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Hydrogen peroxide augments eosinophil adhesion via β_2 integrin

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SUMMARY

During eosinophil (EOS) accumulation at sites of allergic inflammation, an initial step is the binding of EOS to adhesion molecules expressed on vascular endothelial cells (EC). We have previously observed that adhesion of peripheral blood EOS to recombinant human vascular cell adhesion molecule-1 (rh-VCAM-1) stimulates the respiratory burst of EOS. Although the biological consequence of this activation remains to be elucidated, reactive oxygen species such as hydrogen peroxide (H_2O_2) may modify the adhesive property of EOS. In the present study, we examined whether H_2O_2 modifies the adhesive property of EOS. EOS were isolated from the peripheral blood of healthy subjects. Adhesion of the EOS to paraformaldehyde-fixed human umbilical vein EC (HUVEC), stimulated or not stimulated with tumour necrosis factor-α (TNF-α; 100 pM for 24 hr), was examined in the presence or absence of H_2O_2. H_2O_2 significantly enhanced adhesion of EOS to both resting and TNF-α-stimulated fixed HUVEC ($P < 0{\cdot}01$, respectively). Such enhancing effects were inhibited by anti-β_2 integrin antibody or anti-CD11b antibody, but not by anti-CD11a or anti-α_4 integrin antibody. H_2O_2 also enhanced EOS adhesion to rh-intracellular cell adhesion molecule-1 (ICAM-1) but not to rh-VCAM-1. Finally, H_2O_2 enhanced the expression of both CD11b and CD18 on EOS. These results indicate that H_2O_2 directly augments the adhesive property of EOS through β_2 integrin.

INTRODUCTION

Eosinophils (EOS) preferentially accumulate at sites of allergic inflammation and because of this probably play crucial roles in the pathophysiology of diseases, such as asthma, through the release of a variety of inflammatory mediators.[1,2] For EOS to participate in allergic inflammation, it is necessary that they migrate from the peripheral circulation into the tissues. An initial step of this process is the interaction of circulating EOS with adhesion proteins expressed on the surface of vascular endothelial cells (EC). Accumulating evidences have established that EOS integrin adhesion molecules such as α_4 integrins, including $\alpha_4\beta_1$ (very late activation antigen-4 [VLA-4], CD49d/CD29), and β_2 integrins, including $\alpha_L\beta_2$ (lymphocyte function-associated antigen-1 [LFA-1], CD11a/CD18) and $\alpha_M\beta_2$ (Mac-1, CD11b/CD18), play important roles in the adhesion to, and the transmigration through, EC.[3,4] Vascular cell adhesion molecule (VCAM)-1, a counter ligand for $\alpha_4\beta_1$, is a potent inducer of spontaneous adhesion of blood EOS *in vitro*.[5,6] Administration of anti-$\alpha_4\beta_1$ antibodies (Ab) *in vivo* inhibits the accumulation of EOS induced by soluble inflammatory mediators and the antigen-induced EOS infiltration into guinea-pig skin or mouse trachea.[7,8] Monoclonal Ab (mAb) against intracellular cell adhesion molecule-1 (ICAM-1), a ligand for β_2 integrins, also inhibited EOS airway infiltration in a primate model of asthma.[9] Although recombinant human (rh)-ICAM-1 requires second signals, such as granulocyte–macrophage colony-stimulating factor (GM-CSF) or Mn^{2+}, for inducing adhesion of blood EOS,[10–12] it has been shown that the β_2 integrin/ICAM-1 pathway is essentially involved in EOS transmigration across EC monolayers *in vitro*.[13,14]

We have previously reported that adhesion of EOS to rh-VCAM-1 activates a respiratory burst of EOS.[6] This suggests that the interaction between EOS α_4 integrin and endothelial VCAM-1 is an important step, not only for selective EOS recruitment into the tissue but also as an initial action in the activation of EOS effector functions. The biological consequences of the activation of such a specific effector function remains unknown; however, it is possible that the released oxygen species modify either the morphological or functional

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Abbreviations: EC, endothelial cells; EOS, eosinophils; EPO, eosinophil peroxidase; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; H_2O_2, hydrogen peroxidase; HUVEC, human umbilical vein endothelial cells; ICAM-1, intracellular cell adhesion molecule-1; PAF, platelet-activating factor; VCAM-1, vascular cell adhesion molecule-1.

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status of EC, such as the permeability, adhesion molecule expression and adhesiveness to leucocytes.[15–17] Oxygen species also alter the functional status of leucocytes. For example, hydrogen peroxide (H_2O_2) promotes adhesion of neutrophils[18] and monoblastoid U-937 cells[19] via CD11b/CD18 molecules. Here we report that H_2O_2 enhances EOS adhesion to both resting and tumour necrosis factor-α (TNF-α)-stimulated EC through the enhanced expression and activation of β_2 integrin of EOS.

MATERIALS AND METHODS

Reagents

Percoll was purchased from Pharmacia (Uppsala, Sweden). Hanks' balanced salt solution (HBSS), newborn calf serum (NCS), fetal calf serum (FCS), trypsin–EDTA, L-glutamine and penicillin–streptomycin were obtained from Life Technologies (Grand Island, NY). Anti-CD16 mAb-coated magnetic microbeads were obtained from Miltenyl Biotec (Auburn, CA). H_2O_2 was obtained from Wako Pure Medical Co. (Osaka, Japan). Human umbilical vein endothelial cells (HUVEC) and endothelial cell basal medium (EBM) medium were obtained from Clonetics (Palo Alto, CA). rh-TNF-α, rh-VCAM-1 and rh-ICAM-1 were obtained from R & D Systems (Minneapolis, MN). Other reagents were purchased from Sigma (St. Louis, MO) unless stated otherwise.

Separation of EOS

EOS were isolated from peripheral blood specimens, of normal volunteers, that contained less than 5% EOS in the total leucocytes. Subjects ranged in age from 26 to 39 years, and gender distribution was equal. Isolation of EOS was performed by negative immunomagnetic bead selection, as previously described.[14] Briefly, heparinized blood was diluted with HBSS without Ca^{2+} and centrifuged for 20 min at 700 *g* over 1·090 g/ml Percoll. Plasma, the mononuclear cell band and Percoll were removed, and the red blood cells in the pellet were lysed by hypotonic shock. The resulting granulocytes were washed with HBSS (chilled to 4°) supplemented with 2% NCS (HBSS/NCS), then incubated with anti-CD16 Ab-coated magnetic beads for 40 min at 4°. The cells were filtered through a steel wool column in a magnetic field (Miltenyl Biotec) to remove neutrophils bound to magnetic beads. CD16-negative EOS (>98% pure and >99% viable) were collected, washed and resuspended in HBSS supplemented with 5% FCS (HBSS/FCS).

Preparation of HUVEC

HUVEC were cultured on type-IV collagen-coated tissue-culture flasks. When confluent, HUVEC were passaged into collagen-coated 96-well-tissue culture plates, and either medium alone or TNF-α (at 100 pM) was added and culture continued for 24 hr in 5% CO_2 at 37°. Following culture, the incubated mixture was decanted and the HUVEC were washed three times with HBSS. HUVEC were fixed with 100 µl of 1% paraformaldehyde in phosphate-buffered saline (PBS) at ambient temperature for 15 min to block the generation of mediators. After washing these cells three times with HBSS, 200 µl of 1% glycine in HBSS was added and incubated at ambient temperature for 1 hr to quench the residual paraformaldehyde. Plates were then decanted and washed three times with HBSS before use.

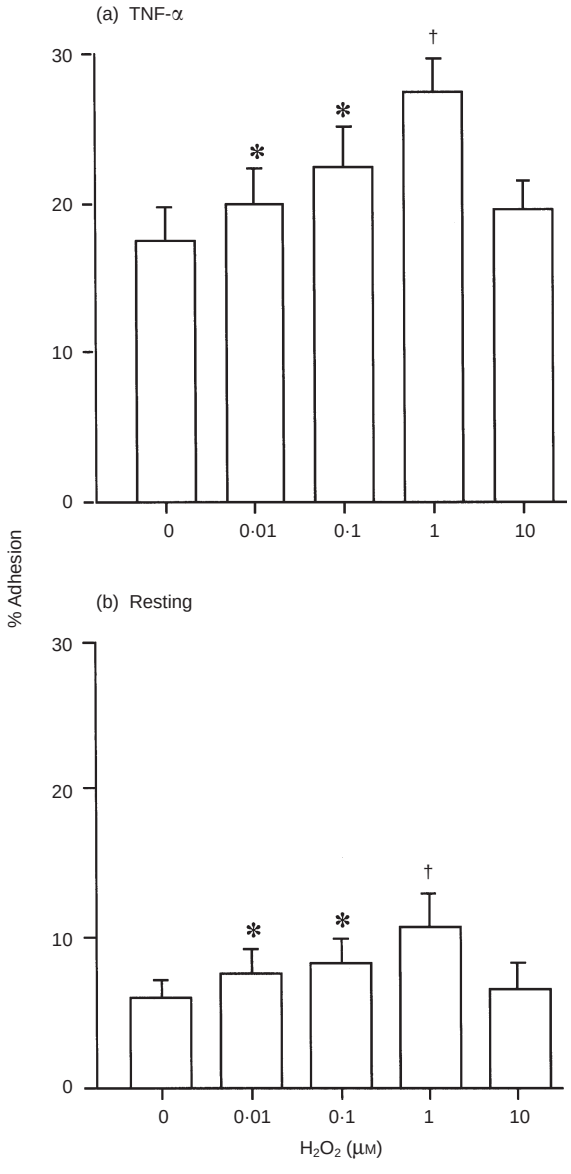

Figure 1. Effect of H_2O_2 on eosinophil (EOS) adhesion to tumour necrosis factor-α (TNF-α)-stimulated (a) and resting (b) fixed, human umbilical vein endothelial cells (HUVEC). EOS were incubated in the presence or absence of H_2O_2 for 30 min at 37°. $*P<0{\cdot}05$, $\dagger P<0{\cdot}01$ versus control (no H_2O_2), $n=8$, respectively.

EOS adhesion assay

EOS adhesion was assessed as residual EOS peroxidase (EPO) activity of adherent EOS, as previously described.[6,10,14] Briefly, EOS (100 µl of 1×10^5 cells/ml in HBSS/FCS) were placed onto HUVEC monolayers or rh-adhesion molecule-coated plates, in the presence or absence of an activator, and incubated for 30 min at 37°. After five washes with HBSS (preincubated to 37°), 100 µl of HBSS/FCS was added to the reaction wells. As standards, 100 µl of serially diluted cell suspension (1×10^3, 3×10^3, 1×10^4, 3×10^4 and 1×10^5 cells/ml) was added to empty wells. EPO substrate (Tris buffer, pH 8·0, containing

1 mM *o*-phenylenediamine, 1 mM H_2O_2 and 0·1% Triton-X-100) was then added to all of the wells. After a 30-min incubation at room temperature, 50 µl of 4-M H_2SO_4 was added to stop the reaction, and the absorbance at 490 nm was measured. Percent EOS adhesion was calculated from the log dose–response curve. EOS viability after the incubation exceeded 98%, as assessed by using Trypan Blue dye exclusion.

Expression of CD11b and CD18 on EOS

The expression of CD11b and CD18 on EOS was examined by flow cytometric analysis. EOS were exposed to either 1 µM H_2O_2 or buffer (HBSS/5% FCS) alone for 30 min at 37°. The cells were washed three times and resuspended in PBS (chilled to 4°) supplemented with 2% bovine serum albumin (BSA) and 0·2% sodium azide (fluorescence-activated cell sorter [FACS] buffer). Cells (1×10^5/100 µl) were incubated with fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD18 (clone MHM23, immunoglobulin G1 [IgG1]; Dako A/S, Glostup, Denmark) and/or phycoerythrin (PE)-conjugated mouse anti-human C3bi (CD11b) (clone 2LPM19c, IgG1; Dako A/S) on ice for 30 min. PE- and FITC-conjugated mouse IgG1 (Dako A/S) were used as isotype-matched controls. Cells were then washed and resuspended in FACS buffer. Mean fluorescence was measured on at least 10 000 events using a fluorescence-activated cell sorter (FACScan; Becton-Dickinson, San Jose, CA, USA). Relative mean fluorescence was determined by subtraction of values for the IgG1 isotype-matched control.

Statistics

Data are presented as mean ± SEM. For statistical analysis, the Student's *t*-test was used for paired comparisons and analysis of variance (ANOVA) for repeated measures; Scheffe constants were used for comparison of more than two variables to determine a significance. A *P*-value of less than 0·05 was considered significant.

RESULTS

Effect of H_2O_2 on EOS adhesion

Initial experiments were conducted to determine whether H_2O_2 modifies adhesion of EOS to paraformaldehyde-fixed HUVEC. After 30 min of incubation, 0·01–1 µM H_2O_2 significantly enhanced adhesion of EOS to TNF-α-stimulated (100 pM, 24 hr) HUVEC (0·01 µM, $P<0·05$; 0·1 µM, $P<0·05$; 1 µM, $P<0·01$; $n=8$, respectively; Fig. 1a). Similarly, H_2O_2 promoted adhesion of EOS to resting HUVEC (0·01 µM, $P<0·05$; 0·1 µM, $P<0·05$; 1 µM, $P<0·01$; $n=8$, respectively; Fig. 1b). In both experiments the maximum effect of H_2O_2 was obtained at 1 µM (% adhesion: TNF-α-stimulated HUVEC, 27·4 ± 2·6%; resting HUVEC 10·8 ± 2·5%) and was comparable to the maximum effect of *N*-formyl-methionyl-leucyl-phenylalanine (FMLP, 1 µM: TNF-α-stimulated HUVEC, 27·0 ± 2·2%; resting HUVEC, 10·9 ± 3·3%, $n=5$). Although 10 µM H_2O_2 did not augment EOS adhesion, the cell viability exceeded 98% under this experimental condition. Moreover, 1–10 µM H_2O_2 did not modify the pH of the assay buffer (data not shown). To confirm the specificity of the H_2O_2 effect, EOS were exposed to 1 µM H_2O_2 in the presence or absence of catalase (from *Aspergillus nigar*; Sigma), which rapidly degrades H_2O_2. The adhesion to both TNF-α-stimulated and resting HUVEC was then determined: EOS adhesion augmented by 1 µM H_2O_2 was blocked by 10 U/ml catalase (% inhibition of adhesion to TNF-α-stimulated HUVEC, 90·7 ± 3·4%, Fig. 2a; % inhibition of adhesion to resting HUVEC, 97·2 ± 2·6%, $n=5$, respectively, Fig. 2b). H_2O_2 at 1 µM also augmented eosinophil adhesion to unfixed TNF-α-stimulated and resting HUVEC, and adhesion to both was blocked by 10 U/ml catalase (data not shown).

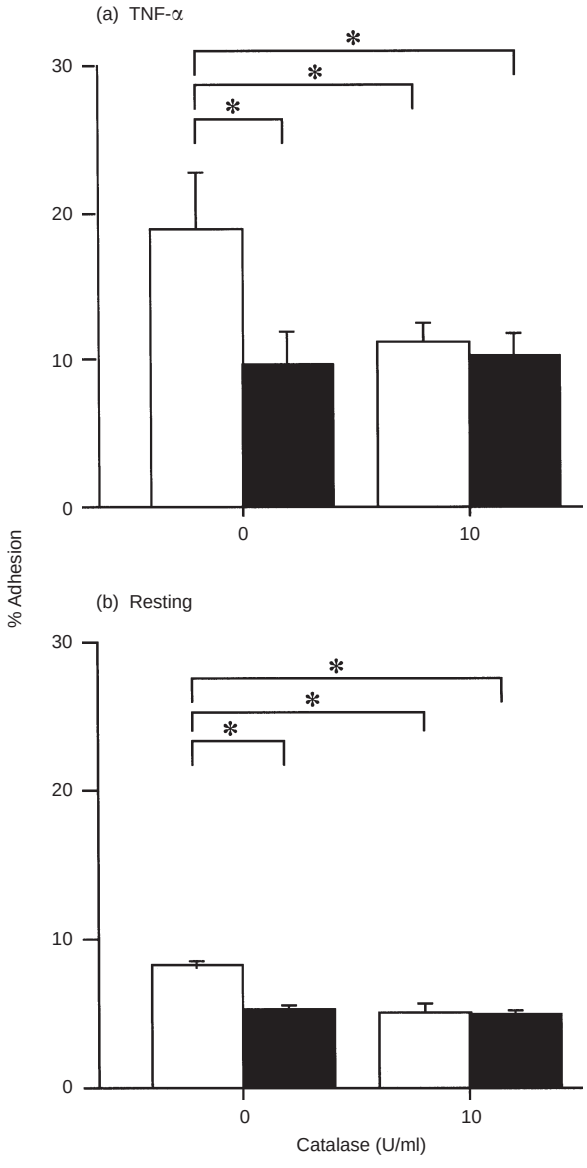

Figure 2. Effect of 10 U/ml of catalase on eosinophil (EOS) adhesion to tumour necrosis factor-α (TNF-α)-stimulated (a) and resting (b) fixed, human umbilical vein endothelial cells (HUVEC). Open bars represent adhesion of EOS in the presence of 1 µM H_2O_2. Closed bars represent adhesion of EOS in the absence of H_2O_2. *$P<0·01$, $n=5$, respectively.

To examine whether H_2O_2 directly affects the adhesive property of EOS, EOS adhesion to 1% BSA-coated microassay plates was examined: 1 µM H_2O_2 augmented the adhesion (buffer control, 16·2 ± 2·5% versus H_2O_2, 25·1 ± 2·3%; $P=0·001$, $n=6$).

The reaction kinetics of the H_2O_2 effect on adhesion of EOS

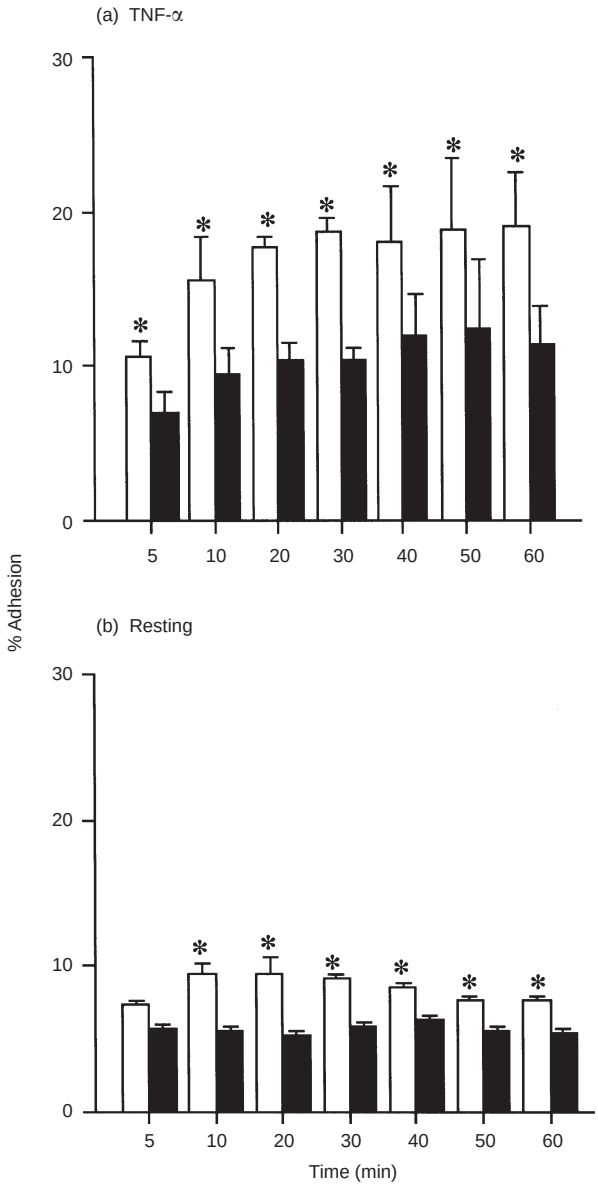

Figure 3. Kinetics of the H_2O_2 effect on adhesion of eosinophils (EOS) to tumour necrosis factor-α (TNF-α)-stimulated-(a) ($n=4$) and resting (b) ($n=4$) human umbilical vein endothelial cells (HUVEC). Open bars represent adhesion of EOS in the presence of 1 μM H_2O_2. Closed bars represent adhesion of EOS in the absence of H_2O_2. *$P<0{\cdot}05$.

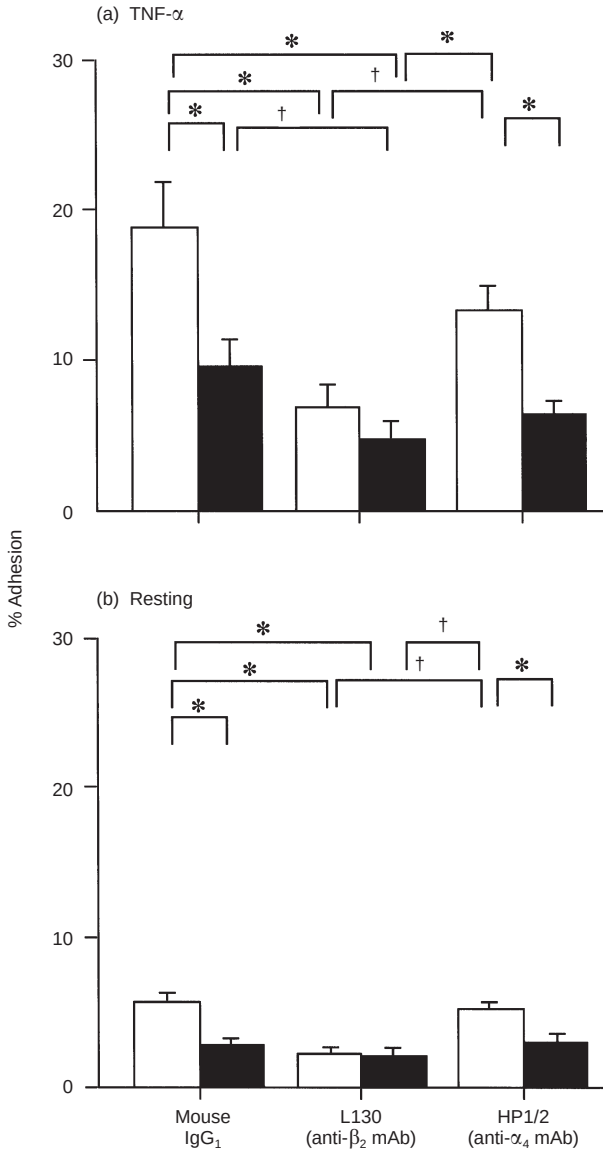

Figure 4. Effects of anti-β_2 integrin monoclonal antibody (mAb) (L130) and anti-α_4 integrin mAb (HP1/2) on adhesion of eosinophils (EOS) to tumour necrosis factor-α (TNF-α)-stimulated (a) and resting (b) human umbilical vein endothelial cells (HUVEC). Mouse immunoglobulin G1 (IgG1) is an isotype-matched control. Open bars represent adhesion of EOS in the presence of 1 μM H_2O_2. Closed bars represent adhesion of EOS in the absence of H_2O_2. *$P<0{\cdot}01$, †$P<0{\cdot}05$, $n=5$, respectively.

was examined using TNF-α-stimulated HUVEC. The H_2O_2 effect was observed at 5 min after addition of H_2O_2 and lasted for at least 60 min ($P<0{\cdot}05$ at each time-point examined, $n=4$; Fig. 3a). The H_2O_2 effect on adhesion of EOS to the resting HUVEC was observed at 10 min after addition of H_2O_2 and lasted for 60 min ($P<0{\cdot}05$ at each time-point examined except 5 min, $n=4$; Fig. 3b).

Effect of anti-integrin mAbs on the H_2O_2-induced adhesion of EOS

To identify the EOS integrin(s) involved in the H_2O_2 effect, EOS were pretreated with either anti-α_4 integrin mAb (clone HP1/2, mouse IgG1, 3 μg/ml; Cosmo Bio., Tokyo, Japan), anti-β_2 integrin mAb (clone L130, mouse IgG1, 3 μg/ml; Becton-Dickinson) or an isotype-matched control mouse IgG1 (3 μg/ml; Cappel, Aurora, Ohio, USA) at ambient temperature for 15 min, and then EOS adhesion was examined. H_2O_2 at 1 μM significantly enhanced the adhesion of EOS to TNF-α-stimulated HUVEC in the presence of mouse IgG1 or anti-α_4 Ab ($P<0{\cdot}01$, $n=5$, respectively, Fig. 4a). In contrast, the H_2O_2 effect was blocked by anti-β_2 mAb (Fig. 4a). Similarly, H_2O_2 enhanced the EOS adhesion to resting HUVEC in the presence of mouse IgG1 or anti-α_4 Ab ($P<0{\cdot}01$, $n=3$, respectively), but the effect was blocked by anti-β_2 Ab (Fig. 4b). To further confirm whether H_2O_2 enhanced the adhesion via β_2 integrin,

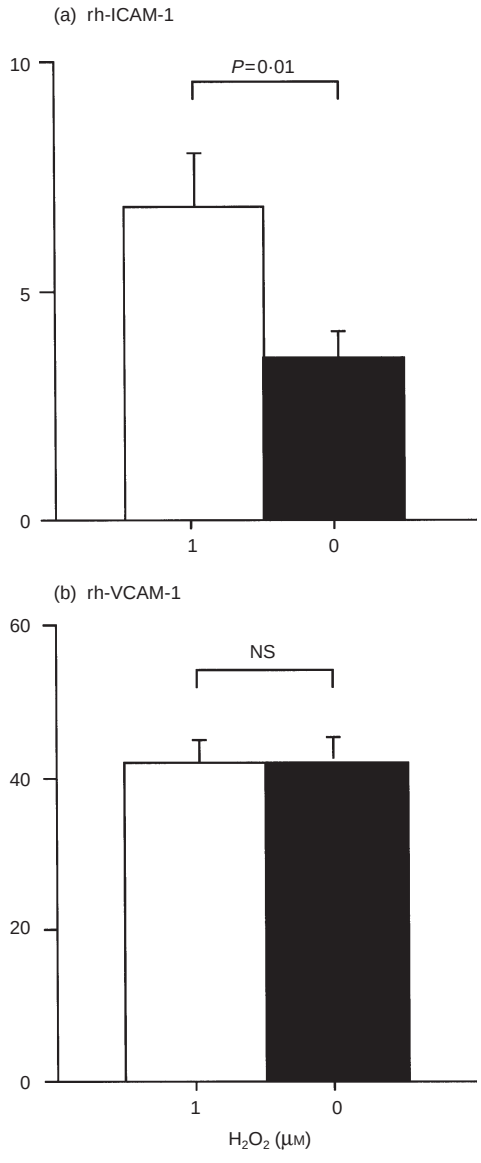

Figure 5. Effect of 1 μM H_2O_2 on adhesion of eosinophils (EOS) to recombinant human intracellular cell adhesion molecule-1 (rh-ICAM-1) (a) and rh-vascular cell adhesion molecule-1 (VCAM-1) (b). Open bars represent adhesion in the presence of 1 μM H_2O_2. Closed bars represent adhesion in the absence of H_2O_2. $n=5$, respectively.

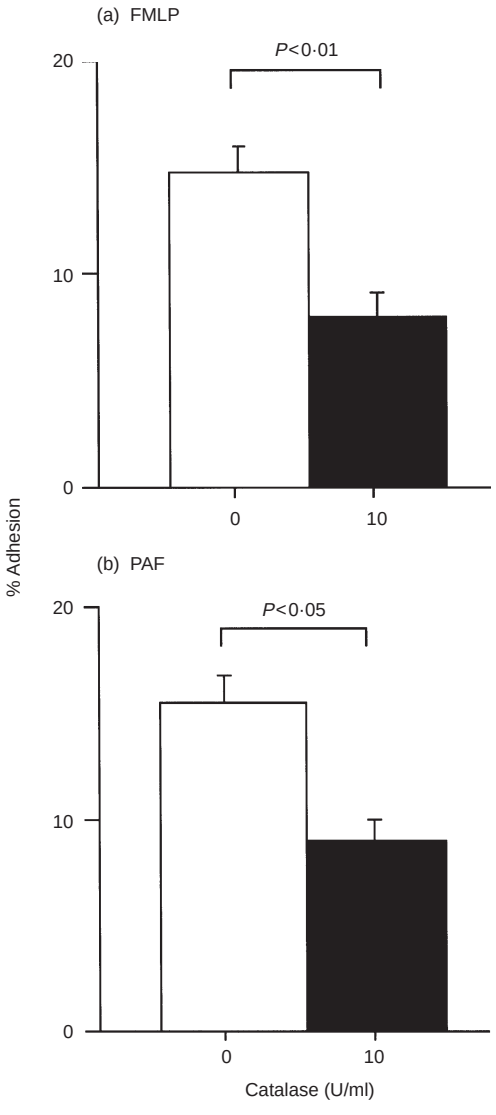

Figure 6. Effect of 10 U/ml catalase on adhesion of eosinophils (EOS) induced by *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) (a) and platelet-activating factor (PAF) (b) (both at 1 μM). Open bars represent adhesion of EOS in the presence of 1 μM H_2O_2. Closed bars represent adhesion of EOS in the absence of H_2O_2. $n=5$, respectively.

adhesion of EOS to immobilized rh-ICAM-1, a ligand for β_2 integrin, or to rh-VCAM-1, a ligand for α_4 integrin, was examined: 1 μM H_2O_2 enhanced adhesion of EOS only to rh-ICAM-1 (rh-ICAM-1, $3{\cdot}5\pm0{\cdot}7\%$ in medium alone versus $6{\cdot}8\pm1{\cdot}3\%$ in medium + H_2O_2, $P=0{\cdot}01$, Fig. 5a; rh-VCAM-1, $41{\cdot}8\pm3{\cdot}6\%$ in medium alone versus $41{\cdot}6\pm4{\cdot}0\%$ in medium + H_2O_2, $P=$NS, $n=5$, Fig. 5b).

To further identify EOS β_2 integrin(s) involved in the H_2O_2 effect, EOS were pretreated with either anti-CD11a mAb (clone G25.2, mouse IgG2a, 5 μg/ml; Becton-Dickinson), anti-CD11b mAb (clone D12, mouse IgG2a, 5 μg/ml; Becton-Dickinson), or isotype-control mouse IgG2a (5 μg/ml; Cappel), and then EOS adhesion was examined in the presence or absence of 1 μM H_2O_2. The EOS adhesion augmented by H_2O_2 was partially inhibited by anti-CD11b antibody (% inhibition: adhesion to TNF-α-stimulated HUVEC, $60{\cdot}3\pm18{\cdot}1\%$; adhesion to resting HUVEC, $68{\cdot}6\pm16{\cdot}0\%$; $P<0{\cdot}01$, $n=5$, respectively; results not shown). On the other hand, neither anti-CD11a antibody nor mouse IgG2a modified the augmented EOS adhesion to TNF-α-stimulated or resting HUVEC (data not shown). The effect of the combination of anti-CD11a and anti-CD11b did not differ from anti-CD11b antibody alone (data not shown).

Effect of H_2O_2 on EOS expression of CD11b and CD18

To examine whether H_2O_2 modifies the number of CD11b or CD18 molecules on EOS, the expression of those molecules was determined by flow cytometric analysis. Following stimulation with 1 μM H_2O_2, the expression of both CD11b and CD18 was enhanced (mean fluorescence index [MFI]: CD11b,

204·0 ± 26·3, control, versus 236·0 ± 24·0, H_2O_2, $P < 0·05$; CD18, 48·0 ± 8·3, control, versus 56·0 ± 6·3, H_2O_2, $P < 0·01$, $n = 3$, respectively; results not shown).

Role of H_2O_2 as an autocrine mediator for EOS adhesion

Because EOS are capable of generating radical oxygen species in response to a variety of inflammatory mediators, endogenous H_2O_2 may play roles in the development of stimulus-dependent adhesion of EOS in an autocrine mechanism. To examine this possibility, adhesion of EOS to paraformaldehyde-fixed resting HUVEC in response to GM-CSF, phorbol myristate acetate (PMA), FMLP, or platelet-activating factor (PAF), was examined in the presence or absense of 10 U/ml of catalase. Catalase did not modify spontaneous-, 100 pM GM-CSF-induced, or 1 ng/ml phorbol 12-myristate 13-acetate (PMA)-induced adhesion (control versus catalase: spontaneous, 5·2 ± 0·9% versus 5·1 ± 0·8%; GM-CSF, 12·2 ± 1·4% versus 12·5 ± 2·8%; PMA, 21·6 ± 3·7% versus 22·2 ± 4·2%; $n = 5$, $P =$ NS, respectively; results not shown). In contrast, EOS adhesion induced by both FMLP and PAF (both at 1 μM) was partially inhibited by 10 U/ml of catalase (control versus catalase: FMLP, 14·8 ± 1·4% versus 8·0 ± 1·3%, $P < 0·01$, Fig. 6a; PAF, 15·4 ± 1·6% versus 9·0 ± 1·4%, $P < 0·05$, $n = 5$, Fig. 6b, respectively).

DISCUSSION

Our findings extend earlier observations concerning interaction between EOS and endothelial adhesion molecules,[3,4,6,10] and suggest the potentially important biological consequence of the EOS respiratory burst during this process. First, nanomolar concentrations of H_2O_2 induced EOS adhesion to resting EC. Second, H_2O_2 also promoted adhesion of EOS to EC activated with a proinflammatory cytokine, TNF-α. The H_2O_2 effects appear to involve EOS β_2 integrins, particularly CD11b/CD18. The counter ligands on HUVEC for the enhanced adhesion are postulated to be ICAMs, because CD11b/CD18 binds to ICAM-1, ICAM-2, and ICAM-3.[3,4] Indeed, H_2O_2 augmented adhesion of EOS to rh-ICAM-1. Although EOS α_4 integrin(s) could also interact with endothelial adhesion proteins such as VCAM-1, the anti-α_4 mAb did not affect the H_2O_2 -induced EOS adhesion. Furthermore, H_2O_2 did not enhance adhesion of EOS to rh-VCAM-1, indicating that α_4 integrin is not involved in the H_2O_2-induced adhesion. Finally, we have provided evidence that H_2O_2 directly enhances eosinophil expression of CD11b and CD18. Although both the number and functional state of integrins can be regulated by a variety of mediators[3,4,20,21] our data suggest that the increased expressions of these molecules are, at least in part, involved in the enhancing effects for EOS adhesion. Intracellular signalling mechanisms associated with the H_2O_2 effect were not evaluated in this study, but should be clarified in future work.

At the sites of allergic inflammation, multiple inflammatory cells, including EOS and macrophages, may generate oxygen species.[22,23] It has been reported that bronchoalveolar lavage (BAL) cells obtained following an antigen challenge generated 8–10 μM superoxide anion.[23] EOS obtained from BAL following a segmental antigen challenge possess a greater ability to generate superoxide anion, as well as to induce adhesion and expression of CD11b, than that shown by EOS in peripheral circulation.[24] H_2O_2 can be detected at micromolar concentrations in normal human sera.[25] Although the precise concentration of oxygen species at the inflammatory sites remains unknown, these findings suggest that it is possible to achieve an H_2O_2 concentration similar to that used in this study at an allergic inflammation site.

As EOS are capable of generating radical oxygen species in response to a wide range of inflammatory mediators, EOS-derived H_2O_2 may play roles in the development of adhesive property of the cells. In this context, we observed that both FMLP- and PAF-induced EOS adhesion was partially inhibited by catalase while such an inhibitory effect was not observed when GM-CSF or PMA was the stimulant. These results suggest that EOS-derived H_2O_2 may be involved in the development of EOS adhesion as an autocrine/paracrine mediator *in vivo* when certain inflammatory mediators, such as FMLP or PAF, are involved.

Our observations agree with earlier work on other types of cells. Skogkund *et al.* reported that 30–300 μM H_2O_2 induced the adhesion of monoblastoid U-937 cells to plastic, and that this adhesion was inhibited by mAb against CD11b and CD18, but not by mAb against CD11a or CD11c.[19] Fraticelli *et al.* reported that H_2O_2 caused the up-regulation of CD11b and CD18 on neutrophils concurrently with a shedding of L-selectin and increased the adherence of neutrophils to EC.[18] These reports and our present observations suggest that H_2O_2 can induce a direct proadhesive effect on certain types of leucocytes, including EOS, via enhanced expression of CD11b/CD18.

Our observations raise possibilities as to the biological significance of the expression and function of EOS β_2 integrin during interaction with H_2O_2. When activated, a variety of inflammatory cells, including EOS, are capable of generating radical oxygen species at sites of allergic inflammation, and therefore EOS will probably be exposed to H_2O_2. Meanwhile, H_2O_2 enhances the expression of ICAM-1 on EC and increases the ability of EC to potentiate leucocyte adhesion.[15,16] Thus, H_2O_2 alters the adhesive property of both EOS and EC, and thereby augments the interaction between these two kinds of cells in a β_2 integrin-dependent manner. The effect of H_2O_2 on EOS β_2 integrin may also modify the subsequent transmigration of EOS across EC, because it has been well documented that the β_2 integrin/ICAM-1 pathway is crucial in this process.[13,14] Thus, H_2O_2 may modify the adhesion and transendothelial migration of EOS, and contribute to the phenotypic change of EOS in the airway. These effects would contribute to the eventual manifestations of inflammation in allergic diseases such as asthma.

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