

E. Fernvik
G. Halldén
J. Lundahl
J. Raud
U. Alkner
M. van Hage-Hamsten
R. Grönneberg

Allergen-induced accumulation of eosinophils and lymphocytes in skin chambers is associated with increased levels of interleukin-4 and sVCAM-1

Authors' affiliations:

E. Fernvik, G. Halldén, J. Lundahl,
M. van Hage-Hamsten, Department of
Laboratory Medicine, Division of Clinical
Immunology, Karolinska Hospital and Institute,
Stockholm, Sweden
J. Raud, Discovery Division, Astra Pain Control
AB, Södertälje, Sweden
U. Alkner, Bioanalytical Chemistry, Astra Draco
AB, Lund, Sweden
R. Grönneberg, Department of Medicine,
Division of Respiratory and Allergic Diseases,
Huddinge University Hospital, Karolinska
Institute, Huddinge, Sweden

Correspondence to:

Eva Fernvik, Med. Lic.
Division of Clinical Immunology
L2:01
Karolinska Hospital
S-171 76 Stockholm
Sweden

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Key words: eosinophils; histamine; interleukin-4; lymphocytes;
 α_2 -macroglobulin; skin chamber; sVCAM-1.

Background: The aim of the study was to characterize the kinetic accumulation of various inflammatory mediators in allergen-challenged skin chambers applied on patients with pollen-related allergic rhinitis/mild asthma.

Methods: Skin blisters were induced on the forearms and challenged with allergen or phosphate-buffered saline (PBS). Peripheral blood was drawn before and 8 h after challenge for analysis of differential cell counts, sVCAM-1, and α_2 -macroglobulin. Chamber fluids, collected at 1, 4, and 8 h after allergen application, were analyzed for differential cell counts, histamine, interleukin (IL)-4, sVCAM-1, and α_2 -macroglobulin.

Results: The number of recruited leukocytes was equal in allergen and PBS chambers; however, the numbers of eosinophils and lymphocytes were significantly ($P \leq 0.05$) elevated in allergen-challenged chambers at 8 h. Compared to PBS chambers, allergen chambers contained significantly ($P < 0.01$ – 0.05) higher levels of histamine (at 1 and 4 h), IL-4 (at 4 and 8 h), α_2 -macroglobulin (at 1 and 8 h), and sVCAM-1 (at 1 and 8 h). In contrast to α_2 -macroglobulin, levels of sVCAM-1 in peripheral blood were significantly ($P < 0.05$) increased at 8 h.

Conclusions: Increased levels of sVCAM-1 and IL-4 in allergen-challenged chambers, in parallel with increased recruitment of eosinophils and lymphocytes, points to the participation of IL-4 and VCAM-1 in the development of the late-phase reaction. Increased levels of sVCAM-1 in allergen-challenged chambers probably reflects a combination of leakage and local production.

Abbreviations EDTA: ethylene diaminetetraacetic acid; FITC: fluorescein isothiocyanate; IL-4: interleukin 4; LCR: late cutaneous reaction; LPR: late-phase reaction; MFI: mean fluorescence

intensity; OCD: optimal challenge dose; OG: *n*-octyl- β -D-glucopyranoside; PBS-EDTA: phosphate-buffered saline with EDTA; SPT: skin prick test; sVCAM-1: soluble vascular cell adhesion molecule; VLA-4: very late antigen 4.

Allergic asthma is characterized by mucosal infiltration of the airways by activated eosinophils and lymphocytes. An increased number of these cells has been associated with enhanced airway hyperreactivity and disease severity (1, 2).

Vascular cell adhesion molecule-1 (VCAM-1), a member of the immunoglobulin supergene-family of adhesion molecules, is thought to be important for eosinophil recruitment in asthma (3). Selective blocking of common endothelial adhesion molecules demonstrated that VCAM-1 is prominently involved in the binding of basophils and eosinophils to the stimulated monolayer (4). This observation is consistent with the fact that basophils, eosinophils, and lymphocytes express the natural ligand for VCAM-1, the very late antigen-4 (VLA-4) (5). Zangrilli et al. have previously demonstrated a large increase in the soluble form of VCAM-1, designated sVCAM-1, in bronchoalveolar lavage fluid from sensitive asthmatic and nonasthmatic subjects 24 h after segmental antigen challenge (6), and endothelial VCAM-1 expression in bronchial mucosa biopsies from asthmatics has been correlated with eosinophil migration into the airways (7, 8).

Another possible mechanism for the increased eosinophil recruitment in asthmatic and allergic reactions is that histamine induces upregulation of P-selectin on the surface of endothelial cells (9–11). However, histamine does not modulate the expression of VCAM-1. Cytokines are thought to play a crucial role in recruiting leukocytes into sites of IgE-mediated late-phase reactions (LPR); for example, interleukin-4 (IL-4) is an important immunoregulatory cytokine with several biologic activities and cellular targets. It is well established that IL-4 acts on T cells, inducing T-cell proliferation and differentiation into the Th2 phenotype (12). IL-4 can also cause isotype switching to IgE in human B cells (13). Additionally, IL-4 has been reported to be chemotactic for fibroblasts (14), and blood eosinophils in atopic dermatitis patients (15). Among other regulatory functions, IL-4 induces VCAM-1 expression on endothelial cells *in vitro*, and Fukuda et al. have recently shown that IL-4 may selectively promote VCAM-1 expression on vascular endothelium also in human airways, an effect which may contribute to the selective recruitment of eosinophils (8).

This study aimed to investigate whether specific mediators, supposed to be involved in the allergen-induced

accumulation of eosinophils and lymphocytes, can be identified by a skin chamber technique. For the first time, levels of IL-4 and sVCAM-1 in skin chambers challenged with allergen, applied on patients with pollen-related allergic rhinitis and mild asthma, have been measured. We also studied early histamine release, and α_2 -macroglobulin was used as a marker for increased vascular permeability.

Material and methods

Subjects

The study group consisted of nine allergic patients, six men and three women, aged 25–41 years (mean age 28 years), with a history of birch and/or timothy pollen-related allergic rhinitis and mild bronchial asthma (FEV 1.0% of predicted 94.0 [83.5–104.5]), and positive skin prick test (SPT) $\geq 3+$ and RAST $\geq$ class 3 (Pharmacia CAP System; Pharmacia & Upjohn, Uppsala, Sweden). Most of the subjects were also allergic to pet dander.

All subjects in the study were drug-free, except for inhaled β_2 -agonist when needed, and all experiments were performed in a symptom-free interval outside the pollen season.

All subjects gave their informed consent to participate in the study, which was approved by the ethics committee of Huddinge Hospital.

Skin testing

During the screening period, the SPT was performed in 23 subjects on the volar aspect of the forearm with freeze-dried birch or timothy pollen (Aquagen SQ ALK) 1000–100 000 SQ/ml, histamine Soluprick 10 mg/ml, and ALK diluent (Aquagen SQ ALK) (human serum albumin 0.3 mg/ml in sodium chloride solution with 0.1% phenol), with registration of 15-min wheals.

Intradermal end-point titration tests (20 μ l) were then performed with a starting dose of approximately 1/100 of SPT 3+ dose with $\times 10$ incremental doses, and were stopped when the optimal challenge dose (OCD) was reached. This dose corresponds to the concentration of allergen required to produce a wheal with a diameter of >10 mm with pseudopods, or of >15 mm without pseudopods and having erythema of >45 mm at 10 min, with a following late induration of >30 mm (mean diameter) at 6 h. The areas of all wheals and flares from SPT and intradermal tests were

outlined on a transparent plastic film by the investigators, whereas late indurations at 1, 2, 4, 6, 10, and 24 h were registered by the test subjects themselves. For theoretic optimization of recruitment of eosinophils into allergen-challenged skin chambers, only individuals (9/23) with a significant late cutaneous reaction (LCR) (>30 mm at 6 h) and RAST of ≥ 3 to selected allergens were included in the subsequent skin chamber experiments (Table 1).

Skin chambers

Skin blisters were raised on the volar aspect of each forearm, two per arm, with a negative pressure cutaneous suction chamber system (Dermavac suction unit, Peter Bjerring, Marselisborg Hospital, DK-8000 Aarhus, Denmark). The suction chamber was placed on a cleaned skin area, and continuous gentle suction of 290 mmHg was applied for 2–3 h until a complete blister of approximately 8 mm in diameter was formed. The blisters were covered overnight with a plastic eye chamber (Poroplast Augenverband S, Bendaggio Oculare S, Lohman GmbH, Munich, Germany), and the next morning the blisters were aseptically unroofed. A transparent sterile adhesive plastic film (Tegaderm, 3M Pharmaceuticals, Loughborough, UK) with a circular hole (9 mm in diameter) was applied around the denuded blister base. The film was used to protect skin from possible irritation by a cutaneous adhesive (Skin Bond Cement, Smith & Nephew United, Inc., Largo, FL, USA). Skin Bond Cement was applied around the bottom edge of sterilized, open-bottom (9-mm diameter), transparent plastic skin chambers (Department of Physiology and Pharmacology, Division of Physiology, Karolinska Institute) with an inner volume of 1 ml, which were placed over the unroofed

blisters and secured with tape. The chambers were then washed four times with sterile phosphate-buffered saline (PBS) containing 30 IU/ml heparin. Two chambers were then filled with relevant allergen in $\times 10$ OCD concentration (Table 1) in a total volume of 0.5 ml. For this purpose, freeze-dried birch/timothy allergen (ALK) was first diluted to 100 000 SQ/ml by adding 4.5 ml ALK diluent according to the manufacturer's instruction, and further diluted with PBS-heparin (5000 IU/ml with a final concentration of 30 IU/ml) to avoid clotting. Two control chambers on the opposite arm were filled only with 0.5 ml PBS-heparin. The four chambers were filled in a randomized order with allergen chambers on one arm and control chambers on the contralateral forearm. After 1 h of incubation, all chambers were emptied and thereafter refilled with PBS-heparin, and the fluid was carefully aspirated. The chambers were then washed twice to allow a complete collection of exudate, and to ensure that no allergen was left in the chamber. All chambers were afterward refilled with PBS-heparin (1 ml). One allergen-challenged and one control chamber were emptied and refilled with PBS-heparin also after 4 h (for mediator analysis), whereas the other pair (for cell analysis) were left untouched for 7 h (8 h after start of challenge) before final removal.

All aspirated chamber fluids were centrifuged at 300 g for 5 min, and the supernatants were stored at -80°C until further analysis.

Leukocyte counts

Leukocytes, more specifically the number and percentage of lymphocytes and eosinophils, were analyzed by flow cytometry in peripheral blood, before application of allergen and at 8 h. In skin chamber fluids, lymphocytes were counted at 1 and 8 h, but the eosinophils were analyzed only at 8 h since there were no detectable eosinophils at 1 h. Due to a low cell number in some chamber fluids, cells from all patients could not be analyzed on all occasions.

Analysis with flow cytometry

The different preparations of leukocytes were analyzed in an EPICS XL (Coulter, Inc., Hialeah, FL, USA) flow cytometer, in which the cells are distinguished by their different light-scattering properties. Forward scatter reflects the cell size, and side scatter reflects the cell complexity (granularity). The instrument was daily calibrated with standardized fluorospheres, Flow Check (Coulter).

Table 1. Individual patient data: RAST class, optimal concentration dose (OCD) for challenge, 6-h peak of late cutaneous reaction (LCR) after prestudy challenge, and chamber challenge dose

Sex	Allergen	RAST (class)	OCD (SQ/ml)	LCR 6-h peak (mm)	Chamber challenge dose (SQ/ml)
F	Birch	3	1000	55×50	10 000
M	Birch	3	1000	65×50	10 000
M	Birch	5	10	110×75	100
M	Timothy	3	1000	140×85	10 000
F	Birch	5	10–100	30×25	1000
M	Timothy	4	1000	35×30	10 000
M	Birch	3	1000	100×45	10 000
M	Timothy	4	100–1000	35×30	10 000
F	Birch	3	100–1000	30×25	10 000

In order to count the eosinophils by flow cytometric methods, the leukocyte preparations from blood and skin exudates were fixed and permeabilized by the FOG method (16). Briefly, the cell membrane was stabilized by incubation of the leukocytes in 200 μ l 4% (w/v) paraformaldehyde for 10 min at 20°C. The cells were then washed once in 3 ml PBS-EDTA at 4°C followed by centrifugation at 400 g for 8 min at 4°C.

Cell-membrane permeabilization was achieved by exposing the cells to 200 μ l 0.74% (w/v) *n*-octyl- β -D-glucopyranoside (OG) (Sigma Chemical, St Louis, MO, USA) dissolved in PBS, for 6 min at 20°C. The permeabilized leukocytes were then washed once more in PBS-EDTA at 4°C. The FOG method decreases the light-scattering properties of all leukocytes except eosinophils. Three leukocyte clusters were thus distinguished; lymphocytes, neutrophils together with monocytes, and eosinophils (Fig. 1). The eosinophils were identified by their high scatter signals and expression of CD9 and intracellular ECP (EG2), as previously described (17, 18).

Analysis of sVCAM-1, IL-4, and histamine in skin chamber fluids and serum

sVCAM-1

sVCAM-1 levels were measured in serum before and 8 h after allergen challenge, and in chamber fluids 1, 4, and 8 h after challenge, with the commercially available immunoassay Cytoscreen (BioSource International, CA, USA). The cutoff for the analysis was determined to be 2.3 ng/ml.

IL-4

IL-4 levels were measured in skin chamber fluids obtained after 1, 4, and 8 h, with the commercially available

immunoassay Cytoscreen US (BioSource). The cutoff for the analysis was determined to be 0.8 pg/ml.

Histamine

Histamine levels were measured in skin chamber fluids obtained after 1 and 4 h, with the commercially available immunoassay Histamine (Immunotech, Marseilles, France). The cutoff for the analysis was determined to be 0.1 ng/ml.

All three assays were performed according to the instructions of the manufacturer.

α_2 -macroglobulin

The levels of α_2 -macroglobulin were measured in serum samples at 0 and 8 h, and in skin chamber fluids at 1, 4, and 8 h. In serum, α_2 -macroglobulin was determined by a turbidimetric method (DAKO A/S, Glostrup, Denmark). The method was applied to an autoanalyzer (Cobas Mira Plus, Roche, France), and the sensitivity was 0.4 g/l. In skin chamber fluids, the levels of α_2 -macroglobulin were measured as described previously with a radioimmunoassay sensitive to 7.8 ng/ml (19).

Fluids from the skin chambers, and serum samples, were collected in plastic tubes and stored at -80°C until analysis.

There was no evidence of interference in these assays by the allergen or the diluent (containing heparin) placed initially in the skin chambers.

Statistical analysis

Data are presented as medians (interquartile range). Statistical evaluations within the patient group were done with Wilcoxon's paired test when sample sizes were equal. In all other cases, the Mann-Whitney U-test was used. Differences were considered statistically significant at $P < 0.05$. When appropriate, correlations between the different parameters were sought by calculating the Spearman correlation coefficient.

Results

Skin testing

Nine subjects fulfilled the criteria for skin chamber experiments and exhibited a positive LCR with mean diameter of >30 mm, all peaking at 6 h after OCD challenge (Table 1).

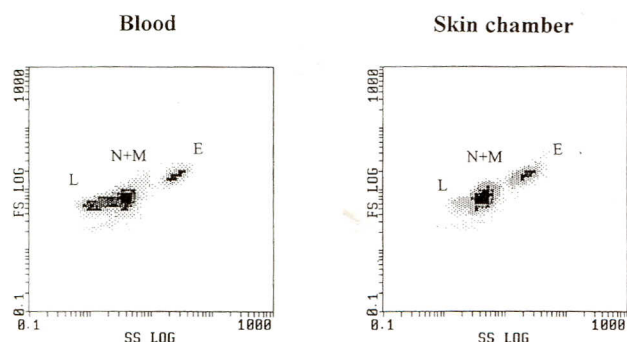


Figure 1. Distribution of leukocyte subpopulations in permeabilized, FOG-treated, blood and skin chamber fluid. Leukocyte populations (L: lymphocytes; N+M: neutrophils and monocytes; E: eosinophils) were detected in two-parameter scatter-plot histogram with logarithmic amplification. Histograms are from two representative experiments.

Leukocytes in peripheral blood and skin chamber fluids

Peripheral blood leukocytes were counted before allergen was applied to the chamber. The concentration of leukocytes in blood from the patient group was $6.4 (4.9-6.5) \times 10^9/l$ ($n=9$), which is in the normal range for healthy subjects at the Karolinska Hospital ($4-11 \times 10^9$ leukocytes/l). The number of recruited leukocytes did not differ between the activated chambers and the control chambers, either at 1 h, $0.03 (0.02-0.04) \times 10^9$ leukocytes/l ($n=9$) vs $0.05 (0.02-0.07) \times 10^9/l$ ($n=7$), or at 8 h, $0.59 (0.32-0.79) \times 10^9$ leukocytes/l ($n=9$), vs $0.48 (0.09-1.20) \times 10^9$ leukocytes/l ($n=9$) (Table 2).

Eosinophils in peripheral blood and skin chamber fluids

The prechallenge number of eosinophils in blood was $0.24 (0.17-0.30) \times 10^9/l$ ($n=9$), which is within the normal range for healthy subjects at the Karolinska Hospital ($0.05-0.40 \times 10^9$ eosinophils/l). The proportion of eosinophils was $3.7 (2.5-6.0)\%$ ($n=9$) in the patient group, a figure which is within the normal range, $3.4\% (1.7-5.1\%)$ ($n=35$) (18).

In contrast to the similar accumulation of total leukocytes in the different chambers, challenged and unchallenged, the proportion as well as the number of eosinophils was significantly increased after 8 h in the challenged chambers. The percentage of recruited eosinophils was $6.4\% (4.9-7.0\%)$ ($n=9$) in the allergen-challenged chambers, in contrast to $1.4\% (0.5-2.0\%)$ ($n=9$) ($P=0.01$) in the control chambers, and the number of eosinophils was $0.06 (0.02-0.11) \times 10^9/l$ vs $0.02 (0.003-0.06) \times 10^9/l$ ($n=9$) ($P=0.05$) (Table 2).

Lymphocytes in peripheral blood and skin chamber fluids

The prechallenge number of lymphocytes in blood was $2.2 (1.8-2.6) \times 10^9/l$ ($n=9$), and the proportion of lymphocytes was $38.5\% (35.0-40.0\%)$, both of which are within the normal range for healthy subjects at the Karolinska Hospital ($1.2-4.5 \times 10^9/l$ and $20-45\%$).

The number of lymphocytes was significantly ($P<0.05$) increased at 8 h in the challenged chambers as compared to the control chambers, $0.02 (0.01-0.05) \times 10^9/l$ ($n=9$), vs $0.006 (0.001-0.01) \times 10^9/l$ ($n=9$). However, at 1 h, there was no difference in accumulation between the challenged, $0.003 (0.002-0.004) \times 10^9/l$ ($n=9$), and the control chamber, $0.003 (0.002-0.005) \times 10^9/l$ ($n=7$) (Table 2).

There was no difference in the percentage of recruited lymphocytes either at 1 or at 8 h; $10.0\% (4.5-14.0\%)$ ($n=9$) in the challenged chamber, in contrast to $7.6\% (3.3-9.0\%)$ ($n=7$)

Table 2. Number of leukocytes, eosinophils, and lymphocytes recruited to allergen-challenged and control chamber at 8 h

	Allergen-challenged chamber $\times 10^9/l$	Control chamber $\times 10^9/l$
Leukocytes	0.59 (0.32-0.79)	0.48 (0.09-1.20)
Eosinophils	0.06 (0.02-0.11)	0.02 (0.003-0.06)
Lymphocytes	0.02 (0.01-0.05)	0.006 (0.001-0.01)

in the control chamber at 1 h, and $3.5\% (2.0-4.0\%)$ ($n=9$) vs $1.8\% (0.3-2.0\%)$ ($n=9$) at 8 h.

Histamine levels in skin chamber fluids

The histamine levels were analyzed at 1 and 4 h in fluids from the allergen-challenged and the control chambers. There were significant differences in the levels of histamine between the challenged and the control chambers at both time points; $19.4 (16.3-27.8)$ ng/ml vs $0.8 (0.6-0.8)$ ng/ml ($P<0.01$) at 1 h, and $4.7 (3.1-11.2)$ ng/ml vs $0.6 (0.6-0.8)$ ng/ml ($P=0.01$) at 4 h ($n=9$) (Fig. 2).

There was a strong correlation (0.78 , $P=0.013$) between the number of eosinophils recruited to the allergen-challenged skin chamber at 8 h and the levels of histamine at 1 h. A moderate correlation (0.64 , $P=0.061$) was observed between the number of lymphocytes recruited to the challenged chamber at 8 h and the levels of histamine at 1 h.

IL-4 levels in skin chamber fluids

The levels of IL-4 were analyzed in fluids from the skin chambers at 1, 4, and 8 h. At 1 h, there were detectable

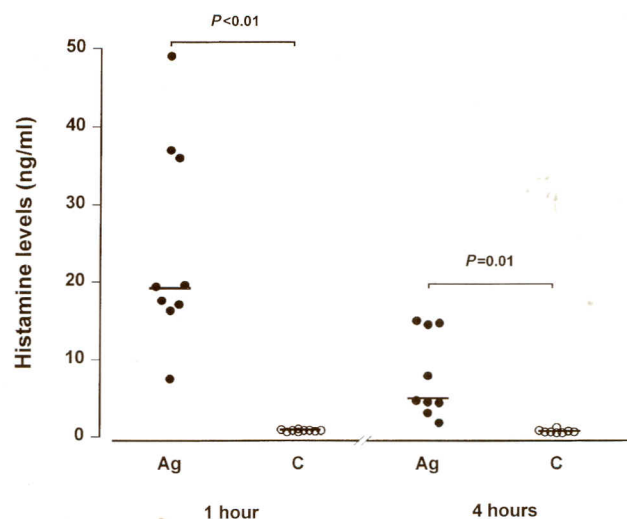


Figure 2. Histamine levels in fluids from allergen-challenged (Ag) and control chambers (C), at 1 and 4 h. Each patient is represented by open/filled circle, and median values are marked by lines.

Table 3. sVCAM-1 and α_2 -macroglobulin (α_2 -MG) levels in skin chamber fluids from allergen-challenged and control chambers, presented as median values and interquartile range (IQR)

	1 h			4 h			8 h		
	sVCAM ng/ml	α_2 -MG μ g/ml	Ratio $\times 10^{-3}$	sVCAM ng/ml	α_2 -MG μ g/ml	Ratio $\times 10^{-3}$	sVCAM ng/ml	α_2 -MG μ g/ml	Ratio $\times 10^{-3}$
Allergen chamber									
Median	35.0	99.3	0.4	56.4	69.4	0.8	62.6	65.5	1.1
(IQR)	(23.2–54.4)	(53.0–124.4)	(0.2–0.5)	(22.2–62.0)	(28.6–74.2)	(0.3–1.1)	(2.2–77.1)	(19.1–72.2)	(0.04–1.2)
Control chamber									
Median	2.2	47.3	0.1	21.9	57.9	0.3	2.2	40.4	0.4
(IQR)	(2.2–2.2)	(15.4–66.6)	(0.03–0.2)	(2.2–48.4)	(17.7–61.8)	(0.05–0.6)	(2.2–28.2)	(13.3–45.8)	(0.05–0.7)

levels of IL-4 in fluid from the allergen-challenged chambers only in three patients, whereas the fluids from the control chambers were negative in all cases. At 4 h, we were able to detect IL-4 in six of the challenged chambers, as compared to only one of the control chambers; 1.1 (0.7–2.4) pg/ml vs 0.7 (0.7–0.7) pg/ml ($n=9$) ($P<0.05$). At 8 h, detectable levels of IL-4 in the allergen-challenged chambers were found in seven patients, still only in one of the control chambers, and the level had increased significantly ($P<0.05$) to 3.6 (0.7–5.0) pg/ml, as compared to that at 4 h.

The median levels of IL-4 increased with time in the allergen-challenged chambers, and were significantly higher at both 4 and 8 h than in the control chambers (Fig. 3). There was a moderate correlation (0.62, $P=0.077$) between the number of lymphocytes and IL-4 at 1 h in the allergen-challenged chamber, but no correlation (0.50–0.56) at any time point between the number of eosinophils and IL-4 levels.

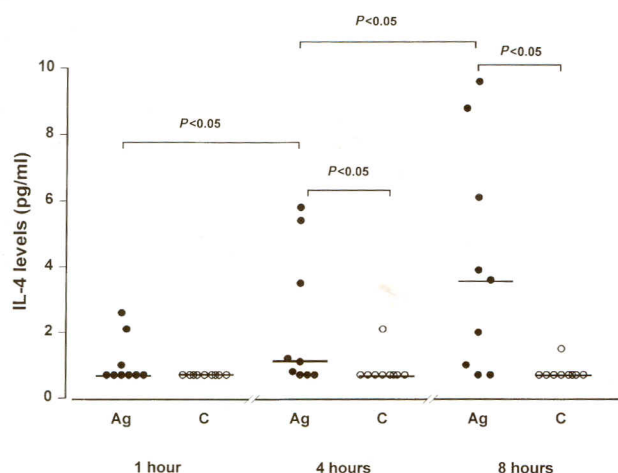


Figure 3. IL-4 levels in fluids from allergen-challenged (Ag) and control chambers (C), at 1, 4, and 8 h. Each patient is represented by open/filled circle, and median values are marked by lines.

sVCAM-1 and α_2 -macroglobulin levels in serum and skin chamber fluids

The α_2 -macroglobulin can be used as a marker for leakage of molecules from the blood circulation into the skin chambers. The levels of sVCAM-1 and α_2 -macroglobulin were analyzed in parallel at 0 and 8 h in serum and at 1, 4, and 8 h in fluids from the allergen-challenged and the control chambers.

The levels of sVCAM-1 in serum were significantly ($P<0.05$) increased at 8 h as compared to the initial value; 648.0 (497.0–1029.0) ng/ml vs 573.0 (465.0–834.5) ng/ml. However, there was no difference in serum α_2 -macroglobulin levels; 2.0 (0.9–2.2) mg/ml at 8 h vs 2.0 (1.1–2.1) mg/ml at 0 h.

The levels of sVCAM-1 were significantly higher in fluid from the allergen-challenged chambers than from the

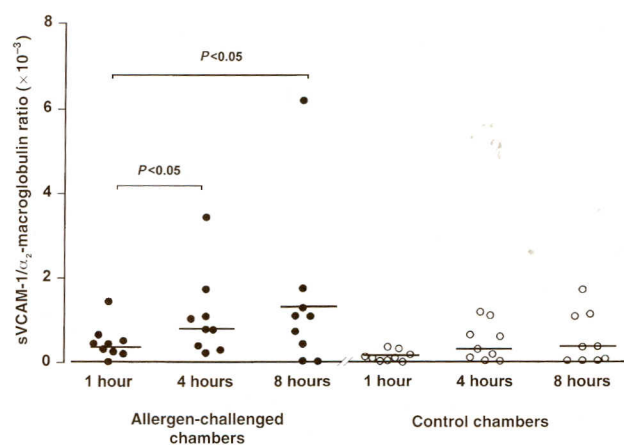


Figure 4. Levels of sVCAM-1 and α_2 -macroglobulin in fluids from allergen-challenged and control chambers. Ratio between sVCAM-1 and α_2 -macroglobulin was calculated at 1, 4, and 8 h. Each patient is represented by open/filled circle, and median values are marked by lines.

control chambers at 1 h ($P=0.01$) and at 8 h ($P<0.05$), but there was no significant difference between the chambers at 4 h. The same pattern was obtained when we measured the α_2 -macroglobulin levels in the different chamber; there was a significant difference at 1 h ($P<0.01$) and at 8 h ($P<0.01$). There was also a significant increase in sVCAM-1 concentrations at 4 h as compared to 1 h in the control chambers ($P=0.043$) (Table 3).

The ratio of sVCAM-1 to α_2 -macroglobulin concentrations in allergen-challenged chamber fluids was significantly ($P<0.05$) increased at 4 h as compared to 1 h. The ratio was also significantly ($P<0.05$) increased at 8 h as compared to 1 h, but there was no difference from 4 h (Fig. 4).

Discussion

We have shown that allergen challenge in skin chambers applied on patients with pollen-related rhinitis and mild asthma results in increased levels of histamine, IL-4, and sVCAM-1, and increased influx of eosinophils and lymphocytes. To our knowledge, the presence of IL-4 and sVCAM-1 in a skin chamber model is a novel finding which has not been reported before.

The histamine levels were analyzed at 1 and 4 h in fluids from both the allergen-challenged and control chambers. At both these time points, we found significantly higher levels of histamine in the challenged chambers. This is in accordance with previously performed skin chamber studies where an early peak of histamine release *in vivo* has been demonstrated (20–24).

It is commonly accepted that an imbalance in the T-helper cell response to common environmental antigens is one of the major underlying mechanisms leading to the development of atopic diseases (25). This imbalance leads to a predominantly Th2-type cytokine response, resulting in an increased production of IL-4 and IL-5. IL-4 is the main isotype switch factor leading to synthesis of IgE by B cells in response to allergen, a characteristic feature in atopic patients (26).

The levels of IL-4 were analyzed in skin chamber fluids at 1, 4, and 8 h. There were significantly higher levels of IL-4 in the allergen-challenged chambers at both 4 and 8 h than in the control chambers. We also demonstrated a time-dependent increase in IL-4 levels. The cellular source for IL-4 is not clear, but IL-4 can be produced either by infiltrating lymphocytes and eosinophils or by resident mast cells, or by all three. It has been shown that purified

eosinophils from allergic patients express both IL-4 mRNA and the protein, and that a calcium ionophore increases the IL-4 expression and causes the eosinophil to release the cytokine (27).

In the present study, there was a moderate correlation (0.62) between the number of lymphocytes and IL-4 at 1 h in the allergen-challenged chamber, but no correlation (0.50–0.56) at any time point between the number of eosinophils and IL-4 levels. These results suggest a relationship between recruited lymphocytes and IL-4, and might indicate that IL-4 in the skin chamber fluids derives from lymphocytes. However, we cannot exclude the possibility that IL-4 also derives from eosinophils and mast cells.

In human airways affected by allergy, it has been demonstrated that selective VCAM-1 induction by IL-4 plays an important role in the preferential recruitment of eosinophils and T cells (3). In line with that observation, several studies have suggested that increased VCAM-1 expression on the vascular endothelium in bronchial mucosa biopsies correlates with IL-4 levels in the bronchial lavage fluid of allergic asthmatics (8).

We found increased levels of sVCAM-1 in skin blister fluids in the allergen-challenged chambers at 1 and 8 h as compared to the control chambers. The time-dependent increase in ratio between sVCAM-1 and α_2 -macroglobulin in fluids from allergen-challenged chambers indicates that the increased levels of sVCAM-1 can be locally produced. However, we cannot rule out a complementary explanation, namely, that sVCAM-1 has leaked into the chamber from the postcapillary venule due to increased vascular permeability. This explanation is supported by our findings in serum, where a significant increase in sVCAM-1 was obtained at 8 h, as compared to the prechallenge value. The increased sVCAM-1 levels in serum could be due to a systemic reaction induced by a leakage of allergen from the challenged skin chambers. The level of α_2 -macroglobulin at 8 h was 60% higher in the allergen-challenged chambers in comparison to the controls, suggesting a marginally higher permeability, induced by allergen. However, at the same time point, there was approximately 2800% more sVCAM-1 in the challenged chambers. These results support the theory that the higher levels of sVCAM-1 in the allergen-challenged chambers are probably derived from cells within the blister, and not only from an increased permeability.

In the present study, the number as well as percentage of both eosinophils and lymphocytes in peripheral blood was within the normal range as compared to healthy controls, and the number of recruited leukocytes did not differ between the allergen-challenged and the control chambers.

We observed, however, that the number as well as the proportion of eosinophils was significantly higher in the allergen-challenged chambers than the control chambers. Histamine is believed to upregulate P-selectin on the surface of endothelial cells (9–11), and this could be one factor contributing to the increased eosinophil recruitment in asthmatic and allergic reactions. In a mouse model, P-selectin has been shown to mediate eosinophil recruitment in skin in acute inflammatory reactions (28). We observed a strong correlation (0.78) between the recruitment of eosinophils to the allergen-challenged chambers at 8 h and the histamine release at 1 h. A higher expression of VCAM-1 on the endothelial cells, eventually induced by IL-4, could also explain the recruitment of eosinophils, although we could not find any correlations between the levels of IL-4 and sVCAM-1 in the skin chamber fluids.

The role of T-cell infiltration in allergic reactions has been the subject of numerous studies, in part because these cells can produce proinflammatory mediators including cytokines, and therefore may play an important role in modulating the disease process. In a skin chamber model, it has previously been demonstrated that it is preferentially activated T cells of the helper-memory type that are recruited during the LPR to allergen (21). In this study, we

found an increased number of lymphocytes in the allergen-challenged chambers as compared to controls, but there was no difference in the percentage of lymphocytes between the two chambers. However, we did not perform any further phenotype characterization as a consequence of the limited cell number.

In summary, our findings point to an accumulation of different mediators associated with the allergic inflammatory process in the allergen-challenged skin chambers. We found an early accumulation of histamine and a late accumulation of IL-4 and sVCAM-1, a finding which may partly explain the significantly higher recruitment of eosinophils and lymphocytes to the challenged chambers.

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