Induction of Intercellular Adhesion Molecule 1 (ICAM-1) Expression in Normal Human Eosinophils by Inflammatory Cytokines

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Intercellular adhesion molecule-1 (ICAM-1) functions as a ligand for lymphocyte function–associated antigen-1 (LFA-1), and thereby plays a crucial role in mediating cell-cell interactions in inflammatory reactions. Human eosinophils represent important effector cells in allergic skin diseases. To gain more insight into the capacity of eosinophils to physically interact with LFA-1–positive inflammatory leukocytes, in the present study ICAM-1 expression in eosinophils was investigated. Using fluorescence-activated cell sorter analysis, it could be shown that highly purified (≥95%) eosinophils from peripheral blood of non-atopic individuals do not constitutively express ICAM-1 molecules. However, stimulation of eosinophils with interferon gamma (IFNγ), tumor-necrosis factor alpha (TNFα), or interleukin 3 (IL-3) markedly upregulated ICAM-1 surface expression in a time- and dose-dependent manner. Cytokine-induced ICAM-1 expression in human eosinophils was corroborated by Northern blot analysis. Accordingly, unstimulated eosinophils did not express significant amounts of ICAM-1 mRNA, but ICAM-1 mRNA expression could be markedly induced in these cells upon stimulation with IFNγ plus TNFα. The combination of TNFα with either IFNγ, IL-3, IL-5, or granulocyte/macrophage colony-stimulating factor (GM-CSF) increased ICAM-1 expression in a synergistic fashion, whereas IL-5 or GM-CSF by itself did not induce ICAM-1 expression. Cytokine-induced ICAM-1 expression was specific, because IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, C5a, and platelet-activating factor did not significantly affect eosinophil ICAM-1 surface expression. In summary, these studies indicate that eosinophils may be activated to express the adhesion molecule ICAM-1 upon stimulation with selected inflammatory cytokines, which may allow adhesion-mediated cross-talk between eosinophils and LFA-1–positive cells. In addition, these data demonstrate for the first time a role for IL-3, IL-5, and GM-CSF in regulation of ICAM-1 expression in human cells. *J Invest Dermatol 100:417–423, 1993*

Blood and tissue eosinophilia are major features of allergic reactions and parasitic infestations [1–3]. Accordingly, in allergic asthma an increased number of low-density eosinophils in the blood and signs of eosinophil degranulation and mediator release into lung tissue, associated with decreased lung function, could be demonstrated [4–7]. In addition, there is evidence for eosinophil activation in lesional skin in urticaria and bullous pemphigoid [8]. In atopic dermatitis, eosinophil accumulation in the dermis after patch testing with aeroallergens has been found to be a prominent feature [9]. Moreover, eosinophil-derived granule proteins such as eosinophil cationic protein (ECP) or major basic protein are present in lesional atopic skin, and ECP serum levels were found to correlate with disease activity in atopic dermatitis. Taken together, these studies indicate an active participation of eosinophils in the pathogenesis of atopic dermatitis [8,10,11]

Intercellular adhesion molecule-1 (ICAM-1) plays a pivotal role for the development of inflammatory responses [12]. ICAM-1 is an immunoglobulin-like 90-kDa transmembrane glycoprotein [13] that is highly inducible in a variety of cells including endothelial cells, fibroblasts, monocytes, melanocytes, and keratinocytes upon appropriate cytokine stimulation [14]. The function of ICAM-1 in immunologic processes is mainly given by its capacity to serve as a ligand for lymphocyte function–associated antigen-1 (LFA-1), and ICAM-1 expression may be an important prerequisite for the capacity of a given cell type to physically interact with LFA-1–positive cells such as cytotoxic or helper T cells, Langerhans cells, monocytes, or granulocytes [14,15]. Studies assessing ICAM-1 expression in human eosinophils are controversial. Previous reports showed that peripheral blood eosinophils by themselves do not express ICAM-1 on their cell surface [16,17]. This is in contrast to a recent report indicating that sputum eosinophils from symptomatic asthmatics express significant amounts of ICAM-1 [18]. To clarify ICAM-1 expression in human eosinophils, in the present study, ICAM-1 surface and mRNA expression in a first attempt was as-

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Abbreviations:
BSA: bovine serum albumin
DMSO: dimethylsulfoxide
ECP: eosinophil cationic protein
GM-CSF: granulocyte/macrophage colony-stimulating factor
HBSS: Hank's balanced salt solution
ICAM-1: intercellular adhesion molecule-1
IFNγ: interferon gamma
Ig: immunoglobulin
IL: interleukin
LFA-1: lymphocyte function–associated antigen-1
MoAb: monoclonal antibody
PAF: platelet-activating factor
PBS: phosphate-buffered saline
PMA: phorbol-myristate acetate
PMN: polymorphonuclear granulocytes
rh: recombinant human
TNF: tumor-necrosis factor

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sessed *in vitro* in highly purified blood eosinophils from non-atopic individuals.

MATERIALS AND METHODS

Control subjects Non-atopic healthy blood donors (n = 5) were investigated. Atopy was excluded by history, skin prick tests, and laboratory findings. Accordingly, total immunoglobulin E (IgE) and specific IgE to timothy, cultivated rye, common silver birch, mugwort, cat, cladosporium herbarum, and dermatophagoides pteronissinus were determined in sera using the CAP IgE FEIA (Kabi-Pharmacia, Freiburg, Germany). The donors did not receive any medication for at least 4 weeks prior to blood collection.

Cytokines and Reagents *Escherichia* coli–derived recombinant human (rh) tumor necrosis factor α (TNFα) and rh TNFβ were produced by BASF, Ludwigshafen, Germany, with a specific activity of 8.5×10^6 U/mg and 6×10^7 U/mg protein for TNFα and TNFβ, respectively [19]. Recombinant human interleukin-5 (IL-5) with a specific activity of 10^3 U/μg was kindly provided by the Glaxo Institute for Molecular Biology, Geneva, Switzerland. Recombinant human IL-8 was a kind gift from the Sandoz Research Institute, Vienna, Austria. Recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF) with a specific activity of 5×10^7 U/mg was kindly provided by Behring-Werke, Marburg, Germany. Recombinant human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, and IL-7 were purchased from Genzyme, Boston, MA. Before testing, all cytokines were diluted in phosphate-buffered saline (PBS) containing 50 μg/ml bovine serum albumin (BSA). *E. coli*–derived rh C5a and platelet-activating factor (PAF) were obtained from Sigma, Munich, Germany. A stock solution of 10^{-2} M PAF was prepared in ethanol dimethylsulfoxide (DMSO) 50:50 [v/v] and stored at −70°C; dilutions were made in PBS containing 1 mg/ml BSA. Phorbol-myristate acetate (PMA) and purified BSA were obtained from Sigma, Munich, Germany. S-form lipopolysaccharide (LPS) from Salmonella abortus equi was a kind gift from Dr. C. Galanos, MPI for Immunobiology, Freiburg, Germany.

Antibodies A mouse monoclonal antibody (MoAb) against CD16 (BW 209/2) was a gift from Dr. R. Kurrle, Behring-Werke, Marburg, Germany. Mouse IgG1 MoAb against ICAM-2 (6D5) was kindly provided by Dr. C.A. Gahmberg, Helsinki, Finnland. Mouse IgG1 MoAb against ICAM-1/CD54 (clone 84H10, Balb/c spleen cells × myeloma MOPC 315), against LFA-1 gp 170/CD11a (clone 25.3.1, myeloma × 63 − Ag 8.653 × Balb/c spleen cells), against LFA-1 β-chain/CD18 (clone BL5, SP2/0 − Ag.14 × Balb/c spleen cells) and mouse IgG1 isotype control antibody (myeloma × 63 Ag.8 × Balb/c spleen cells) were purchased by Immunotech S.A., Marseille, France. Fluorescein-conjugated goat anti-mouse Fab_2 (anti-mouse IgG) purified by immunoaffinity chromatography was obtained from Dianova, Hamburg, Germany.

Isolation and Purification of Human Eosinophil Granulocytes Human granulocytes were isolated from heparin-anticoagulated venous blood of normal non-atopic blood donors as described [20] by ficoll-gradient centrifugation (Kabi-Pharmacia, Freiburg, Germany) and three 30-second cycles of 0.2% NaCl treatment followed by the addition of an equal volume of 1.6% NaCl to lyse red blood cells. Granulocyte preparations of non-atopic controls contained approximately 4.6 ± 0.4% eosinophil granulocytes as judged by Pappenheim stain of cytospin preparations. Cells were more than 95% viable by trypan blue exclusion. For further purification of eosinophils, granulocytes were resuspended at a concentration of 10^7 ml in HEPES-buffered Hanks' balanced salt solution, pH 7.4, containing 1 mg/ml BSA (HBSS/BSA).

Eosinophils were purified from granulocyte preparations in a modification of a method described previously [21]. For this purpose immunomagnetic beads (Dynabeads M-450, Dynal, Norway) were coated with monoclonal αCD16 antibodies. In brief, 2-ml beads (4×10^8 beads/ml) were mixed with 50 μl αCD16 (1 mg/

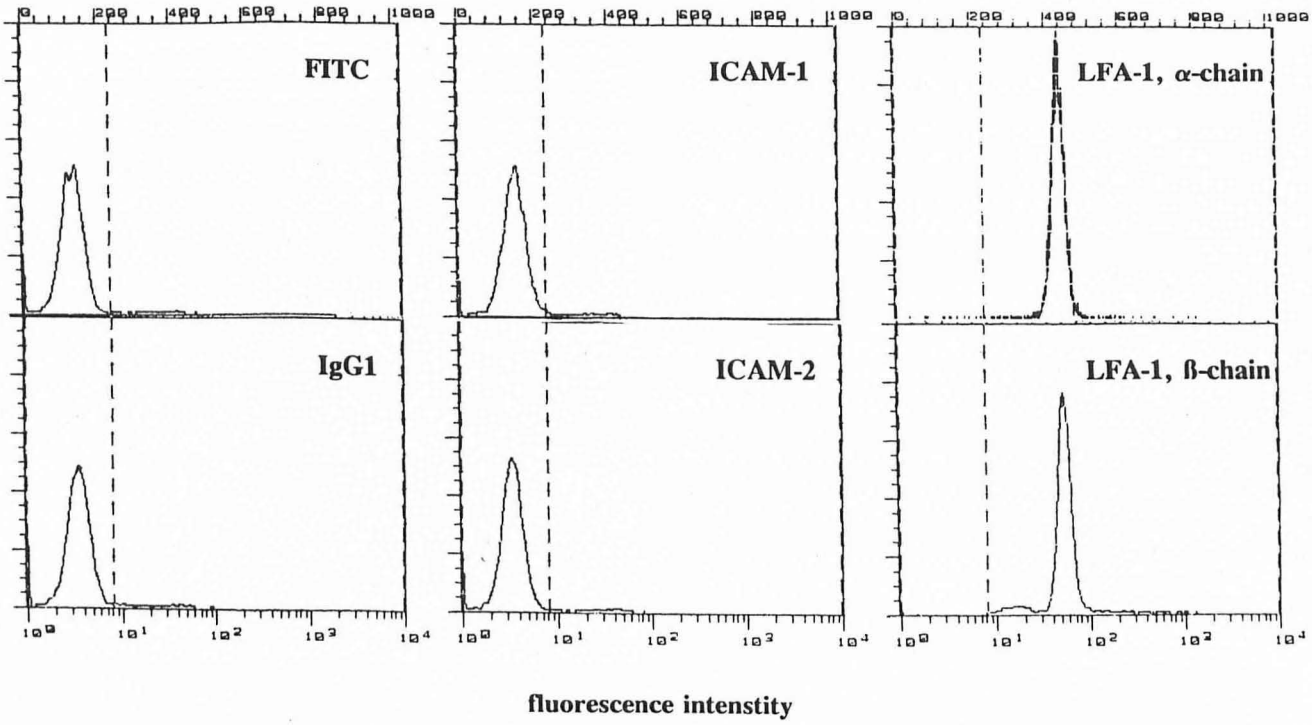

Figure 1. FACS analysis of constitutive expression of ICAM-1, ICAM-2, and LFA-1 in isolated peripheral blood eosinophils. Staining of eosinophils with fluorescein isothiocyanate (FITC)–Fab_2 without a primary antibody *(left top)* and staining with monoclonal IgG1 isotope control antibody plus FITC-Fab_2 *(left bottom)*. Staining of eosinophils with MoAb against ICAM-1 and ICAM-2 *(middle)* and staining of eosinophils with MoAb against LFA-1/α-chain as well as LFA-1/β-chain *(right)* as described in *Materials and Methods*. Data are given as histograms of cell number versus fluorescence intensity. Data represent one of five nearly identical experiments. *Vertical bar,* cutoff for the IgG1 control.

ml) and incubated for 24 h/4°C in Minisorp tubes (Nunc, Roskilde, Denmark) on a rotary mixer. Coated beads were washed three times in HBSS/BSA and the beads retrieved using a Dynal Magnetic Particle Concentrator MPC-6. αCD16-coated beads were stored at a concentration of 2×10^8 beads/ml HBSS/BSA under sterile conditions for a maximum of 4 weeks at 4°C.

Five-hundred microliters granulocytes were centrifuged in Minisorp tubes for 7 min/820 *g* at 4°C and the supernatant was aspirated; subsequently 250μl of the αCD16-coated beads were added to the pellet. The mixture was incubated for 1 h/4°C on a rotary mixer. Thereafter, HBSS/BSA was added and PMNs, which were coupled to the beads, were removed magnetically using the MPC-6 device. Supernatant was aspirated and residual beads were removed by a second magnetic separation step. Eosinophils in the supernatant were washed as described above and resuspended at a concentration of 10^6/ml in HBSS/BSA. Eosinophils were quantitated with Kimura stain [22] in a Neubauer counting chamber. If the purity of the cells was less than 95%, the magnetic purification procedure was repeated. Cytospin preparations of the cells used in the experiments described below had a purity of ≥95% as shown by Pappenheim stain.

Cell Culture For culture human eosinophils were plated in six-well tissue-culture plates (Costar, Cambridge, MA) at a density of 10^5/ml in a final volume of 2 ml cell suspension. Cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (Gibco, Berlin, Germany) with or without cytokines as indicated. Culture was performed under defined conditions in a humidified atmosphere at 37°C containing 5% CO_2. At the indicated time points, cells were collected and centrifuged at 820 *g* for 7 min at 4°C. Viability was more than 95%, tested by trypan blue exclusion.

The human carcinoma cell line KB (American Type Culture Collection, Rockville, MD) was maintained with or without cyto-

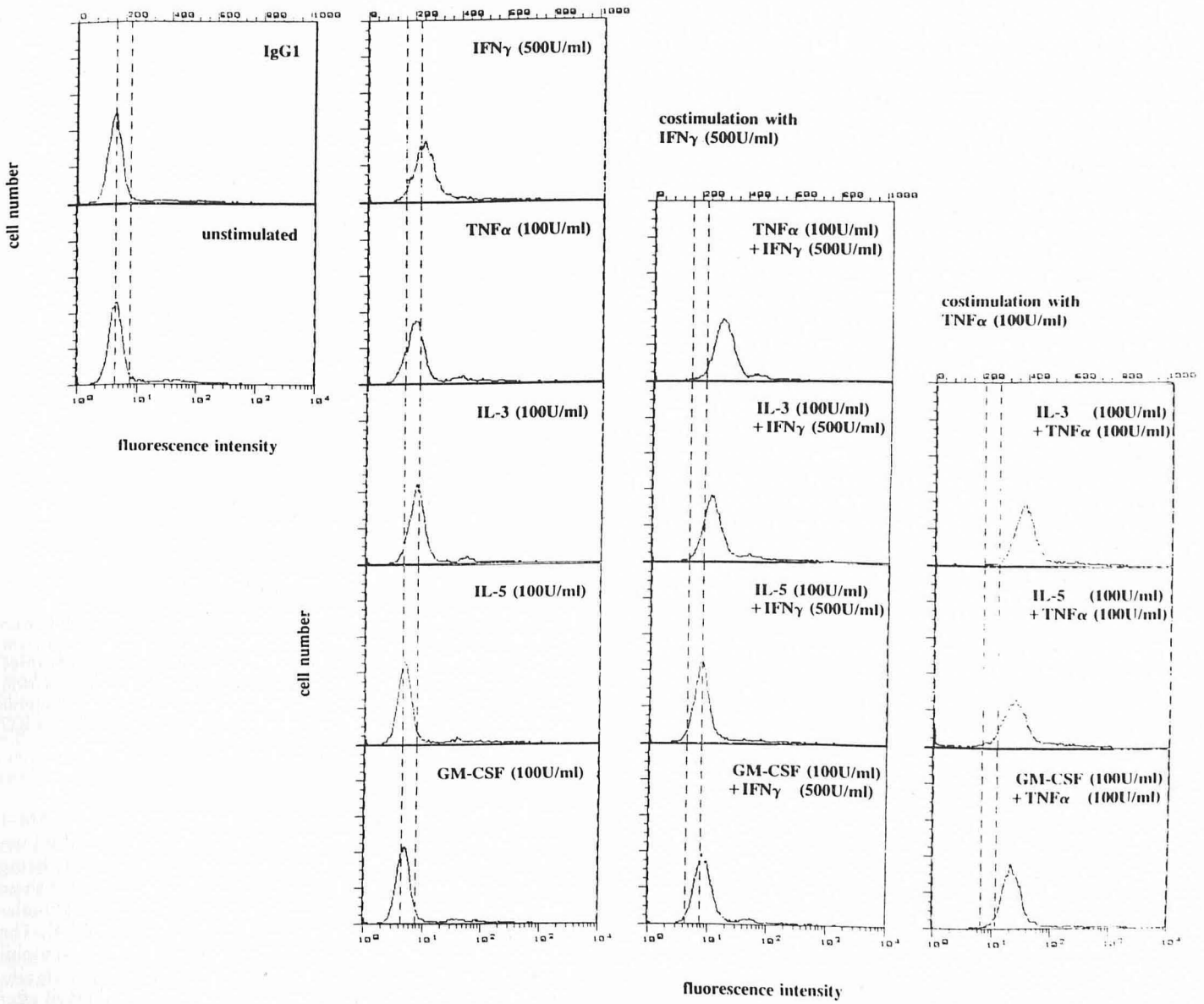

Figure 2. ICAM-1 surface expression in eosinophils. Eosinophils were incubated for 24 h without stimulus *(first column)*, stimulated with indicated cytokines *(second column)* or co-stimulated either with IFNγ plus indicated cytokines *(third column)* or TNFα plus indicated cytokines *(fourth column)*. ICAM-1 surface expression was determined by FACS analysis as described in *Materials and Methods* using MoAb 84H10. Staining of unstimulated eosinophils with mouse IgGl isotype control antibody *(first column, top)*. Data are given as histograms of cell number versus fluorescence intensity. Histograms represent one of five essentially identical experiments. *Right vertical bar,* cutoff for the IgGl control. *Left vertical bar,* peak deviation of ICAM-1–positive cells.

kine stimulation as indicated in monolayer cultures in Dulbecco's modified Eagle's medium (Seromed, Berlin, Germany) containing 5% fetal calf serum in a humidified atmosphere containing 5% CO_2 as previously described [23]. Cells were grown in 140 × 20 mm culture petri dishes as indicated (Becton Dickinson, Mountain View, CA) until subconfluency (approximately 3×10^7 cells/dish).

Flow Cytometry Surface marker expression was determined by flow cytometry using a FACScan (Becton and Dickinson, Mountain View, CA). Eosinophils (2×10^5) were incubated with the primary monoclonal antibody (MoAb) or the equivalent amount of a mouse isotype control antibody for 30 min at 4°C. Optimal staining was obtained with the following antibody dilutions or concentrations: MoAb αICAM-1/CD54 (1:40), αLFA-1, α-chain/CD11a (1:40), αLFA-1, β-chain/CD18 (1:40). The αICAM-2 MoAb was used at a concentration of 100 μg/ml. Subsequently, cells were washed twice in PBS, containing 0.01% sodium azide, resuspended and incubated with a 1:20 dilution of goat-anti mouse fluorescein isothiocyanate-(FAB_2) for another 30 min at 4°C. Thereafter, cells were washed twice and analyzed immediately. Data are given either as histograms of fluorescence intensity versus cell number or as percent reactive cells in comparison to unstimulated control cells as previously described [23].

Northern Blot Analysis Northern blot analysis was performed as previously described [23]. Briefly, 6 h after culture, eosinophils, and, 4 h after culture, KB cells were harvested and total RNA isolated by extraction with an acid guanidinium thiocyanate-phenol-chloroform mixture as described [24]. The concentration of RNA was determined from A260 nm, and A260/A280 ratios were greater than 1.7. Ten micrograms of RNA was electrophoresed in 1.2% agarose gels containing formaldehyde (2.2 M) followed by transfer to nylon membranes (Hybond N, Amersham-Buchler, Braunschweig, Germany). Equivalent loading and uniform RNA transfer were assured by ethidium bromide staining of the gels before and after northern transfer. Membranes were prehybridized (4 h) and hybridized (overnight) at 42°C with 6 × SSPE/5 × Denhardt's solution/200 μg/ml salmon sperm DNA. ICAM-1-specific mRNA was detected using a 3.0-kB cDNA, kindly provided by Dr. T.A. Springer, Boston, MA. The cDNA fragments were Biotin-14-dATP labeled by nick translation using the Bio-Nick labeling system (Gibco/BRL, Berlin, Germany) [25]. Northern blot analysis was performed under stringent washing conditions (2 × 5 min using 2 × SSC/0.5% sodium dodecyl sulfate (SDS) at 60°C and 1 × 40 min using 0.1% SSC/1% SDS at 50°C). Signals were detected using the Photogene detection system (Gibco/BRL, Berlin, Germany). Autoradiography was carried out for 15 min at 25°C by using Kodak XAR films.

Statistical Analysis Statistical significance of the data was calculated using the Mann-Whitney U test.

RESULTS

Constitutive Expression of Adhesion Molecules ICAM-1, ICAM-2, and LFA-1 on Human Eosinophils In initial experiments, constitutive expression of the adhesion molecules ICAM-1, ICAM-2, and LFA-1 was assessed in freshly isolated eosinophils by fluorescence-activated cell sorter (FACS) analysis. Eosinophils significantly expressed the LFA-1 molecule (α-chain/CD11a and β-chain/CD18) as previously reported [17] (Fig 1). In contrast, neither ICAM-1 nor ICAM-2 molecules could be detected on the cell surface of eosinophils. MoAb against ICAM-2 were capable of binding to positive control cells, because in the same experiment ICAM-2 expression could be demonstrated on peripheral blood monocytes (data not shown) [26].

Upregulation of ICAM-1 Surface Expression by Cytokine Stimulation ICAM-1 expression is known to be inducible by incubation with selected cytokines including IFNγ and TNFα. Therefore, the effect of cytokine stimulation on eosinophil ICAM-1 expression was examined *in vitro*. ICAM-1 surface expression could be significantly upregulated *in vitro* upon a 24-h stimulation with rhIFNγ (10–500 U/ml) or rhTNFα (1–100 U/ml). In addition, IL-3 (1–100 U/ml) was found to induce eosinophil ICAM-1 expression (Fig 2). Cytokine-induced upregulation of ICAM-1 occurred in a time- and dose-dependent manner with rhIFNγ being the most potent stimulus. Time dependence was similar for these cytokines showing maximal ICAM-1 expression after 24 h stimulation; representative data were shown for IFNγ (Figs 3 and 4). The combination of IFNγ with TNFα was found to induce eosinophil ICAM-1 expression in a synergistic fashion. In contrast, no significant effect on ICAM-1 surface expression could be observed after incubation with rhIL-1α (1–100 U/ml), rhIL-1β (1–100 U/ml), rhIL-2 (1–100 U/ml), rhIL-4 (1–100 U/ml), rhIL-6 (1–100 U/ml), rhIL-7 (10–500 ng/ml), rhIL-8 (0.01–1 μg/ml), or the stimuli PAF (10^{-6}–10^{-8} M), C5a (10^{-6}–10^{-8} M) and LPS (0.01–0.5 μg/ml), which were tested for different time points (data not shown). Expression of ICAM-2 on cytokine-stimulated eosinophils was not determined in the present study.

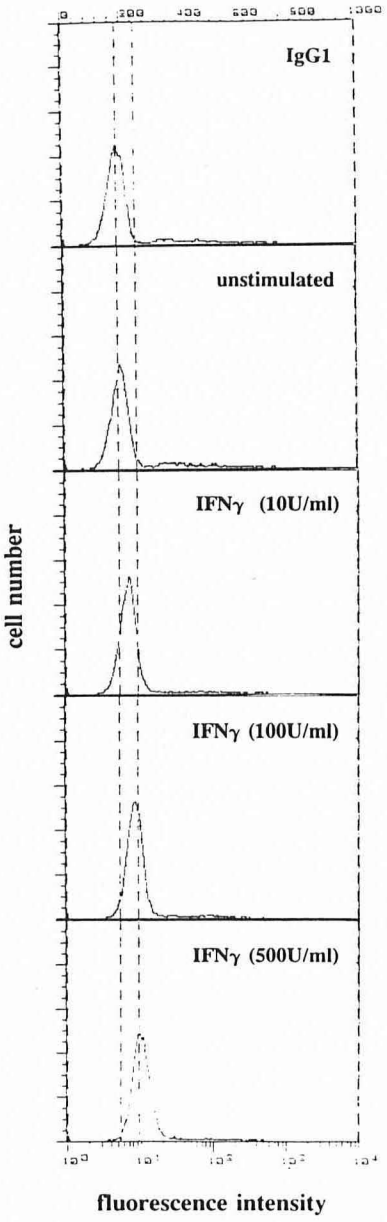

Figure 3. ICAM-1 surface expression in eosinophils. Eosinophils were incubated for 24 h without stimulus (unstimulated) or with different concentrations of IFNγ (10–500 U/ml) as indicated. ICAM-1 surface expression was determined by FACS analysis as described in *Materials and Methods* using MoAb 84H10. *Top* (IgGl), staining of unstimulated eosinophils with a mouse IgGl isotype control antibody. *Vertical bar*, cutoff for the IgGl control.

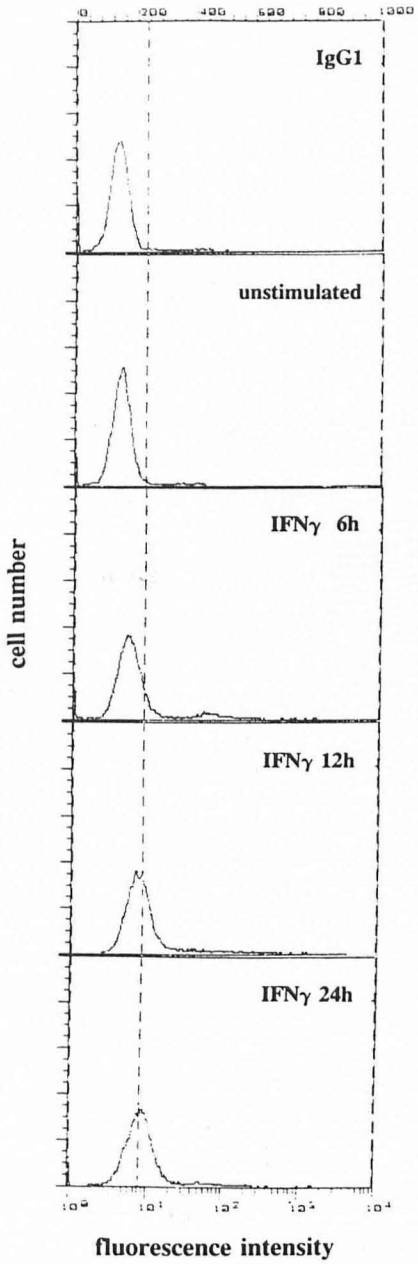

Figure 4. ICAM-1 surface expression in eosinophils. Eosinophils, either unstimulated or stimulated with IFNγ (500 U/ml), were incubated for different time intervals (6–24 h) as indicated. ICAM-1 surface expression was determined by FACS analysis as described in *Materials and Methods* using MoAb 84H10. *Top* (IgGl), staining of unstimulated eosinophils with a mouse IgGl isotype control antibody. *Vertical bar*, cutoff for the IgGl control.

Cytokines Induce ICAM-1–Specific mRNA Cytokine-induced eosinophil ICAM-1 expression was confirmed by Northern blot analysis using a 3.0-kB cDNA specifically encoding for ICAM-1. As shown in Fig 5, unstimulated cells did not express ICAM-1 mRNA. In contrast, ICAM-1 mRNA expression could be significantly upregulated after 6 h stimulation with rhIFNγ (500 U/ml) plus rhTNFα (100 U/ml).

In addition, Northern blot analysis was performed in KB cells. Accordingly, low amounts of ICAM-1–specific mRNA could be detected in unstimulated KB cells. In contrast, ICAM-1 mRNA expression could be significantly upregulated after 4 h stimulation with rhIFNγ (500 U/ml) plus rhTNFα (100 U/ml).

The observed differences in ICAM-1 mRNA expression in eosinophils or KB cells were not due to the variations in the amounts of total mRNA, because there was a similar staining pattern of the gels, with ethidium bromide or β-actin gene was found to be equally expressed for all lanes (data not shown).

Co-stimulatory Effects of Cytokines on Eosinophil ICAM-1 surface expression To investigate co-stimulatory effects of cytokines, eosinophils were cultured in the presence of several cytokine combinations. As is shown in Fig 2, a synergistic ICAM-1 upregulation could be observed after the treatment of TNFα-stimulated eosinophils with IFNγ, IL-3, IL-5, or GM-CSF. The latter two cytokines by themselves did not exert any significant effect on ICAM-1 surface expression. Co-stimulation of IFNγ-treated eosinophils with IL-5 or GM-CSF did not increase ICAM-1 surface expression above levels obtained after stimulation with IFNγ alone (Fig 2).

DISCUSSION

The present study demonstrates that expression of the cell adhesion molecule ICAM-1 may be induced in normal human eosinophils upon *in vitro* cytokine stimulation. Controversial results concerning ICAM-1 surface expression in human eosinophils have been published. Previous studies showed that ICAM-1 is not expressed in peripheral blood eosinophils [16,17]. The present report confirms that ICAM-1, in contrast to its ligand LFA-1, is not expressed constitutively in normal human eosinophils. In addition, ICAM-2, which is a newly recognized membrane protein closely related to the two most N-terminal domains of ICAM-1 and which functions as a second ligand for LFA-1, is also absent from the eosinophil surface [27]. In contrast, it could be shown recently that sputum eosinophils from asthmatic patients express ICAM-1 on their cell surface [18]. The present observation, that eosinophil ICAM-1 expression is inducible by cytokines, strongly indicates that eosinophil ICAM-1 expression in sputum eosinophils from asthmatics is due to increased levels of ICAM-1 inducing cytokines in these patients. Accordingly, sera of asthmatics were found to contain increased amounts of IFNγ [28].

The present data identify the cytokines IFNγ and TNFα as potent mediators upregulating ICAM-1 surface expression in human eo-

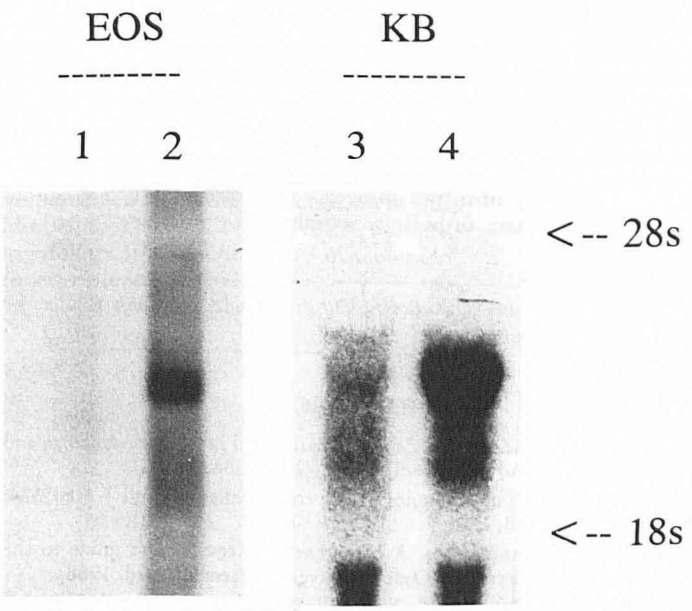

Figure 5. ICAM-1 mRNA expression in cytokine-stimulated cultured human eosinophils and KB cells. Northern blot analysis of total cellular RNA (10 μg) using a Biotin-labeled cDNA probe encoding for ICAM-1. Eosinophils (EOS) were incubated for 6 h without stimulus *(lane 1)* or stimulated with IFNγ (500 U/ml) plus TNFα (100 U/ml) *(lane 2)*. KB cells (KB) were incubated for 4 h without stimulus *(lane 3)* or stimulated with IFNγ (500 U/ml) plus TNFα (100 U/ml) *(lane 4)*. Representative Northern blots of three experiments, respectively, are shown.

sinophils *in vitro*. Moreover, stimulation of eosinophils by IFNγ plus TNFα led to an increase of ICAM-1-specific steady-state mRNA levels, as has been previously also shown for KB cells [23]. Interferon γ has been shown to induce ICAM-1 surface expression in several other cell types including dermal fibroblasts [13], endothelial cells [29], melanocytes [30], monocytes [31], and keratinocytes [32]. In addition, TNFα significantly up-regulates ICAM-1 surface expression *in vitro* in endothelial cells [29], melanocytes [30], and keratinocytes [32]. Moreover, the combination of TNFα and IFNγ was found to upregulate ICAM-1 surface expression in human eosinophils in a synergistic fashion, and a similar synergistic effect could be previously demonstrated for keratinocytes [32].

The present study provides evidence that IL-3 may represent another cytokine capable of inducing ICAM-1 expression in human eosinophils. Moreover, synergistic ICAM-1 induction could be observed upon stimulation of eosinophils with a combination of TNFα plus IL-3, IL-5, or GM-CSF. These data further support the concept that IL-3, IL-5, and GM-CSF are potent activators and modulators of eosinophil functions, as was previously shown by their capacity to induce cytotoxicity, mediator release, and oxydative metabolism [33-36].

The mechanisms responsible for synergistic ICAM-1 induction achieved by cytokine stimulation is unclear. For IFNγ and TNFα it has been proposed that IFNγ may induce TNF receptors [37,38]. However, IFNγ recently was found to have no influence on TNF-receptor surface expression in keratinocytes, although a synergistic induction of ICAM-1 expression by IFNγ plus TNFα stimulation could be observed in this cell type [39]. Further studies are currently underway to determine the effect of cytokines on TNF-receptor expression in human eosinophils. Receptors for the eosinophil-activating cytokines IL-3, IL-5, and GM-CSF share a common β-chain [40,41]. Therefore, it is tempting to speculate, that the observed synergistic effects of TNFα with these cytokines on eosinophil ICAM-1 expression may be due to TNFα-modulated signal-transduction through the common β-subunit of this cytokine receptor family.

Eosinophils are believed to be important effector cells in several skin diseases, because a series of mediators released by eosinophils capable to induce tissue damage could be detected in lesional skin [8]. However, the mechanisms involved in the generation and maintenance of an eosinophilic infiltrate are only purely understood. The present study provides clear evidence that cytokine-stimulated eosinophils are capable to express the adhesion molecule ICAM-1, and this may allow ICAM-1-positive eosinophils to physically interact with LFA-1-positive inflammatory cells such as lymphocytes, monocytes, Langerhans cells, or granulocytes. Further studies will determine the *in vivo* relevance of cytokine-induced eosinophil ICAM-1 expression in inflammatory skin diseases, such as atopic dermatitis, urticaria, or bullous pemphigoid.

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ANNOUNCEMENT

The 42nd annual Symposium on the Biology of Skin will be "Genetics of Skin Disease." The symposium will be held at Snowmass Lodge in Snowmass, Colorado on July 24–28, 1993. The meeting will be held just before the annual meeting of the Society for Pediatric Dermatology. Those wishing to present a poster at the SBS may submit an abstract to the Symposium Director on a standard SID or ESDR abstract form. All correspondence regarding participation in this meeting, and all abstract submissions, should be addressed to: David A. Norris, M.D., Symposium on the Biology of Skin, Department of Dermatology B-144, University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, Colorado 80262.