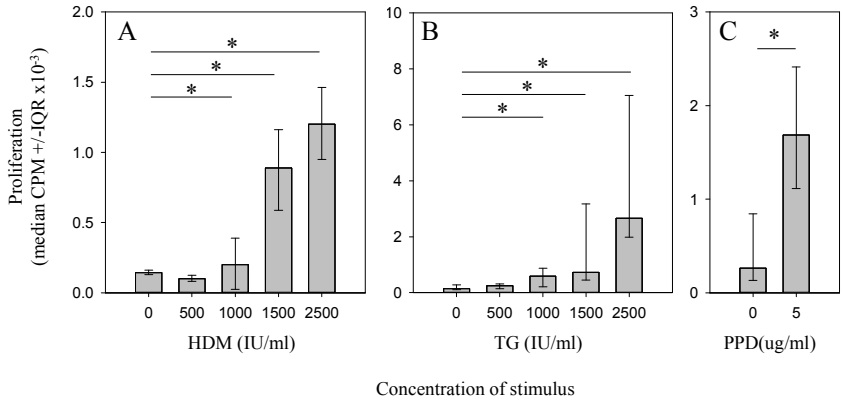


Figure 1. Eosinophils pulsed with allergen stimulate Th cell proliferative responses. Panels show proliferation in co-cultures of peripheral blood CD4⁺ Th cells and autologous eosinophils that have been pulsed with the antigens HDM (A), TG (B), or PPD (C). Results are expressed as median CPM and IQR from 9 independent experiments (*p < 0.01, Mann-Whitney U-test with Bonferroni correction).

FIG 2

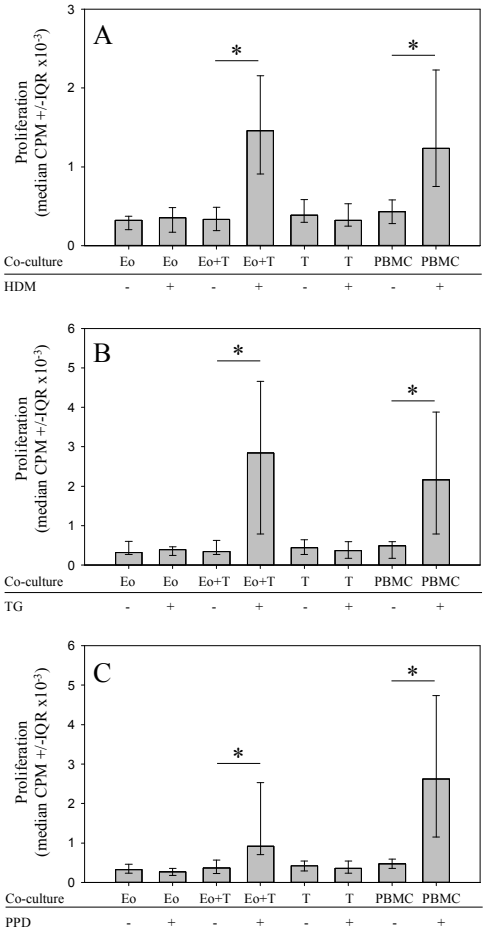
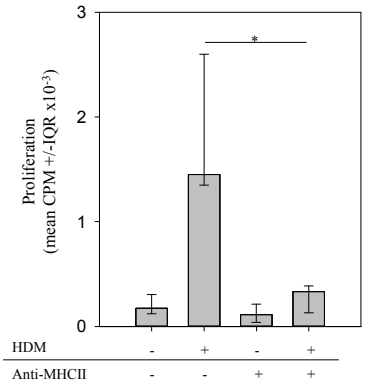


Figure 2. Eosinophils pulsed with antigen stimulate Th proliferation comparable to PBMC responses. Panels show proliferation in cultures containing peripheral blood CD4⁺ Th cells and autologous eosinophils, either alone or together, with or without pulsing of the eosinophils with the antigens HDM (A), TG (B) or PPD (C). Proliferation in PBMC cultures, either untreated or antigen stimulated, is included for comparison. Eo = purified eosinophils, T = purified CD4⁺ T cells (*p<0.05, Mann Whitney U-test with Bonferroni correction).

Supplemental Figure 1



Supplemental Figure 1. Blockade of HLA-DR/DP/DQ inhibits HDM induced Th cell proliferation. Panels show proliferation in co-cultures of peripheral blood $CD4^+$ Th cells and autologous eosinophils that have been pulsed with different combinations of HDM or blocking anti-HLA-DR/DP/DQ monoclonal antibody. Results are expressed as median CPM and IQR from 9 independent experiments (* $p < 0.01$, Mann-Whitney U-test).

Eosinophil expression of MHC class II and co-stimulatory molecules

Effective APC require expression of both MHC class II and co-stimulatory molecules, so we next tested whether cultured eosinophils display HLA-DR/DP/DQ and CD40, CD80 and CD86. Examples of flow cytometric analyses, and graphical summaries of data from 9 independent experiments, demonstrate eosinophil expression of HLA-DR/DP/DQ (Figure 3) and CD40, CD80 and CD86 (Figure 4) during 5 day cultures, with or without HDM addition. It can be seen that, within 24 hours, eosinophil expression of MHC class II, and the co-stimulatory molecules CD40, CD80 and CD86, was significantly increased in all cultures, an effect that may be at least partly due to the presence of GM-CSF added to the medium to prevent eosinophil apoptosis.[9] However, there was a further effect of HDM. Addition of the antigen sustained the elevated levels of MHC class II, CD40, CD80 and CD86 over the course of the incubation, since, without HDM, expression of all the markers fell back by day 5 to levels not significantly above those seen at the beginning of the culture.

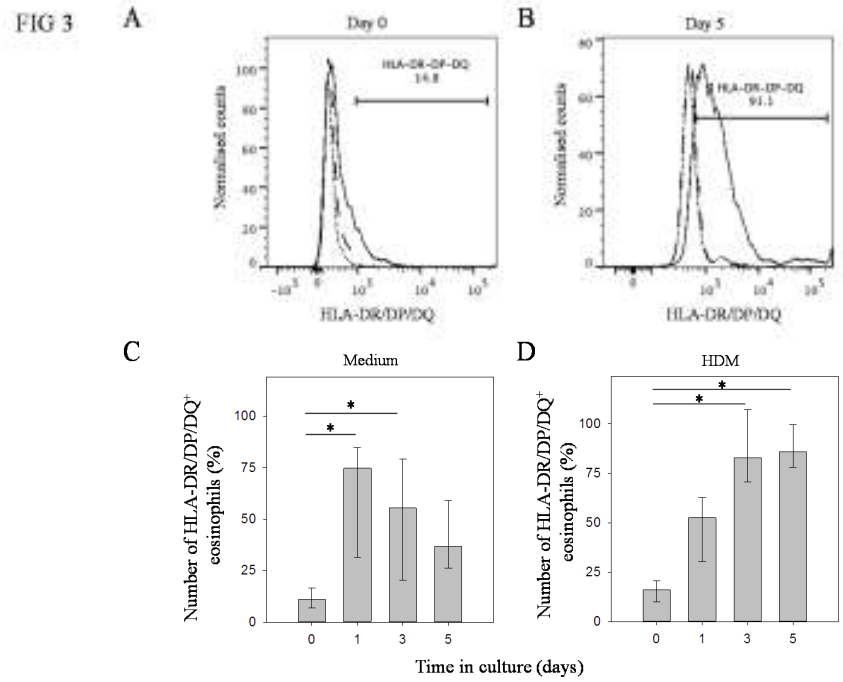


Figure 3. Cultured eosinophils express MHC class II. Representative flow cytometric histograms (n=9) demonstate HLA DR/DP/DQ expression by purified eosinophils incubated with HDM at day 0 (A) and day 5 (B) of culture (solid line = stained cells, dotted line = unstained cells, dashed line = isotype control). The gate indicates the percentage of eosinophils staining positively for HLA-DR/DP/DQ. HLA-DR/DP/DQ expression on unstimulated (C) or HDM stimulated (D) eosinophils over 5 days of culture is summarized in bar charts, with results expressed as median and IQR of % eosinophils positive for HLA DR\DP\DQ staining from 9 independent experiments (*p < 0.01, Mann Whitney U-test with Bonferroni correction).

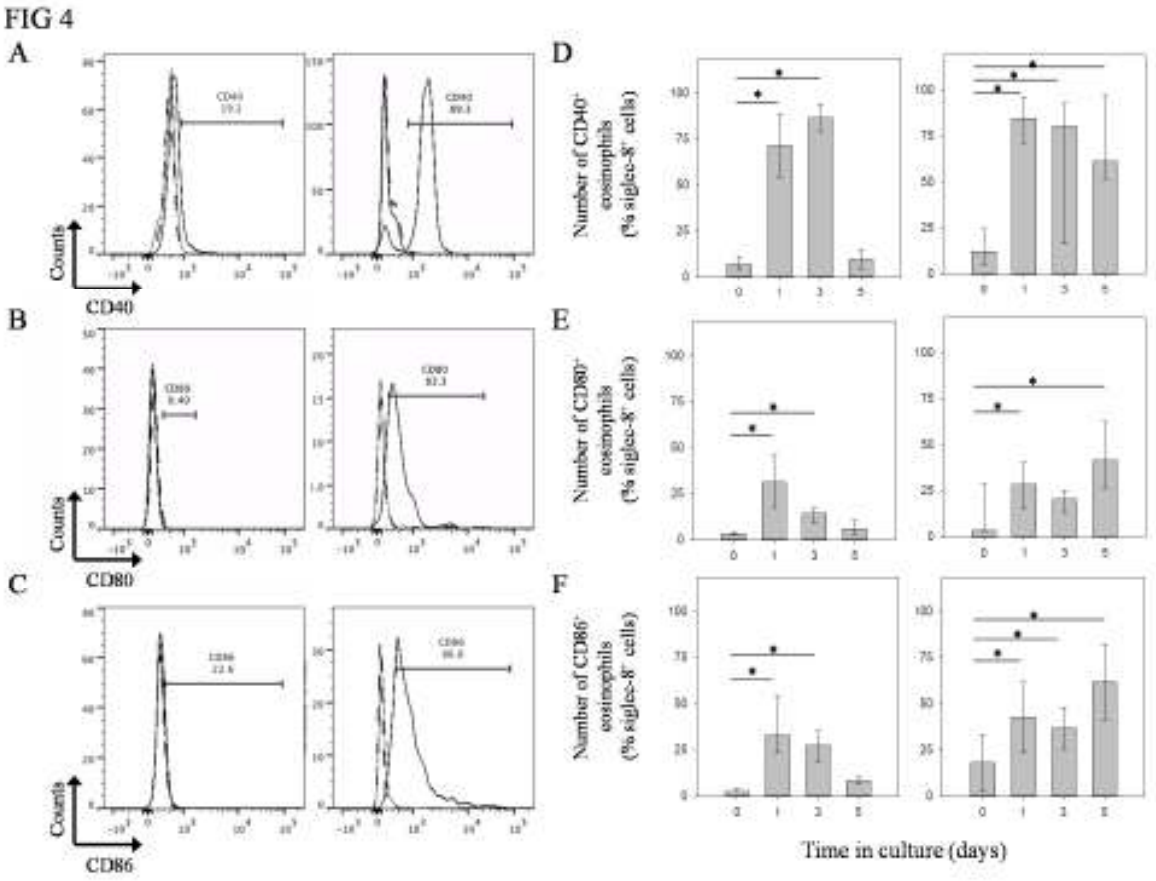


Figure 4. Cultured eosinophils express costimulatory molecules. Representative flow cytometric histograms (n=9) demonstate CD40 (A), CD80 (B) and CD86 (D) expression by purified eosinophils incubated with HDM at day 0 (left panels) and day 5 (right panels) of culture (solid line = stained cells, dotted line = unstained cells, dashed line = isotype control). The gate indicates the percentage of eosinophils staining positively for each co-stimulatory molecule. CD40 (D), CD80 (E) and CD86 (F) expression by unstimulated (left panels) or HDM stimulated (right panels) eosinophils over 5 days of culture is summarized in the bar charts, with results expressed as median and IQR of % eosinophils positive for each marker from 9 independent experiments (*p < 0.01, Mann Whitney U-test with Bonferroni correction).

The HDM preparation is a simple extract, so we next tested whether the major allergens it contains, Der p 1 and Der p 2, recapitulate its ability to sustain enhanced expression of MHC class II and co-stimulatory molecules by eosinophils. Eosinophils incubated with Der p 1 or Der p 2 exhibited similar sustained increases in expression of HLA-DR\DP\DQ, CD40, CD80 and CD86 compared with those elicited by the crude HDM preparation (Figure 5).

FIG 5

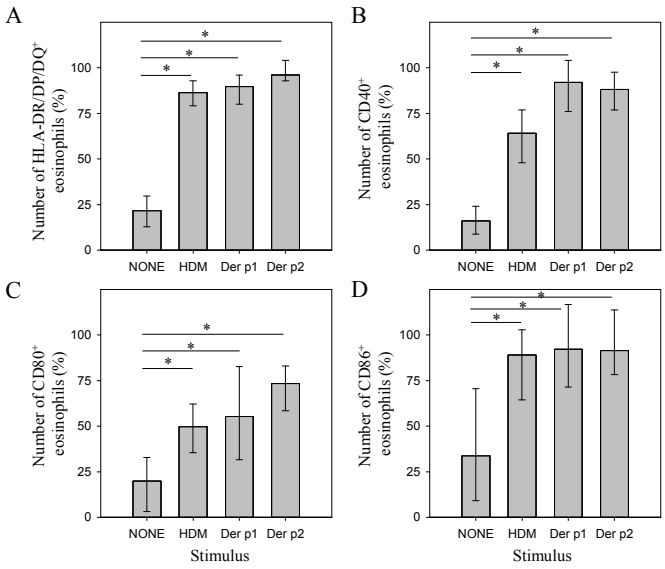


Figure 5. Der P1 and Der P2 antigens share the ability of HDM to sustain eosinophil surface expression of MHC class II and costimulatory molecules. Comparision of the effects of incubation with the purified allergens Der p 1 or Der p 2, or the allergen extract HDM, on numbers of eosinophils that express of HLADR\DP\DQ (A), CD40 (B), CD80 (C) and CD86 (D) after 5 days of culture. Results are expressed as median and IQR of % eosinophils positive for each marker from 6 independent experiments (* p < 0.01, Mann-Whitney U-test with Bonferoni correction).

Th cytokine responses induced by eosinophils pulsed with antigen

Having demonstrated the ability of eosinophils to present antigen to drive Th proliferative responses, the question arises as to whether cytokines associated with any particular $CD4^+$ subset are produced. Signature cytokines for the major subsets Th1 (IFN-γ), Th2 (IL-13), Th9 (IL-9), Treg (IL-10), Th17 (IL-17A) and the

inflammatory cytokines TNF-α, IL-3 and IL-6, were measured in co-cultures of $CD4^+$ Th cells and eosinophils with, or without, pulsing with the antigens HDM, TG or PPD. Different patterns of cytokine response to the antigens were seen in cultures from each of the donors tested (n=6), with examples illustrated in Figure 6, and all results summarized in Table 1. It can be seen that antigen-pulsed eosinophils are capable of eliciting a wide range of cytokines tested. Although IL-6 production was the most frequently seen response, cultures of antigen-pulsed eosinophils could also contain complex mixtures of both pro- and anti-inflammatory cytokines, and, overall, there was no clear or consistent bias towards any particular Th subset.

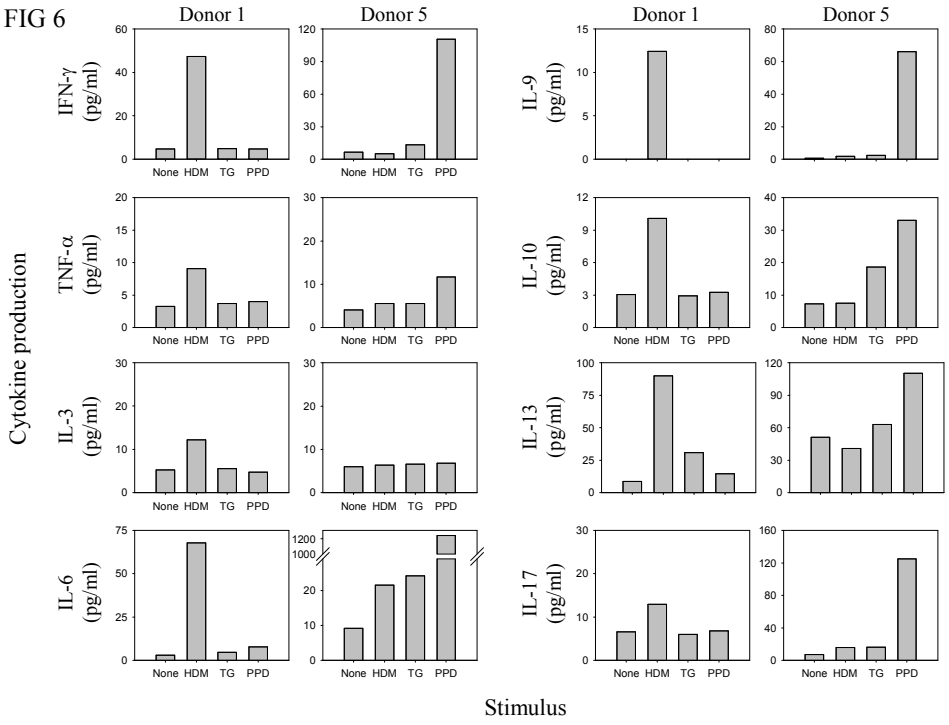


Figure 6. Production of multiple cytokines in co-cultures of $CD4^+$ Th cells and antigen-pulsed eosinophils. Examples are shown (donors 1 and 5, n=6) of different patterns of cytokine secretion by co-cultures of $CD4^+$ Th cells and eosinophils pulsed with the antigens HDM, TG or PPD.

	Stimulus	S1	S2	S3	S4	S5	S6
IFN-γ	HDM	↑	↔	↔	↔	↔	↔
	TG	↔	↔	↔	↔	↑	↔
	PPD	↔	↔	↔	↑	↑	nt
TNF-α	HDM	↑	↔	↔	↔	↔	↔
	TG	↔	↔	↔	↔	↔	↔
	PPD	↔	↔	↔	↑	↑	nt
IL-3	HDM	↑	↔	↔	↔	↔	↔
	TG	↔	↔	↔	↔	↔	↔
	PPD	↔	↔	↔	↔	↔	nt
IL-6	HDM	↑	↔	↔	↑	↑	↑
	TG	↔	↔	↔	↑	↑	↑
	PPD	↑	↔	↔	↑	↑	nt
IL-9	HDM	↑	↔	↔	↔	↑	↔
	TG	↔	↔	↑	↔	↑	↔
	PPD	↔	↔	↔	↑	↑	nt
IL-10	HDM	↑	↔	↔	↔	↔	↔
	TG	↔	↔	↔	↔	↑	↔
	PPD	↔	↔	↔	↑	↑	nt
IL-13	HDM	↑	↔	↓	↔	↔	↑
	TG	↑	↑	↔	↑	↔	↑
	PPD	↔	↔	↑	↔	↑	nt
IL-17	HDM	↔	↔	↔	↔	↑	↓
	TG	↔	↔	↔	↔	↑	↔
	PPD	↔	↔	↔	↑	↑	nt

Table 1: Cytokine production by co-cultures of Th cells and eosinophils presenting antigen. The table shows whether there is an increase (up arrow, SI>2), decrease (down arrow, SI<0.5) or no change (horizontal arrows, SI range 0.5-2) in cytokine production in Th cell-eosinophil co-cultures when eosinophils are pulsed with HDM, TG or PPD, compared to unpulsed control. nt=not tested

Discussion

The present study has established the ability of human eosinophils to present a wide variety of protein antigens, including allergens, to stimulate proliferative and cytokine responses by $CD4^+$ Th cells. In line with their ability to elicit responses to antigen, eosinophils exhibited upregulated MHC class II and costimulatory molecules in culture. Since the donors tested here had no clinical history of allergy, the results raise the possibility that eosinophils acting as APC can help determine whether Th responses to allergen are elicited or become pathogenic.

There is now a substantial body of evidence demonstrating that murine eosinophils presenting antigen have the ability to stimulate Th cells, including the induction of primary responses, and that they act as true professional APC in homing to lymph nodes, where they can interact with Th cells.[21] We show here that human eosinophils are also effective APC for a variety of antigens *in vitro*, able to evoke Th proliferative

responses comparable in magnitude to those seen when paired PMBC samples are challenged with the same antigen. Thus, human eosinophil presentation to Th cells is limited neither to peptide antigens nor superantigens that do not require processing, as has been suggested,[11, 16] but may instead contribute to responses against many different proteins. Whether such activity as APC *in vitro* is replicated *in vivo* remains to be established, but would be consistent with the increasingly accepted view that human eosinophils have immune regulatory, as well as effector, roles.[20, 21]

APC function is dependent on display of MHC class II and costimulatory molecules.[28] We not only confirm that cultured human eosinophils express HLA-DR/DP/DQ, but also show expression of all three major co-stimulatory molecules, CD40, CD80 and CD86 by these cells. Eosinophils in culture require addition of GM-CSF to prevent apoptosis, and the presence of this cytokine is likely to have contributed to the APC phenotype seen here, since eosinophils purified from the spleens of IL-5 transgenic mice were also observed to express MHC Class II, CD40, CD80 and CD86 when stimulated with GM-CSF.[20] Previous studies of human eosinophils have described upregulation of MHC class II and CD86 in response to cytokine or superantigen exposure[14, 19, 29], but, to our knowledge, we are the first to report expression of such a complete APC surface phenotype in cultured human eosinophils. Although it could be argued that the precise conditions *in vitro* do not reflect those *in vivo*, the results nevertheless establish that eosinophils have the potential to present antigen very effectively, and the induction of APC function by stimuli such as GM-CSF *in vivo* may well represent an important mechanism by which eosinophils influence immune responses to allergens. In addition, GM-CSF was not the only factor upregulating APC surface markers, since we demonstrated that the increases in the expression of HLA-DR/DP/DQ, CD40, CD80 and CD86 were sustained for up to 5 days of co-culture by eosinophils stimulated with whole HDM extract or the major HDM allergens Der p 1 and Der p 2. A number of studies have identified similar effects of HDM extract or Der p 1 on other cell types. For example, Der p 1 stimulation of monocyte-derived DC isolated from donors allergic to HDM increased CD86 expression, while control non-allergic subjects had significant increases in CD80 expression[30], and another study showed that Der p 1 stimulated human peripheral blood DC to increase expression of HLA-DR, CD80 and CD86.[31] The underlying mechanisms remain to be established, but may include proteolytic activity of the allergen,[32] or its interaction with pattern recognition receptors.

Th cytokines elicited during responses to antigen play a key role in determining both protection from infection, and immune pathology. Here, eosinophils acting as APC supported production of a wide range of Th cytokines that differed between individuals and antigens, but with no clear preference for any response type. However, our study was of cells from donors with no clinical allergic disease, and so the possibility remains open that eosinophils may skew helper responses towards the pathogenic Th2 subset in patients with overt allergy, or a susceptibility to atopic disease. The notion that eosinophils can drive a variety of Th subsets is supported by a previous study, which also tested co-cultures of human peripheral blood eosinophils and autologous $CD4^+$ Th cells, and demonstrated both Th1 and Th2 cytokine responses to the super-antigen staphylococcal enterotoxin B (SEB). These workers also demonstrated HLA-DR expression by peripheral blood eosinophils isolated from 50% of the subjects, attributed to GM-CSF added to cultures, but stimulation with SEB did not induce eosinophil expression of CD80 or CD86.[33] The reasons for the differences between this result and the present study may well reflect the use of antigens versus polyclonal activator for stimulation. Others have described the effect of stimulation with HDM antigen on eosinophil function. For example, HDM stimulation of eosinophils *in vitro* led to production of IL-9 that may promote a Th2 immune response[34], but, although we detect this cytokine in some co-cultures of Th cells and antigen-pulsed eosinophils, HDM did not elicit the response more frequently than other antigens, and IL-9 was not associated with any clear Th subset bias.

Taken together, our data demonstrate that human eosinophils can act as effective APC to stimulate Th responses against a variety of antigens, including the allergens HDM or TG: a property that may contribute to the regulation of responses *in vivo* and to induction or control of pathology in allergic disease, depending on the cytokines elicited. The findings add to the accumulating evidence that eosinophils possess more complex immunomodulatory roles in allergic disease than previously suspected. Furthermore, any ability of HDM, Der p 1 or Der p 2 to act not only as antigens, but also to increase eosinophil co-stimulatory molecule expression, may enhance their immunogenicity. Having demonstrated the ability of human eosinophils to present antigen, this study opens up new questions as to how important they are in initiating, skewing, amplifying or regulating allergic responses in patients.

Acknowledgements

The authors wish to thank Alison Scaife for invaluble technical assistance and we are also grateful for the willingness of those who volunteered to give blood for this study.

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