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IL-5 enhances the *in vitro* adhesion of human eosinophils, but not neutrophils, in a leucocyte integrin (CD11/18)-dependent manner

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SUMMARY

In an attempt to explain the preferential accumulation of eosinophils at sites of allergic tissue reactions, we have studied the effects of interleukin-5 (IL-5) on the adherence of human eosinophils and neutrophils to plasma-coated glass (PCG) or human microvascular endothelial cells (HMVEC). IL-5 was compared with IL-3, granulocyte–macrophage colony-stimulating factor (GM-CSF) and platelet-activating factor (PAF), since all these agents have biological properties associated with eosinophil activation and/or survival *in vitro*. IL-5, IL-3 and GM-CSF induced a time-dependent increase in adherence of normal density eosinophils to PCG optimal at 60 min, whereas the effect of PAF was greater at 15 min. Similar results were obtained with neutrophils, with the exception that IL-5 had minimal and non-significant effects on this cell type. Unstimulated eosinophils and neutrophils also adhered to PCG or HMVEC, but in low numbers. Preincubation of eosinophils with IL-5, GM-CSF or PAF resulted in dose-dependent increases in the numbers of adherent cells to PCG. IL-3 had a smaller but significant effect on enhanced eosinophil adhesion to PCG, while IL-2 and lyso-PAF were ineffective. Neutrophils gave similar levels of baseline and stimulated adhesion to PCG as eosinophils, IL-5 again had no significant stimulatory effect. IL-5 also increased eosinophil, but not neutrophil, adherence to HMVEC in a concentration-dependent manner. Preincubation with the protein synthesis inhibitor cycloheximide had no effect on IL-5-, GM-CSF- or PAF-stimulated eosinophil adhesion. The contribution of the CD11/18 leucocyte integrins to IL-5- and PAF-induced eosinophil hyperadherence was investigated by inhibition experiments utilizing monoclonal antibodies (mAb). Enhanced adhesion to PCG (by PAF) or HMVEC (by IL-5) was inhibited by (ranked in order of potency) anti-CR3α = common β-chain > LFA-1α. Anti-p150,95α had no measurable effect. Baseline adhesion by unstimulated eosinophils was not significantly influenced by prior incubation with these mAb. Using flow cytometry, IL-5 and IL-3 were found to up-regulate eosinophil but not neutrophil CR3 expression. These findings demonstrate that IL-5 enhances eosinophil, but not neutrophil, adherence reactions, by a mechanism dependent, at least in part, on the CD11/18 family of adhesion glycoproteins.

INTRODUCTION

Eosinophil leucocytes are known to preferentially accumulate and persist at the site of allergic tissue reactions (Gleich & Adolphson, 1986; Spry, 1988). For example, Frew & Kay (1988), employing skin biopsies from late-phase cutaneous reactions (LPCR) in human atopic subjects, demonstrated activated ($EG2^+$) eosinophils 48 hr after challenge with specific allergen. In contrast, neutrophils appeared early and their numbers decreased with time. The mechanism of local tissue eosinophilia is largely unexplained. Although platelet-activating factor (PAF) is a potent eosinophil chemoattractant, it has been shown to have even greater effects on neutrophil locomotion (Wardlaw *et al.*, 1986). Interleukin-5 (IL-5) has been shown to be a selective chemotactic agent for the eosinophil, but the activity was very weak compared to that of PAF (Wang *et al.*, 1989). Furthermore, the general concept that the elaboration of selective chemotactic factors explains the local accumulation of various cell types in inflammatory reactions remains largely unproven. For this reason other mechanisms need to be

Abbreviations: ELAM-1, endothelial-leucocyte adhesion molecule-1; GM-CSF, granulocyte–macrophage colony-stimulating factor; HMVEC, human microvascular endothelial cells; HPF, high power field; HVEC, human vascular endothelial cells; ICAM-1 (2), intercellular adhesion molecule-1 (2); LAD, leucocyte adhesion deficiency; LPCR, late-phase cutaneous reactions; PAF, platelet-activating factor; PCG, plasma-coated glass; rh, recombinant human; tumour necrosis factor.

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considered to explain the local eosinophilia observed in allergy, asthma and helminthic infections. Using *in situ* hybridization, we have recently identified mRNA for IL-5 in both allergen-induced LPCR and bronchial mucosal biopsies from allergic asthma (Q. Hamid, M. Azzawi, R. Moqbel, S. Ying, P. Jeffrey and A. B. Kay, manuscript in preparation). In addition, IL-5 (Yamaguchi *et al.*, 1988), IL-3 (Rothenberg *et al.*, 1988) and granulocyte–macrophage colony-stimulating factor (GM-CSF) (Lopez *et al.*, 1986b) prolong the survival of human eosinophils *in vitro* and this may provide a partial explanation for the eosinophilic response *in vivo*. IL-5 has a number of selective effects on eosinophils. These include terminal differentiation of the committed eosinophil precursor (Clutterbuck, Hirst & Sanderson, 1988; Saito *et al.*, 1988), enhanced cytotoxicity and increased respiratory burst activity (Lopez *et al.*, 1988).

A further hypothesis, which is the subject of the present study, is that IL-5 promotes preferential hyperadhesion of eosinophils compared to the neutrophil. Lamas, Mulroney & Schleimer (1988) reported that eosinophil adherence to cultured human vascular endothelial cells (HVEC) or gelatinized plates could be enhanced by prior incubation with FMLP or tumour necrosis factor (TNF). Furthermore, a number of cytokines acted directly on cultured HVEC to give enhanced adhesion of untreated eosinophils. PAF was also shown to stimulate increased eosinophil adherence to HVEC or plastic surfaces. Enhanced adhesion was inhibited by prior incubation with a monoclonal antibody (mAb) directed against the common β-chain of the CD11/18 leucocyte integrin family (Kimani, Tonnesen & Henson, 1988). These glycoproteins consist of three distinct but related α subunits (LFA-1α, CR3α and p150,95α; CD11a, b and c, respectively) in a non-covalent heterodimeric association with a common β-chain subunit (CD18) (reviewed by Kuypers & Roos, 1989). The importance of the leucocyte integrins (CD11/18) is emphasized by an autosomal recessive heritable disorder designated leucocyte adhesion deficiency (LAD), characterized by deficient expression of the CD11/18 complex, resulting in life-threatening recurrent bacterial infections (reviewed by Anderson & Springer, 1987).

Thus, we have compared IL-5 with IL-3, GM-CSF and PAF to determine whether this cytokine preferentially influences eosinophil, as opposed to neutrophil, hyperadhesiveness to plasma-coated glass (PCG) or human microvascular endothelial cells (HMVEC). Furthermore, the contribution of the CD11/18 complex to this process was assessed using both flow cytometry and inhibition assays.

MATERIALS AND METHODS

Materials were obtained as follows: chamber slides from ICN Biochemicals, High Wycombe, Bucks, U.K.; RPMI-1640 from Gibco, Paisley, Renfrewshire, U.K.; dextran 110 from Fisons, Loughborough, Leics, U.K.; metrizamide from Nyegaard, Birmingham, U.K.; Ficoll–Paque from Pharmacia, Milton Keynes, Bucks, U.K.; BSA grade V, sodium azide and propidium iodide from Sigma Chemical Co., Poole, Dorset, U.K.; PAF and lyso-PAF (C-16) from Bachem, Saffron Walden, Essex, U.K.; rhIL-2 from Amersham International Ltd, Harefield, Middlesex, U.K.; rhIL-3 from Bio-trans Corp., Los Angeles, CA; and rhGM-CSF from Biogen Res. Corp., Cambridge, MA. The activities of these cytokines were defined by the supplier. rhIL-5 was produced as previously described and its activity measured by the human eosinophil colony assay (Campbell *et al.*, 1987). The concentrations of cytokines used in this study are expressed as units per ml (U/ml) throughout.

Monoclonal antibodies

Anti-CR3 (CD11b) was purchased from Becton-Dickinson U.K. Ltd, Oxford, U.K. mAb to LFA-1α (CD11a), p150,95α (CD11c) and the common β-chain (CD18) (TS1/22, SHCL3, TS1/18, respectively) were generously provided by Dr T. Springer of the Dana-Farber Cancer Institute, Boston, MA. All of these mAb were IgG and were of murine origin. FITC-conjugated $F(ab')_2$ fragments of rabbit antibody to mouse immunoglobulins was purchased from Dako Ltd, High Wycombe, Bucks, U.K.

Cell isolation

Eosinophils and neutrophils were isolated from donors with a moderate eosinophilia (range 7–16%, median 10%) associated with allergic rhinitis or mild asthma. Eosinophils and neutrophils were purified from heparinized peripheral blood using dextran sedimentation and discontinuous metrizamide gradients, as described previously (Vadas *et al.*, 1979). Neutrophils were isolated from the 20/22% interface and normal density eosinophils recovered from the lower three layers. Some experiments were performed with neutrophils isolated from heparinized blood of normal healthy individuals using dextran sedimentation and centrifugation on Ficoll–Paque cushions.

Microvascular endothelial cell culture

Human microvascular cells were purchased as sub-confluent secondary cultures from Clonectics Corp., San Diego, CA. These cells were derived from human foetal omental fat pad and cultured as described previously (Kern, Knedler & Eckel, 1983). Their identity as endothelial cells was confirmed by their typical 'cobblestone' morphology and staining with a mAb against Von-Willebrand factor. HMVEC were used in the adhesion assay on the 3rd or 4th passage after they were grown to confluence in glass chamber slides.

Adhesion assay

For the glass adhesion assay, chamber slides were pretreated with 10% pooled human plasma in RPMI-1640 for 20 min at room temperature and each well washed with RPMI-1640. HMVEC were washed twice with RPMI prior to use in the assay. The required concentration of mediator or control substance was added to each well before addition of the purified eosinophils or neutrophils (0·2 ml at 2×10^6/ml). For time-course studies the optimal concentration of each mediator/cytokine was used. Chambers were incubated for the required time at 37° in a humidified 5% CO_2 atmosphere for both PCG or HMVEC assays. Each well was filled with RPMI-1640 until a slight meniscus formed, the wells were capped with a single cover slip and the whole chamber inverted for 15 min at 20° to allow the detachment of non-adherent cells. No washing step was required, thus a high level of reproducibility was attained. Following non-adherent cell detachment, cells were fixed by the addition of 0·2 ml of 1% formyl saline to each well for 3 min at room temperature. The superstructure of the chamber was removed and the slides dehydrated in methanol before staining with haematoxylin/chromotrope 2R for eosinophils or May–Grunwald–Giemsa for neutrophils. The number of adherent

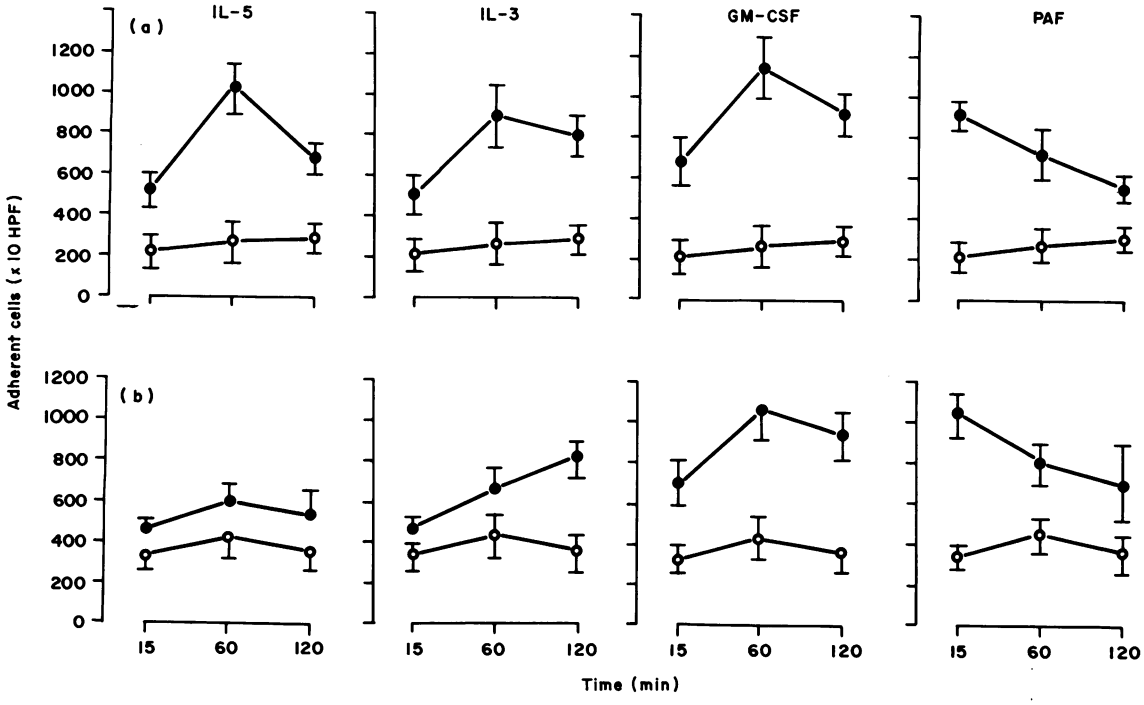

Figure 1. Time–course of the effect of IL-5, IL-3, GM-CSF and PAF on eosinophil (a) and neutrophil (b) adherence to PCG. Each point represents the mean ± SEM of five experiments or more. Compared with diluent, significant increases in eosinophil adherence were observed for PAF and GM-CSF at all time-points tested ($P < 0.01$, respectively). IL-5 gave significant enhanced adherence at 15 min ($P < 0.05$), 60 min ($P < 0.001$), 120 min ($P < 0.05$) and IL-3 at 60 and 120 min ($P < 0.01$). Neutrophil hyperadherence was significant at all time points for GM-CSF ($P < 0.01$); at 15 ($P < 0.01$) and 60 min ($P < 0.05$) for PAF; at 60 ($P < 0.05$) and 120 min ($P < 0.01$) for IL-3.

cells in 10 high power fields (HPF) was counted in a blind fashion.

For inhibition of adherence to PCG, eosinophils were preincubated with diluent or PAF (10^{-6} M) for 15 min at 37°. Resting or stimulated cells were then added to chamber slide wells, which contained previously determined saturating concentrations of the mAb or the negative isotypic control, and incubated for 15 min at 37°. Non-adherent eosinophils were detached and the slides fixed and stained as described above. The assay for inhibition of eosinophil adherence to HMVEC was identical, with the exception that the stimulus used was IL-5 (60 U/ml for 60 min at 37°).

Validation of visual counting

The accuracy of the visual counting assay was assessed using a specific radioimmunoassay for eosinophil cationic protein (ECP; Pharmacia Ltd, Uppsala, Sweden). Adherent control cells and eosinophils stimulated with increasing concentrations of PAF were lysed with 2% hexadecyltrimethylammonium bromide. The lysates were assayed for ECP and the concentrations determined by comparison with known standards. Blinded visual assays were performed in parallel and the percentage increases in stimulated adherent eosinophils compared with baseline calculated for both assays. Comparison of the determination of the numbers of adherent eosinophils by visual counting or measuring the ECP levels of the lysed cells revealed a significant correlation between the two methods ($r = -0.98$, $P < 0.01$, $n = 10$).

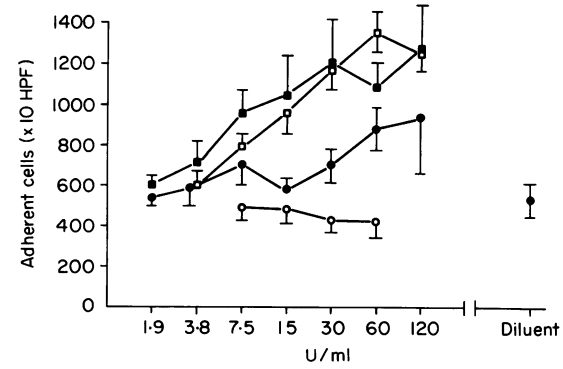

Figure 2. A comparison of the effect of increasing concentrations of IL-2 (○), IL-3 (●), IL-5 (□) and GM-CSF (■) on eosinophil adhesion to PCG. Each point represents the mean ± SEM of five experiments. The incubation time was 60 min. GM-CSF gave significant increases from 3·8 to 120 U/ml ($P < 0.01$, respectively), IL-3 and IL-5 from 7·5 to 120 U/ml ($P < 0.01$, respectively).

Immunofluorescence and flow cytometry

Purified eosinophils (50 μl at 1×10^7/ml) in RPMI supplemented with 10% FCS were stimulated with PAF (10^{-6} M), GM-CSF, IL-3 or IL-5 (60 U/ml in each case) for 60 min at 37°. Control cells were incubated with medium alone. Immunofluorescence with mAb against LFA-1α or CR3α and analysis by flow cytometry (performed on a FACS Analyzer, Becton-Dickinson, Mountain View, CA) were carried out as described previously

Table 1. Effect of increasing concentrations of PAF on eosinophil and neutrophil adhesion to PCG ($n=9$)

	Diluent	Lyso PAF 10^{-6} M	PAF (M) 10^{-12}	10^{-10}	10^{-8}	10^{-6}
Eosinophils	171±31	117±22	261±55	280±25**	437±19**	934±98**
Neutrophils	269±56	185±104	462±83*	646±120**	572±75**	750±127**

* $P<0.01$; ** $P<0.001$.

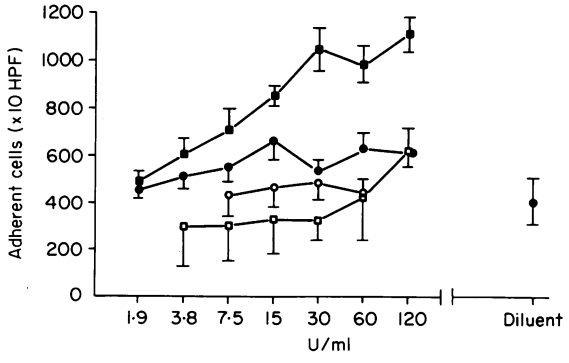

Figure 3. A comparison of the effect of increasing concentrations of IL-2 (○), IL-3 (●), IL-5 (□) and GM-CSF (■) on neutrophil adhesion to PCG. Each point represents the mean±SEM of five experiments. The incubation time was 60 min. GM-CSF gave significant hyperadherence at concentrations from 15 to 120 U/ml ($P<0.01$). IL-3 was significant at 15 ($P<0.02$), 60 and 120 U/ml ($P<0.05$, respectively), while IL-5 gave no significant increase at any concentration.

(Hartnell *et al.*, 1990). Fluoresence intensity was determined on 10,000 cells from each sample using logarithmic amplification, which was converted to the linear equivalent by a Hewlett-Packard Consort 30 computer. Non-specific binding of the mAb was determined by incubating the cells with identical concentrations of mouse myeloma proteins of the same antibody isotype, but with irrelevant specificity. The mean fluorescence of the population was calculated and the value for the control antibody was subtracted from the value of the specific mAb to give a specific mean fluorescence value.

Statistical analysis

All results are expressed as mean±SEM and were analysed for statistical significance using the Student's *t*-test.

RESULTS

Adhesion to PCG: time–course

IL-5, IL-3 and GM-CSF induced optimal eosinophil hyperadherence to PCG at 60 min. Although less adherent cells were observed at 120 min, this was still significantly above the values observed for unstimulated cells. In contrast, PAF gave optimal enhanced adhesion of eosinophils and neutrophils to PCG at 15 min. GM-CSF produced elevated numbers of adherent neutrophils, with IL-3 exerting a lesser effect, while no significant increase in neutrophil adherence to PCG was observed following IL-5 treatment. There were no significant increases in numbers of adherent non-stimulated eosinophils or neutrophils at either the 60 or 120 min time-points (Fig. 1). Neither IL-2 nor lyso-PAF had any effect on eosinophil or neutrophil adhesion (data not shown). In some experiments longer time-points were performed (4 hr or more). However, both eosinophil and neutrophil integrity was disrupted, thus preventing accurate counting.

Table 2. Effect of cycloheximide on resting and stimulated eosinophil adherence to PCG (cells per × 10 HPF). Cells were stimulated for 60 min at 37° ($n=3$)

		Cycloheximide (10^{-6} M)
Resting	307±122	280±113
Stimulated		
PAF (10^{-6} M)	833±105	755±71
IL-5 (60 U/ml)	750±70	720±87
GM-CSF (60 U/ml)	813±178	731±47

Adhesion to PCG: dose–response

The effect of increasing concentrations of IL-5, GM-CSF, IL-3 and IL-2 on eosinophil adhesion to PCG is shown in Fig. 2. Dose-dependent, significant enhancement of eosinophil adhesion to PCG was observed with (ranked in order of potency) IL-5=GM-CSF >IL-3. IL-2 had no measurable effect on eosinophil adhesion. Eosinophil and neutrophil adhesion was also enhanced in a dose-dependent manner by PAF, but not lyso-PAF (Table 1). In addition, hyperadherence was accompanied by marked eosinophil flattening and elongation on the PCG.

The same experiments were performed with neutrophils (Fig. 3). GM-CSF gave large increases in the numbers of adherent neutrophils, whereas IL-3 had lower but significant effects at three concentrations tested. Neither IL-5 nor IL-2 had any significant effect at any of the doses tested.

Adherence to PCG: effect of cycloheximide

The contribution of protein synthesis to enhanced adhesion by eosinophils to PCG was evaluated using the protein synthesis inhibitor cycloheximide. No significant inhibition of resting or stimulated eosinophil adherence was observed (Table 2).

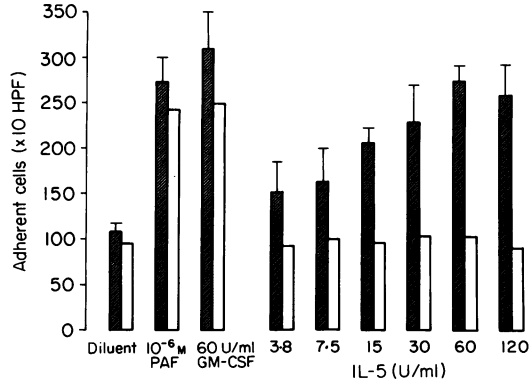

Figure 4. Eosinophil (■) and neutrophil (□) adherence to cultured HMVEC before and after stimulation with increasing concentrations of IL-5. Each bar represents the mean ± SEM of four eosinophil and the mean of two neutrophil experiments. Significant increases in adherence eosinophils were observed at 30 ($P<0.05$), 60 ($P<0.01$) and 120 ($P<0.02$) U/ml of IL-5 and for both PAF ($P<0.01$) and GM-CSF ($P<0.02$).

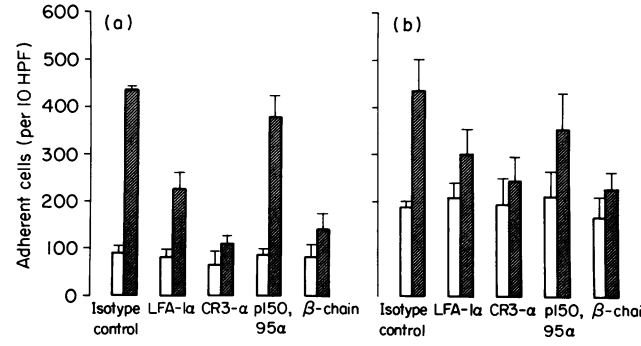

Figure 5. Inhibition of resting or stimulated eosinophil adherence to (a) PCG (PAF, 10^{-6} M, ■) or (b) cultured HMVEC (IL-5, 60 U/ml, ■) by monoclonal antibodies against the leucocyte integrins (CD11/18). Each bar represents the mean ± SEM of four (PCG) or three (HMVEC) experiments. For adherence to PCG, significant inhibition of stimulated eosinophils was given by anti-CR3α ($P<0.01$), anti-β-chain ($P<0.01$) and anti-LFA-1α ($P<0.05$) and for HMVEC by anti-CR3α ($P<0.02$) and anti-β-chain ($P<0.05$). (□) Diluent.

Adherence to HMVEC

The effect of IL-5 on the adhesion of eosinophils to HMVEC is summarized in Fig. 4. Under comparable conditions the number of cells adhering to HMVEC was lower than that observed with the PCG assay. Stimulation with IL-5 gave dose-dependent increases in eosinophil adhesion (optimal concentration 60 U/ml), similar to that observed with PAF (10^{-6} M). In the two experiments performed, IL-5 had negligible effects on neutrophil adherence to HMVEC.

Inhibition of eosinophil adherence with anti-CD11/18 mAb

The contribution of the leucocyte integrins (CD11/18) to the adhesion of eosinophils to PCG or HMVEC was evaluated in inhibition assays using specific mAb (Fig. 5). Enhanced adhesion to PCG (PAF, 10^{-6} M) was significantly inhibited by anti-CR3α ($P<0.01$), anti-common β-chain ($P<0.01$), anti-LFA-1α ($P<0.05$); and for HMVEC (IL-5, 60 U/ml) by anti-β-chain ($P<0.05$) and anti-CR3α ($P<0.02$). Anti-p150,95α and the isotype negative control had no measurable inhibitory effect in either assay. No significant inhibition by mAb of adhesion by unstimulated eosinophils to PCG or HMVEC was observed with any of the mAb tested.

FACS analysis of eosinophil and neutrophil CR3 and LFA-1 expression

Flow cytometry experiments were performed to determine whether eosinophil hyperadherence was associated with up-regulation of CR3α or LFA-1α (Table 3). Significant increases in eosinophil CR3α expression, but not LFA-1α (compared with unstimulated cells maintained at 37°), were observed following preincubation with PAF (10^{-6} M, $P<0.05$), IL-3, IL-5 or GM-CSF (60 U/ml, $P<0.01$, $P<0.05$, $P<0.05$, respectively). In contrast, whereas neutrophil CR3α expression was increased significantly by preincubation with PAF ($P<0.05$) or GM-CSF ($P<0.01$), neither IL-3 nor IL-5 had any significant effect on this cell type. Furthermore, no significant increase in LFA-1 expression by stimulated neutrophils was observed.

DISCUSSION

IL-5, in addition to stimulating eosinophil proliferation from bone marrow-derived precursors (Clutterbuck *et al.*, 1988; Saito *et al.*, 1988), has been shown to enhance various human eosinophil, but not neutrophil, biological activities (Yamaguchi *et al.*, 1988; Lopez *et al.*, 1988). Here we show that IL-5 (and to a lesser extent IL-3) was potent and selective in the enhancement of eosinophil adhesion to PCG and HMVEC. The specific effect of IL-5 on eosinophil adherence and other biological functions presumably reflects the absence of receptors for this cytokine on human neutrophils. The demonstration of a substance specific for eosinophil hyperadherence may have implications for diseases such as allergic asthma and helminthic infections. Asthma is characterized by eosinophil accumulation in and around the airways, while neutrophil numbers are similar to those observed in the normal bronchi (Dunnill, Masseralla & Anderson, 1969). IL-5 has selective, but only weak, chemotactic activity for eosinophils (Wang *et al.*, 1989). Thus, IL-5 may exert its effect by promoting the selective adhesion and extravasation of eosinophils into the tissues, where non-specific, but more potent chemoattractants (such as PAF) might complete their migration into inflammatory foci. Our observations regarding the effect of GM-CSF on neutrophil adherence are at variance with those of Lopez *et al.* (1986b), who reported no increase in the numbers of adherent neutrophils to plastic or HVEC following incubation with GM-CSF. The reasons for this discrepancy are unclear, but may be related to differences in methodology and/or source of materials.

Our findings with PAF agree closely with those of Kimani *et al.* (1988), in both the dose-response and the kinetics of the response. These workers conducted their studies utilizing HVEC and serum-coated plastic plates and also demonstrated inhibition of PAF-induced eosinophil hyperadherence (but not baseline adherence) by an anti-common β-chain (CD18) mAb. We have extended these observations to include the contribution of the individual α-chains of the leucocyte integrin family (LFA-1α, CR3α and p150,95α) to eosinophil adherence. Anti-CR3 and anti-β-chain mAb gave marked inhibition of IL-5- and PAF-induced enhancement of adhesion to HMVEC and PCG.

Table 3. CR3 and LFA-1 expression by resting or stimulated eosinophils and neutrophils ($n=3$)

	Mean fluorescence ($\bar{x} \pm$ SEM)				
	Diluent	PAF (10^{-6} M)	GM-CSF†	IL-3†	IL-5†
Eosinophil CR3	90±12	128±12*	128±3*	113±10**	120±7*
Neutrophil CR3	155±23	257±24*	253±22**	168±39	133±25
Eosinophil LFA-1	41±9	43±9	41±9	42±8	41±9
Neutrophil LFA-1	36±0·3	41±6	38±2	29±8	33±4

* $P<0{\cdot}05$, ** $P<0{\cdot}01$.
† 60 U/ml.

The use of anti-leucocyte integrin mAb has indicated that stimulated neutrophils appear to utilize CR3 (Anderson *et al.*, 1986; Wallis *et al.*, 1986) and, to a lesser extent, LFA-1 (Smith *et al.*, 1988) in adherence reactions. CR3 is known to bind to HVEC, plastic and PCG via a 'lectin-like' site, which is distinct from its passive C3bi binding site (Anderson *et al.*, 1986; Wallis *et al.*, 1986). The adhesive ligand for CR3 on endothelial cells has yet to be identified and the exact nature of the moiety on PCG or plastic to which eosinophils and other phagocytic cells bind is also unclear. Treatment with anti-LFA-1α resulted in approximately 50% inhibition of stimulated adhesion to PCG and approximately 30% to HMVEC, suggesting a role for LFA-1 in PAF and IL-5-induced eosinophil hyperadherence. LFA-1 involvement has been demonstrated in neutrophil hyperadherence (Smith *et al.*, 1988). Two ligands for LFA-1 have been identified, ICAM-1, which is present on stimulated HVEC (Dustin *et al.*, 1986; Marlin & Springer, 1987), and ICAM-2, which is constitutively expressed by HVEC (Staunton, Dustin & Springer, 1989). ICAM-1 has recently been shown to be involved in the adhesion of PAF-stimulated primate eosinophils to stimulated HVEC. Moreover, intravenous treatment with an anti-ICAM-1 mAb attenuated primate allergen-induced airway eosinophilia (Wegner *et al.*, 1990). However, the ligand for LFA-1 involved in eosinophil hyperadherence to PCG and HMVEC is unclear. Anti-p150,95 had no significant effect on adhesion in either assay. Resting normal density human eosinophils and neutrophils express similar levels of the leucocyte integrins (Hartnell *et al.*, 1990). Thus, the similarities observed between eosinophil and neutrophil adhesion reactions are perhaps not surprising.

The baseline adhesion of resting cells to PCG or HMVEC was not significantly influenced by prior incubation with mAb, suggesting that unstimulated cells may adhere via other, perhaps non-specific, mechanisms. Furthermore, evidence of the involvement of other, as yet unknown, ligands was provided by the observation that complete inhibition of stimulated eosinophil adherence was not obtained with the anti-leucocyte integrin mAb. One possibility is the unidentified ligand for endothelial leucocyte adhesion molecule-1 (ELAM-1), an HVEC-specific structure transiently expressed after activation by a number of cytokines, including IL-1 and TNF (Bevilacqua *et al.*, 1987). Our observations suggest a major role for the leucocyte integrins, particularly CR3, in eosinophil hyperadherence to PCG and HMVEC.

We also observed that, as for the neutrophil (Arnaout & Colten, 1984), eosinophils do not increase LFA-1 expression following PAF or cytokine prestimulation over the time-course studied. In addition, eosinophil p150,95 expression showed no measurable up-regulation by PAF (data not shown). There were significant increases in eosinophil CR3 expression of approximately 40% following prestimulation with PAF, IL-3, IL-5 or GM-CSF. This is somewhat lower than that observed for the neutrophil whose CR3 expression is known to be increased two- to 10-fold following cell stimulation with a number of substances (Arnaout & Colten, 1984; Berger *et al.*, 1984), including PAF (Shalit *et al.*, 1988) and GM-CSF (Buckle & Hogg, 1989). Eosinophil CR3 numbers have also been shown to be up-regulated following stimulation with IL-5 (Lopez *et al.*, 1986a). We have extended this observation to include IL-3, which was also found to be eosinophil-specific, and GM-CSF. Up-regulation of neutrophil CR3 expression appears to be a result of recruitment from intracellular stores in the peroxidase-negative granules (Bainton *et al.*, 1987). This may be true for the eosinophil, although granule-associated CR3 has not been demonstrated in this cell.

Although significant, the changes in eosinophil CR3 expression were small and may not account for the marked increase in adhesion. Recent reports have cast doubt on the role of increased receptor expression in neutrophil aggregation and hyperadherence to HVEC (Phillips *et al.*, 1988; Buyon *et al.*, 1988; Vedder & Harlan, 1988). Leucocyte integrin-dependent enhanced neutrophil adhesion may require an increase in affinity due to conformational change in the receptor or as a result of receptor 'clustering' (Detmers *et al.*, 1988), and this may be true for the eosinophil. Indirect evidence for this was provided by the observation that anti-LFA-1α partially inhibited enhanced eosinophil adhesion to PCG and HMVEC, although no enhancement of LFA-1α expression was observed in stimulated eosinophils or neutrophils. Moreover, increased LFA-1-dependent adherence in a number of systems is not associated with increased expression and may be due to a phosphorylation-induced conformational change (Chatila, Geha & Arnaout, 1989).

The optimal incubation time for enhanced eosinophil adhesion by IL-3, IL-5 and GM-CSF was 60 min, whereas that for PAF was much shorter (15 min). The reasons for this difference are unclear, but may be related to variations in stimulator/response coupling. The relatively short optimal time-point (60

min) and the inability of cycloheximide to impair hyperadherence suggest that *de novo* protein synthesis is not required for the up-regulation of adherence by these inflammatory mediators.

In conclusion, our data suggest that *in vitro* eosinophil hyperadherence is similar to that of the neutrophil in terms of their dependence on CD11/18 and involves the utilization of CR3 and, to a lesser extent, LFA-1. Although we observed enhanced expression of CR3α by stimulated eosinophils, the possibility that enhanced eosinophil adhesion is dependent upon an affinity or conformational change in the receptor and not increased expression cannot be excluded. The observation that IL-5 selectively enhanced eosinophil adherence to PCG and HMVEC may provide some insight into the preferential eosinophil accumulation observed in atopic allergic inflammation and infection with helminthic parasites.

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