Anti-IgE–induced accumulation of leukocytes, mediators, and albumin in skin chamber fluid from healthy and atopic subjects

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The aim of this study was to examine potential differences between healthy and atopic subjects with regard to IgE-mediated cutaneous inflammation. For this purpose, we analyzed histamine, tryptase, leukotriene B_4, albumin, eosinophils, and total leukocytes in skin chamber fluid after challenge with anti-human IgE. We also measured gross skin reactivity (wheal, flare, and late-phase reactions), circulating IgE, and eosinophils, as well as the state of eosinophil activation. It was found that despite having more circulating IgE, the skin responsiveness of the atopic subjects did not differ significantly from that of the nonatopic subjects with respect to mediator release, albumin extravasation, or total recruitment of leukocytes. Moreover, the sizes of anti-IgE–induced wheal, flare, and late-phase reactions were very similar in the two groups. On the other hand, significant recruitment of eosinophils during the IgE-mediated reaction was more or less restricted to the atopic group. Yet the recruited eosinophils, of which the majority was in an early state of activation before degranulation, did not seem to contribute significantly to the IgE-mediated delayed skin edema. Furthermore, the eosinophil count in anti-IgE chambers of the atopic subjects did not correlate with any of the other parameters monitored. Thus because the anti-IgE–induced recruitment of eosinophils appeared to be unrelated to factors such as the number of peripheral blood eosinophils, the degree of mast cell activation, the intensity of inflammatory skin changes, and the level of circulating IgE, it is apparent that the mechanisms for and pathophysiologic role of IgE-mediated dermal eosinophil accumulation in atopic subjects require further investigation. (J Allergy Clin Immunol 1996;97:1151-63.)

Key words: *Allergic skin reaction, atopy, anti-IgE, skin chamber, leukocytes, eosinophils, histamine, tryptase, leukotriene B_4, albumin extravasation*

Immediate-type allergic inflammation in the skin is triggered by cross-linking of IgE antibodies on the surface of dermal mast cells.[1] This leads to release of inflammatory mediators such as histamine and different chemotactic factors that cause an immediate wheal and flare response (WFR), usually followed by a slowly developing erythematous induration, termed the late-phase reaction (LPR). The WFR is a direct consequence of rapidly induced hyperemia and increased vascular

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Abbreviations used

ECP:	Eosinophil cationic protein
HETE:	Hydroxy-eicosatetraenoic acid
LPR:	Late-phase reaction
LT:	Leukotriene
MFI:	Mean fluorescence intensity
OG:	n-octyl-beta-D-glucopyranoside
PBE:	Peripheral blood eosinophil
PBS:	Phosphate buffered saline
WFR:	Wheal and flare reaction

permeability, whereas the subsequent edema or induration of the LPR is characteristically associated with gradual leukocyte infiltration.[2, 3] Regarding tissues other than skin, late allergic reactions are also commonly observed in the lungs and nose where the LPR is believed to cause a delayed increase in airway resistance and hyperresponsiveness.[3-7] Although the symptoms differ depending on the target organ, the type of mediators released and the sequence of microvascular inflammatory events follow a rather stereotypical pattern regardless of the tissue in which an allergic reaction manifests.[3-7] Thus the accessibility of the skin makes it an attractive tissue for studies of basic mechanisms, including drug intervention, in IgE-dependent inflammatory reactions. For this purpose, cutaneous anti-IgE challenge in nonatopic subjects appears to be a useful and reproducible alternative to allergen provocation in atopic subjects.[8, 9] Although the skin reactions caused by anti-IgE in nonatopic subjects cannot be distinguished macroscopically from those caused by allergen or anti-IgE in atopic subjects, differences between atopic and nonatopic subjects in this regard may be present at the level of mediator release, leukocyte recruitment, and/or protein extravasation. One approach to directly investigate these inflammatory changes is to use the suction–blister chamber technique.[3] However, to date, there are no skin chamber studies in which healthy and atopic subjects have been thoroughly compared regarding their reactivity to anti-IgE.

Among the different subsets of leukocytes recruited to sites of allergic LPRs, the eosinophil, a minor constituent of the circulating leukocyte count, has been subject to extensive investigation because accumulation of eosinophils is a conspicuous feature of allergic inflammation and is implicated as an important effector mechanism in the pathogenesis of allergic disease.[10, 11] The molecular basis for eosinophil recruitment in allergic disorders is just beginning to be elucidated, and recent studies suggest that in contrast to neutrophil recruitment, eosinophil recruitment may be triggered by specific stimuli such as IL-4 and IL-5 and involves the very late antigen-4–vascular cell adhesion molecule-1 pathway.[7, 11] Yet these findings are not sufficient to explain the intriguing observations by Henocq et al.[12, 13] that dermal mast cell activation with anti-human IgE caused pronounced eosinophil recruitment in atopic subjects but not in healthy subjects. Hypothetically, there are several mechanisms that may explain this difference between atopic and nonatopic subjects, some of which have recently been discussed by Gleich.[10] For example, as compared with nonatopic subjects, atopic subjects may express more mast cell IgE or may have an increased number of mast cells, leading to enhanced mediator release. Moreover, atopic subjects may have "primed" or a greater number of circulating eosinophils.

In this study we have addressed some of the potential differences between atopic and nonatopic subjects with regard to IgE-mediated skin inflammation. Thus with the use of the suction blister–chamber technique, mast cell mediator release and eosinophil recruitment in healthy and atopic subjects were analyzed after local challenge with anti-human IgE. We also measured gross skin reactivity (diameters of WFRs and LPRs), levels of circulating IgE and eosinophils, and the state of eosinophil activation in blood and chamber fluid. Furthermore, to assess the potential role of inflammatory cells for the edema formation associated with the LPR, albumin accumulation in chambers was determined.

METHODS

Subjects

Nine atopic patients, six men and three women aged 18 to 46 years (mean age, 29 years) with a history of allergic rhinitis, total IgE 141.0 ± 46.9 kU/L (range, 12 to 410 kU/L), and positive prick test and RAST (Pharmacia CAP Systems; Pharmacia Diagnostics, Uppsala, Sweden) responses to a standard panel of 10 inhalant allergens, were recruited for the study. The atopic patients were compared with eight healthy age-matched (19 to 45 years; mean age, 30 years) volunteers; three men and five women with no history of atopy, total IgE 11.3 ± 5.8 kU/L (range, 2 to 49 kU/L), and negative CAP Phadiatop (Pharmacia Diagnostics) test results with specificity of 0.94 and sensitivity of 0.96 for atopic allergy.[14] All subjects in the study were drug-free and had negative test responses to intradermal injection of normal goat IgG. In addition, all atopic subjects had negative RAST responses to goat epithelium (Pharma-

cia Diagnostics). All subjects gave their informed consent to participate in the study, which was approved by the ethical committee of the Karolinska Hospital.

Reagents for skin testing

Affinity-purified goat anti-human IgE (ε-chain–specific) and chromatographically purified normal goat IgG were obtained from AB Kemila-Preparat (Sollentuna, Sweden). The lyophilized antibodies were reconstituted in phosphate-buffered saline (PBS) and dialyzed against sterile PBS for 24 hours. The antibodies were then passed through a Millipore filter (0.22 μm); Millipore Corp., Bedford, Mass.), analyzed for protein content according to the method of Bradford,[15] diluted to 0.1 mg/ml (=titer 1:10) in sterile PBS, divided into aliquots, and stored frozen at −20° C. The LPRs induced by the anti-IgE developed in a dose-dependent manner, and the concentration of anti-IgE chosen for intradermal injection (1:100) was found to cause distinct, continuously growing LPRs, peaking at 6 to 10 hours in five healthy subjects (data not shown). Histamine phosphate (British Drug House, Poole, U.K.) was dissolved in sterile PBS at a concentration of 60 μmol/L and passed through a Millipore filter (0.22 μm).

We have used anti-IgE challenge in human skin for a number of years, and we have no evidence that the subjects become sensitized to the small amounts of animal IgG administered. Thus repeated (with intervals of 2 weeks to several years) injection or instillation into skin chambers of "normal" goat/rabbit IgG (which is always used as negative control) has not been found to cause skin reactions of greater size than those caused by PBS (R. Grönneberg. Unpublished observations).[16]

Skin chambers

Skin blisters were induced on the volar aspect of each forearm with a negative pressure cutaneous suction chamber system (Electronic Diversities, Finksburg, Md.). The suction chamber was placed on a cleaned skin area, followed by continuous gentle suction (300 mm Hg) and heating (39° C) for 2 to 3 hours until a complete blister (9 mm in diameter) was formed. The blisters were covered overnight with a plastic chamber (Augenverband S; Lohmann GmbH, Munich, Germany), and the next morning, the blisters were aseptically unroofed. A transparent sterile adhesive plastic film (Tegaderm; 3M Pharmaceuticals, Loughborough, England) with a circular hole was applied around the denuded blister base, 3 mm away from the blister area. The film was used to protect intact skin from possible irritation by a cutaneous adhesive (Skin-Bond cement; Smith & Nephew United, Inc., Largo, Fla.). The Skin Bond Cement was applied around the bottom edge of sterilized open-bottom plastic skin chambers (volume ≈1 ml), which were placed over the unroofed blisters and secured by tape. The chambers were then washed four times with PBS containing 30 IU/ml heparin. One hundred fifty microliters of anti-IgE (1:10 in PBS-heparin) was infused into one chamber, whereas the contralateral control chamber received only PBS-heparin in accordance with previous skin chamber studies of allergen-induced dermal inflammation.[17, 18] After 1 hour of incubation, the chambers were filled with PBS-heparin (1 ml, same volume in atopic and nonatopic subjects), and the fluid was carefully aspirated and reinstilled two times to allow a complete collection of exudate. The chambers were then refilled with PBS-heparin (1 ml), and chamber contents were removed hourly for the next 3 hours. This was followed by incubation with PBS-heparin for 4 hours (4 to 8 hours after start of challenge) before final removal. All aliquots were spun at 300 *g* for 5 minutes at 4° C. The supernatants were immediately frozen and stored at −70° C until analysis of mediator and albumin content was performed. Storage times were identical for atopic and nonatopic subjects. The cell pellets were resuspended in PBS and used for flow cytometric determination of inflammatory cells as described below. Chamber exudates from the first three subjects (two atopic and one nonatopic) in the study were analyzed with respect to inflammatory cells only.

Skin testing

Immediately after the start of incubation with anti-IgE in the chamber, anti-IgE (1:100 in PBS, 50 μl) was injected intradermally on the same forearm midway between the chamber site and the elbow. Histamine (3 nmol in 50 μl of PBS), normal goat IgG (1:100 in PBS, 50 μl), and PBS (50 μl) were similarly injected on the contralateral arm, incubated with PBS-heparin in the chamber. The WFRs (at 10 minutes) and the subsequent delayed edema or induration (LPR, 1 to 6 hours) to anti-IgE were delineated on transparent film by one of the investigators. The 10- to 24-hour LPR was similarly recorded by the test subjects themselves after thorough instructions were given. The areas (in square centimeters) of the skin responses were determined by the use of computer-assisted planimetry. "Total LPR" was estimated by adding the areas for all LPR time points from 1 to 24 hours.

Mediator and albumin assays

Histamine was analyzed with a commercially available radioimmunoassay (Histamine radioimmunoassay kit; IMMUNOTECH International S.A., Marseille, France). In this assay modified histamine in the sample competes with iodinated histamine tracers for binding to an anti-histamine antibody, coated on a test tube (detection limit: 0.05 ng/ml histamine). Standard solution or sample were mixed with acylation buffer and incubated in the presence of acylating reagent for 30 minutes at room temperature. Acylated standard or sample and iodine 125–radiolabeled histamine were then added to anti-histamine antibody–coated tubes. Uncoated tubes with tracer alone were used for determination of total radioactivity. After incubation for 18 hours at 2 to 6° C, the

coated tubes were emptied, and the bound radioactivity was measured.

Tryptase was measured by a modification of a commercially available solid-phase immunoradiometric assay (Pharmacia Tryptase RIACT, Pharmacia Diagnostics AB) based on two tryptase-specific monoclonal antibodies (detection limit: 0.5 ng/ml tryptase). The tryptase in the skin chamber fluid was allowed to react simultaneously with anti-tryptase antibody bound to the wall of the test tube and soluble ^{125}I-anti-tryptase. After overnight incubation at room temperature, the tubes were washed three times, and the remaining radioactivity was measured.

Leukotriene (LT) B_4 was measured with a commercially available enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, Mich.) (detection limit: 3.9 pg/ml LTB_4). This assay is based on competition between free LTB_4 in the sample and acetylcholinesterase-linked LTB_4 tracer for limited specific rabbit LTB_4-antiserum binding sites. The rabbit antiserum-LTB_4 complex (free or tracer) then binds to a monoclonal anti-rabbit antibody attached to wells in a microtiter plate. The plates were incubated for 18 hours at room temperature, washed, and developed by adding Ellman's reagent. The acetylcholinesterase tracer cleaves the Ellman's reagent to form a yellow substance, the density of which was determined photometrically at 412 nm. The cross-reactivity for the LTB_4 antiserum used was less than 0.1% for LTC_4, LTD_4, LTE_4, arachidonic acid, 5(S)-hydroxy-eicosatetraenoic acid (HETE), 12(S)-HETE, 12(R)-HETE, and less than 0.3% for 5(S),12(S)-DiHETE (Cayman Chemical Co.). The levels of LTB_4 in skin chambers were too low for further high-performance liquid chromatography purification before the enzyme immunoassay. However, we have indirect evidence that other components of complex inflammatory exudates do not give false LTB_4-like signals in this particular enzyme immunoassay; that is, we have found that the marked increase in LTB_4-like immunoreactivity in peritoneal exudates of mice with experimental allergic peritonitis is suppressed below the level of detection by the potent and selective leukotriene synthesis inhibitor MK-886 in both purified (high-performance liquid chromatography) and unpurified samples (J. Raud. Unpublished observation).

Albumin was measured by using a radioimmunoassay with rabbit anti-human albumin (Dakopatts, Copenhagen, Denmark) and commercial standard (Calbiochem, San Diego, Calif.) (detection limit: 6.25 ng/ml albumin). Iodination was performed using a lacktoperoxidase method described by Thorell and Johansson.[19] The dilution buffer was 0.05 mol/L sodium phosphate, 0.9% NaCl, 0.01% bovine serum albumin (BSA Fraction V, Wilfrid Smith, Middlesex, U.K.), and 0.01% NaN_3 at pH 7.6. Tracer and standards (or samples) were mixed with antiserum and incubated overnight at room temperature before addition of goat anti-rabbit antiserum (Astra Draco AB, Lund, Sweden) diluted in assay buffer with 5% polyethlyene glycol 6000 (Kebo Lab, Stockholm, Sweden), after which the radioactivity of the bound fraction was measured.

The mediators and albumin were all measured in duplicate. No unspecific histamine, tryptase, LTB_4, or albumin immunoreactivity was detected in buffer containing only anti-IgE and/or PBS-heparin.

Analysis of leukocytes

Peripheral blood leukocytes from the eight nonatopic subjects, the nine atopic subjects, and 25 healthy blood donors were analyzed within 1 hour after sampling. Erythrocytes in ethylenediamine tetraacetic acid blood were lysed with NH_4Cl, and the leukocytes were used without further isolation procedures as previously described.[20] Leukocytes in skin chamber fluid were obtained after centrifugation at 300 *g* for 5 minutes (4° C). To enable flow cytometric analysis of eosinophils without prior purification procedures, the leukocyte preparations from blood and skin exudates were treated according to the previously described cell membrane permeabilization method.[21, 22] Briefly, the permeabilization is achieved by treating parafomaldehyde-fixed leukocytes with n-octyl-β-D-glucopyranoside (OG). This treatment results in scatter-distinguishable eosinophils and permits analysis of both intracellular and surface antigens by flow cytometry. The monoclonal antibody EG2 (Kabi Pharmacia Diagnostic, Uppsala, Sweden) was conjugated with fluorescein isothiocyanate and incubated with permeabilized leukocytes as previously described.[21, 23]

In some experiments, when a sufficient number of cells could be harvested, the surface expression of CD9 was measured. To obtain optimal binding of monoclonal antibodies to surface antigens and to avoid intracellular binding, antibodies to CD9 (Sera-Lab, Crawley Down, Sussex, U.K.) were incubated with untreated leukocytes. The surface immunostained cells then underwent paraformaldehyde fixation and permealization with OG to obtain the separated eosinophil population by flow cytometry.

The final leukocyte preparations were analyzed in an EPICS Profile II flow cytometer (Coulter Corp., Miami, Fla.). The data were collected in list mode and further analyzed by using the Elite workstation software (Coulter, Corp.). The analyses performed in this study have been previously described.[21, 22] The eosinophils were identified by their preserved high scatter signals, intracellular binding of EG2, and in some experiments by their surface expression of CD9. The percentage eosinophils was determined, and the mean EG2-dependent fluorescence intensity of the EG2-positive population was measured to quantify intracellular expression of eosinophil cationic protein (ECP), reflecting eosinophil activity. As previously described, the selected eosinophil gate contains no or minimal numbers of lymphocytes ($CD2^+$, $CD4^+$, $CD8^+$), monocytes ($CD14^+$), neutrophils ($CD16^+$), or platelets.[21, 22]

Statistical analysis

Statistical analysis of paired observations was performed by using the Wilcoxon signed-rank test (two-tailed), and independent samples were analyzed by the Wilcoxon-Mann-Whitney test (two-tailed). When appropriate, correlations between the different parameters were sought by calculating the Spearman rank-order correlation coefficient (r_s). All values are expressed as means ± SEM.

RESULTS

Anti-IgE–induced skin inflammation

Intradermal injection of anti-human IgE (titer 1:100) or histamine (3 nmol) induced WFRs of similar magnitude in atopic and healthy subjects (Fig. 1). In both groups cutaneous responses to normal goat IgG (titer 1:100) were indistinguishable from the minor reaction induced by vehicle (PBS) (not shown). LPRs developed only after anti-IgE injection and were characterized by distinct, somewhat irregular, erythematous induration and edema, reaching maximum intensity between 4 and 10 hours after challenge (Fig. 2). The LPRs in the group of healthy subjects tended to be larger than those in the group of atopic subjects; however, the difference was not statistically significant when values for total LPR (0 to 24 hours) were compared ($p = 0.20$). None of the patients complained or showed signs of systemic allergic symptoms (e.g., distant skin rash, airway obstruction) after anti-IgE challenge (applies to all parts of the study).

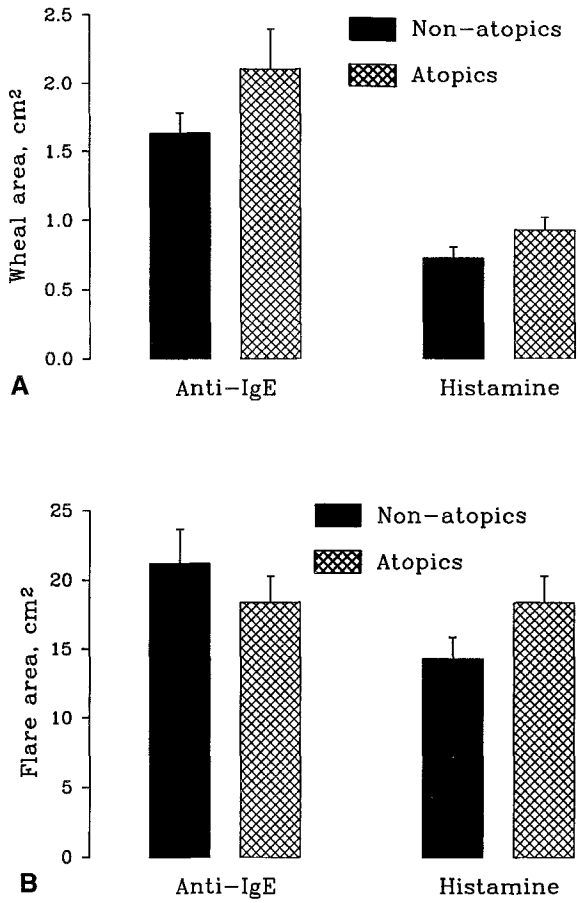

FIG. 1. Wheal area **(A)** and flare area **(B)** 10 minutes after intradermal injection of anti-IgE (titer, 1:100) or histamine (3 nmol) on the volar aspect of the forearms of atopic and nonatopic subjects (mean values ± SEM, $n = 7$ in each subject group).

Anti-IgE–induced mediator release in skin chambers

Challenge with anti-IgE (titer, 1:10) in skin chambers induced a clear-cut release of histamine and tryptase in both healthy and atopic subjects (Figs. 3 and 4). The release patterns for the two mediators were almost identical (i.e., peak release during the first hour of incubation, followed by a gradual decrease during the subsequent 3 hours), and there were no statistical differences between the two groups of subjects ($p > 0.1$ at all time points). In the control chamber only small amounts of histamine and tryptase were detected (Figs. 3 and 4); that is, the total amounts of histamine and tryptase (nanograms during 0 to 4 hours) released in inactive chambers were less than 7% and less than 1%, respectively, of the total release in active anti-IgE chambers.

The atopic subjects had significantly higher levels of circulating IgE than the nonatopic subjects (141.0 ± 46.9 vs 11.3 ± 5.8 kU/L, $p = 0.002$). However, it should be noted in this context that serum IgE in the two groups did not correlate with either the anti-IgE–induced total release of histamine or tryptase, the size of the WFRs, or total LPRs (r_s between −0.571 and 0.714, p values between 0.07 and 0.64).

The levels of LTB_4 during the first 4 hours after anti-IgE challenge were very low in both atopic and nonatopic subjects, with no statistical differences between the two groups ($p > 0.3$ at all time points) (Fig. 5). During the subsequent 4- to 8-hour period, on the other hand, LTB_4 was markedly elevated (Fig. 5). In healthy subjects this late increase in LTB_4 was significantly greater in the anti-IgE chamber as compared with the control site ($p = 0.031$), whereas in the atopic subjects the level of LTB_4 in the active chamber, which tended to be lower than that in the nonatopic subjects (NS, $p = 0.43$), was not statistically different from

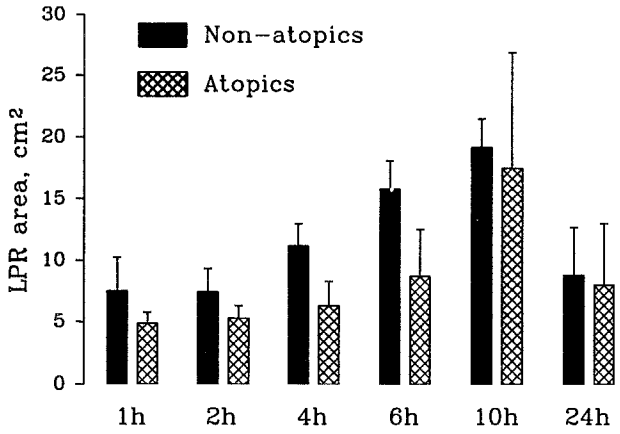

FIG. 2. Size of cutaneous induration (LPR) at indicated time points after intradermal injection of anti-IgE (titer, 1:100) on the volar aspect of the forearms of atopic and nonatopic subjects (mean values ± SEM, $n = 7$ in each subject group).

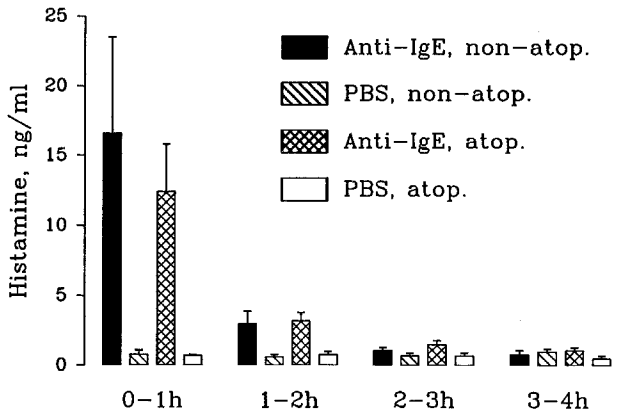

FIG. 3. Histamine levels (in nanograms per milliliter) in skin chamber fluid at indicated time points after local challenge with anti-IgE (titer, 1:10) or PBS in atopic and nonatopic subjects (mean values ± SEM, $n = 7$ in each subject group).

the level in inactive control chambers ($p = 0.34$). There were no statistical differences between the two groups with regard to LTB_4 levels in unstimulated control chambers ($p > 0.05$ at all time points).

Anti-IgE–induced albumin extravasation in skin chambers

Albumin accumulation in inactive control chambers was relatively high in both atopic and nonatopic subjects during the 0 to 8 hours of observation (Fig. 6). Yet, after challenge with anti-IgE, the albumin concentrations rose by approximately 100% (Fig. 6), and this increase was statistically significant at all time points in both groups (p values between 0.008 and 0.023). There were no statistical differences in induced albumin extravasation between the two subject groups ($p > 0.1$ at all time points). Moreover, at no time point did the concentration of albumin after anti-IgE challenge in the two groups correlate with the corresponding concentrations of histamine, tryptase, or LTB_4 or with the accumulation of eosinophils or total number of leukocytes (described below) in the chambers (r_s between −0.393 and 0.607, p values between 0.15 and 0.91). A similar lack of correlation was observed when values in the control chambers were subtracted from active and anti-IgE chambers and when total accumulation of albumin, mediators, and leukocytes during 0 to 4 hours or 0 to 8 hours were considered (not shown).

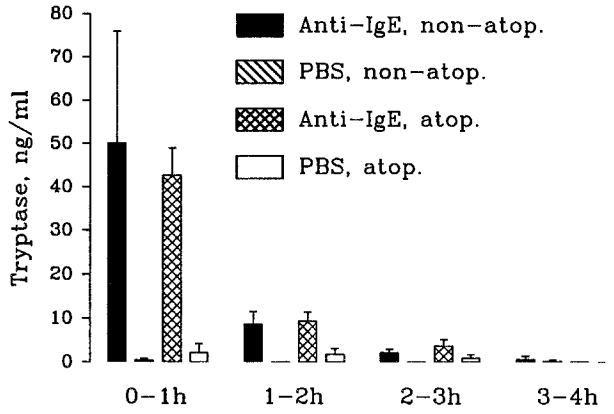

FIG. 4. Tryptase levels (in nanograms per milliliter) in skin chamber fluid at indicated time points after local challenge with anti-IgE (titer, 1:10) or PBS in atopic and nonatopic subjects (mean values ± SEM, $n = 7$ in each subject group).

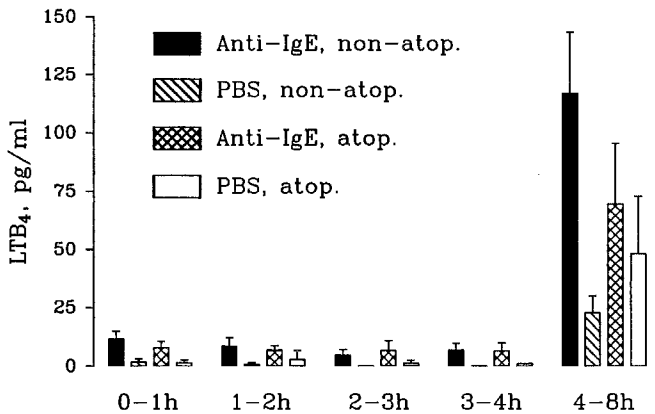

FIG. 5. LTB_4 levels (in picograms per milliliter) in skin chamber fluid at indicated time points after local challenge with anti-IgE (titer, 1:10) or PBS in atopic and nonatopic subjects (mean values ± SEM, $n = 7$ in each subject group).

Anti-IgE–induced leukocyte recruitment in skin chambers

Fig. 7 shows total recruitment of all subtypes of leukocytes ("mixed leukocytes") over time into control and anti-IgE chambers in both subject groups. During the 0- to 8-hour period as a whole, the total leukocyte count in control and active chambers was $5.1 \pm 3.2 \times 10^4$ and $32.0 \pm 12.6 \times 10^4$, respectively, in nonatopic subjects and $11.9 \pm 4.4 \times 10^4$ and $47.8 \pm 15.4 \times 10^4$, respectively, in atopic subjects. In both groups the total leukocyte accumulation in anti-IgE chambers was significantly greater than that in the control chambers ($p < 0.01$); however, there were no significant differences in this respect between the two subject groups ($p = 0.27$ for control chambers and $p = 0.08$ for active chambers). In fact, at no specific time point did any of the chamber leukocyte counts in atopic and nonatopic subjects differ significantly (p values between 0.07 and 0.88).

Anti-IgE–induced recruitment and activation of eosinophils in skin chambers

In contrast to the very similar accumulation of mixed leukocytes in the two subject groups, eosinophil recruitment after anti-IgE challenge was more or less restricted to the atopic group as measured in the 4- to 8-hour samples (eosinophil numbers at earlier time points were too low for reliable identification by flow cytometry (Fig. 8). Thus eosinophil recruitment occurred in all nine atopic subjects (mean number of eosinophils = $47.7 \pm 16.6 \times 10^3$), whereas only two of eight chambers from healthy subjects contained detect-

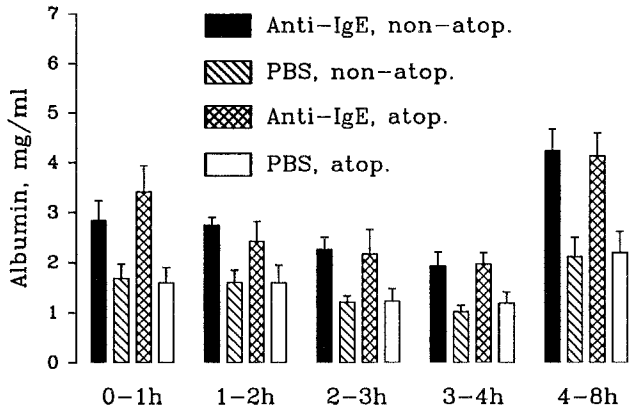

FIG. 6. Albumin levels (in milligrams per milliliter) in skin chamber fluid at indicated time points after local challenge with anti-IgE (titer, 1:10) or PBS in atopic and nonatopic subjects (mean values ± SEM, $n = 7$ in each subject group).

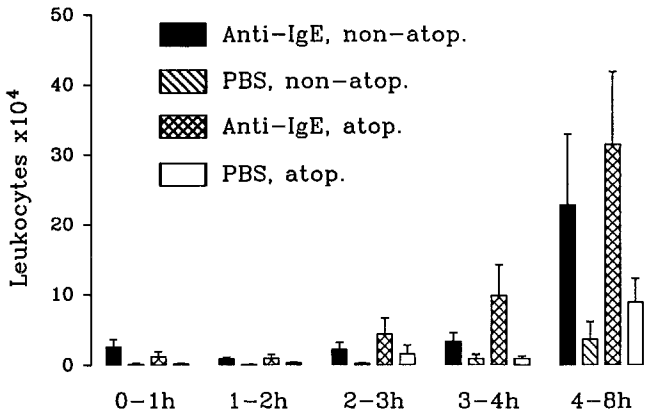

FIG. 7. Total number of mixed leukocytes ($\times 10^4$) in skin chamber fluid at indicated time points after local challenge with anti-IgE (titer, 1:10) or PBS in atopic ($n = 9$) and nonatopic ($n = 8$) subjects. Mean values ± SEM.

able eosinophils (mean = 4.4 ± 3.2 × 10^3, p = 0.003 vs atopic subjects). With respect to control chambers, eosinophil recruitment was only detected in one atopic patient (3.9 × 10^3 eosinophils).

We have previously shown that almost all (≈95%) cells within the defined eosinophil gate bind the monoclonal antibody EG2, which recognizes the intracellular eosinophil-specific ECP.[21, 22] This binding increases when eosinophils are activated, and the degree of activation can be quantified by measuring the mean fluorescence intensity (MFI) of EG2-positive eosinophil populations.[21, 22] In this study 94.7% ± 4.5% (range, 86.1% to 99.7%) of the gated eosinophils were EG2-positive, and the EG2-dependent MFI level for nonactivated eosinophils was found to be 32.9 ± 1.52 U in 25 healthy blood donors. When eosinophils in skin chambers in atopic subjects were analyzed, the MFI values ranged from 64.8 to 120.9 U (mean, 83.8 ± 7.7 U). In the two nonatopic subjects with detectable eosinophils in the anti-IgE chamber, the MFI values were 49.3 and 68.2 U, respectively.

In peripheral blood the percentage of eosinophils in nonatopic subjects was 3.95% ± 0.72% before and 3.08% ± 0.51% 8 hours after the start of anti-IgE challenge with a mean value of 3.36% ± 0.53%. The corresponding data for atopic subjects was 5.58% ± 0.84% before and 5.26% ± 0.75% at the end of the experiment, with a mean value of 5.42% ± 0.74%. Thus the mean peripheral blood eosinophil (PBE) percentage was 61.3% greater in atopic subjects than in nonatopic subjects (p = 0.038). However, there was no tendency for correlation between the PBE count or percentage and number of

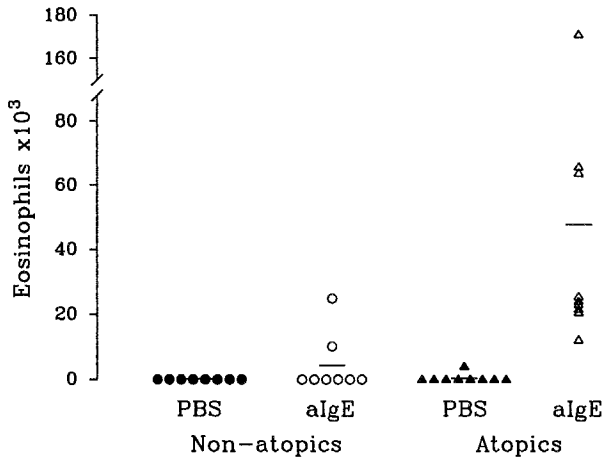

FIG. 8. Recruitment of eosinophils ($\times 10^3$) into skin chamber fluid 4 to 8 hours after local challenge with anti-IgE (titer, 1:10) or PBS in atopic ($n = 9$) and nonatopic ($n = 8$) subjects. *Horizontal bars* indicate group means. A highly significant difference in anti-IgE–induced chamber eosinophilia was observed between atopic and nonatopic subjects ($p = 0.008$).

TABLE I. Correlations between anti-IgE induced skin chamber eosinophilia and other parameters of skin inflammation and serum IgE in atopic subjects

	Total LPR (0-24 hr)	Albumin*	Total serum IgE	PBE count	Histaminet	Tryptase‡	LTB_4§	Leukocytes‖
r_s	0.250	−0.107	0.126	0.008	0.571	0.143	0.252	0.218
p value	0.589	0.819	0.748	0.983	0.180	0.766	0.548	0.574

Data are based on experiments with atopic subjects ($n = 7$ to 9). Correlations between the number of skin chamber eosinophils and the parameters indicated in the table were determined by calculating Spearman's rank-order correlation coefficient.
*Chamber fluid albumin concentration 4 to 8 hours.
†Total skin chamber histamine 0 to 4 hours.
‡Total skin chamber tryptase 0 to 4 hours.
§Total skin chamber LTB_4 4 to 8 hours.
‖Total number of mixed leukocytes in chambers 4 to 8 hours.

chamber eosinophils after anti-IgE challenge in the atopic subjects (Table I). The MFI values of the EG2-positive PBEs before anti-IgE challenge were 39.6 ± 2.83 and 36.2 ± 2.26 U in healthy and atopic subjects, respectively, and 33.6 ± 3.55 and 34.2 ± 2.98 U, respectively, at the end of the experiments.

To further confirm the identity of the cells within the defined eosinophil gate, monoclonal antibodies to CD9 were incubated with chamber leukocytes recruited by anti-IgE when a sufficient number of cells could be harvested ($n = 4$ atopic subjects). CD9 is known to be highly expressed on eosinophils,[24] and we found that the percentage of CD9-positive cells within the given eosinophil gate was 99.5% ± 0.3% and the MFI was 86.4 ± 4.65 U. Furthermore, almost all (>99%) CD9-positive cells exhibited preserved high scatter signals (complexity/granularity), indicating a state of activation before degranulation.

Correlations between chamber eosinophils and other parameters

To investigate potential relationships between eosinophil recruitment and the degree of mast cell activation and intensity of the inflammatory response, possible correlations with other parameters were evaluated. However, the eosinophil count during the 4- to 8-hour period in anti-IgE chambers of atopic subjects did not correlate with any of the other parameters monitored, including the size of the LPRs; the amount of albumin extravasation; the serum IgE levels; the PBE count; the magnitude of histamine, tryptase, and LTB_4 release; and the total number of mixed chamber leukocytes (Table I).

DISCUSSION

Using the suction–blister chamber technique, we have shown that atopic and nonatopic subjects have many features in common regarding skin reactivity to challenge with anti-human IgE. Thus we found no significant differences between the two subject groups with respect to mediator release, plasma protein extravasation, or recruitment of mixed leukocytes. Moreover, in line with previous observations,[8, 13] the size of anti-IgE–induced WFRs and LPRs did not differ significantly between healthy and atopic subjects. If anything, the responsiveness to anti-IgE tended to be weaker in the atopic subjects.

The only major difference between the two groups was that recruitment of eosinophils into chambers was much more pronounced in the atopic group. In other words, a significant number of chamber eosinophils was detected in all nine atopic subjects, but in only two of eight nonatopic subjects. The mean chamber eosinophil count in the atopic group was 10 times higher than that in the group of healthy subjects. These findings confirm and extend the observations by Henocq and Rihoux,[13] who used the Rebuck skin window method to demonstrate that anti-IgE challenge was followed by significant eosinophil accumulation in atopic subjects only. In the latter study a relatively low dose of anti-IgE, which did not result in LPRs, was used in order to discriminate between the two populations.[25] We now demonstrate that higher concentrations of anti-IgE, sufficient to produce clear-cut LPRs in both subject groups, also cause significant eosinophil recruitment in atopic subjects only. This apparent selectivity for eosinophil recruitment is intriguing, and the following series of findings suggest that this feature of atopic subjects is unrelated to factors such as the number of PBEs, the degree of mast cell activation, the intensity of inflammatory skin changes, and the level of circulating IgE. The mean number of PBEs was somewhat elevated (by ≈60%) in the atopic subjects as compared with nonatopic subjects; however, such a small difference in PBEs cannot explain the striking difference in chamber eosinophils. This notion is further supported by the observed lack of correlation between the PBE and chamber eosinophil counts. A lack of correlation between PBEs and skin eosinophils has also been described for intradermal allergen challenge.[26] It has been hypothesized that increased mast cell activity may account for the eosinophil recruitment in atopic subjects.[10] However, we observed no differences in histamine or tryptase release between the two subject groups, and as mentioned previously, the same applies to the size of anti-IgE–induced WFRs and LPRs. That the intensity of the inflammatory responses was similar in the two groups was also indicated by the very similar degree of albumin extravasation and total leukocyte recruitment into chambers. As compared with the nonatopic subjects, the atopic subjects had significantly higher levels of circulating IgE (mean values, 141.0 vs 11.3 kU/L), a feature that can be assumed to increase skin mast cell IgE and to make the mast cells more responsive to anti-IgE challenge.[27, 28] However, as described previously, the results of the anti-IgE challenge do not support a correlation between serum IgE and mast cell reactivity in skin of atopic subjects. Furthermore, there was no correlation between circulating IgE and chamber eosinophils in the atopic group. Taken together, the lines of evidence discussed indicate that the pronounced IgE-mediated eosinophil recruitment into skin of atopic subjects (as compared with nonatopic subjects) must be related to factors such as increased sensitivity of the eosinophils to chemotactic signals and/or selective release of some mediator(s) with eosinophil-specific chemotactic activity.

In vitro studies provide evidence that isolated blood eosinophils from atopic subjects exhibit enhanced migratory activity in response to different chemotactic factors, possibly as a result of eosinophil "priming" caused by increased circulating levels of cytokines such as IL-5.[29-31] Yet these studies do not directly prove that eosinophils in the circulation of atopic subjects are "primed" before isolation, and one cannot exclude the alternative or additional possibility that in comparison with nonatopic subjects, mast cells (or other tissue residing cells) of atopic subjects may release greater amounts of some eosinophil chemotactic factor. In this context LTB_4 has been suggested to represent "low molecular weight eosinophil chemotactic activity of anaphylaxis" (ECF-A).[32, 33] However, increased LTB_4 formation was not the likely cause for eosinophil recruitment in the atopic subjects, because we found that LTB_4 levels in chambers of atopic subjects were equal to or even lower than those in nonatopic subjects. Moreover, the chemotactic activity of LTB_4 on human leukocytes is not selective for eosinophils.[34] Another possibility would be that, as compared with "normal" mast cells, mast cells of atopic subjects may contain or liberate relatively more cytokines with eosinophil-specific activity, for example, IL-4 or IL-5. Interestingly, eosinophils, but not neutro-

phils, are activated by IL-5[35] and use the very late antigen-4–vascular cell adhesion molecule-1 pathway in the process of infiltrating tissues.[36, 37] Moreover, release and/or increased expression of both IL-4, which selectively induces vascular cell adhesion molecule-1 expression on endothelial cells,[11] and IL-5 has been indicated in allergic inflammation in skin and airways.[5] Yet it remains to be shown that cytokine release and/or adhesion molecule expression differs in atopic and nonatopic subjects after anti-IgE challenge.

To enable the analysis of eosinophils in unseparated peripheral blood and in mixed leukocyte suspensions from the skin chambers, we used a recently described cell membrane permeabilization method (i.e., paraformaldehyde fixation and permealization with OG).[21] With this technique, it was found that most chamber eosinophils exhibited both high scatter signals (preserved granularity) and high intracellular expression of ECP, indicating that the eosinophils recruited by anti-IgE were in an early state of activation before degranulation and release of intracellular ECP. This seemingly low degree of eosinophil degranulation is in contrast to previous reports indicating considerable eosinophil degranulation in skin challenged with allergen.[38-40] However, further studies are needed to determine whether these apparent differences in eosinophil degranulation are related to the different stimuli used (anti-IgE vs allergen) or other methodological factors.

Based mainly on temporal associations, it is commonly suggested that the development of delayed allergic cutaneous edema (LPR) is dependent on influx of inflammatory cells.[18, 41-43] This is in agreement with results of animal studies showing that skin edema in response to chemotatic factors is more or less abolished in leukopenic animals or after blockade of leukocytic β_2-integrins.[44, 45] In this study, however, the anti-IgE–induced leukocyte recruitment (considered as total number of cells or eosinophils separately) showed no tendency to correlate with either the development of LPRs or the continuous delayed albumin extravasation in chambers. In further support of a dissociation between the late allergic cutaneous edema and leukocyte or eosinophil infiltration, some investigators have reported lack of correlation between allergen-induced leukocyte recruitment and LPRs,[17, 46, 47] and others have suggested that significant dermal influx of eosinophils and other leukocytes after allergen or anti-IgE challenge may occur without development of late induration or edema.[12, 13, 42] One possible implication of these observations is of course that late allergic cutaneous edema is dependent on factors other than leukocyte extravasation. However, in this context it is also important to emphasize that it may be impossible to find a positive correlation between a single effector mechanism and a target effect at an inflamed site where the chemical and cellular complexity provides a basis for multiple interactions. Thus as indicated in numerous animal studies,[48, 49] it is likely that the leukocyte extravasation during allergic LPRs in skin is indeed of importance for promoting edema formation by acting synergistically with locally formed vasodilating or edema-promoting mediators.

Histamine is a major mediator of the immediate anti-IgE–induced wheal formation.[13, 50] Yet we did not find a correlation between histamine release and the early anti-IgE–induced albumin extravasation. It is possible that this lack of correlation again illustrates mediator interactions, for example, between histamine and LTC_4, which also causes WFRs in human skin and is formed early after both allergen and anti-IgE challenge.[51, 52] This is supported by a recent study in the skin-like hamster cheek pouch, in which very low concentrations of exogenous histamine and LTC_4 act in striking synergism to cause plasma extravasation.[53]

Extravasation of albumin in control chambers was approximately 50% of the corresponding values in active anti-IgE chambers throughout the experiments, suggesting that induction of blisters is somewhat traumatic to the tissue. However, the basal release of mediators and spontaneous influx of leukocytes was comparatively small and did not seem to parallel the basal albumin leakage. This suggests that the basal extravasation of albumin is due to mechanical rather than inflammatory preparative microvascular disturbance. This is in agreement with a study by Atkins et al.,[54] showing that basal IgG extravasation into skin chambers was high in comparison with spontaneous histamine release.

We conclude that with the exception of pronounced cutaneous eosinophil recruitment in the atopic subjects, the investigated dermal indices of IgE-mediated inflammation were very similar in atopic and nonatopic subjects. Moreover, the recruitment of eosinophils in atopic subjects appeared to be unrelated to factors, such as the number of PBEs and the level of circulating IgE. This suggests that selective eosinophil accumulation in atopic skin is not simply a result of quantitative differences between atopic and nonatopic subjects with regard to common features of the

allergic effector response. The use of anti-IgE challenge in skin chambers is likely to be a very useful approach in the further analysis of the apparent differences between healthy and atopic subjects with regard to IgE-mediated eosinophil recruitment.

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REFERENCES

1. Ishizaka T, Ishizaka K. Activation of mast cells for mediator release through IgE receptors. Prog Allergy 1984;34: 188-235.
2. Gleich GJ. The late phase of the immunoglobulin E-mediated reaction: a link between anaphylaxis and common allergic disease? J Allergy Clin Immunol 1982;70:160-9.
3. Zweiman B. Mediators of allergic inflammation in the skin. Clin Allergy 1988;18:419-33.
4. Togias A, Naclerio RM, Proud D, et al. Studies on the allergic and nonallergic nasal inflammation. J Allergy Clin Immunol 1988;81:782-90.
5. Kay AB. Asthma and inflammation. J Allergy Clin Immunol 1991;87:893-910.
6. Holgate S. Mediator and cytokine mechanisms in asthma. Thorax 1993;48:103-9.
7. Montefort S, Holgate ST, Howarth PH. Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. Eur Respir J 1993;6:1044-54.
8. Weiss ME, Trent P, Fisher R, Norman PS, Waterbury WE, Adkinson NF Jr. Rabbit F(ab')2 antihuman IgE is a universal skin test reagent in the evaluation of skin mast cell degranulation in vivo. J Allergy Clin Immunol 1989;83: 1040-8.
9. Grönneberg R, Zetterström O. Eosinophils in late cutaneous reactions [Letter]. Clin Exp Allergy 1991;21:377-8.
10. Gleich GJ. Why do allergic individuals accumulate more eosinophils at the site of an IgE-mediated reaction than do normal subjects? [Editorial]. Clin Exp Allergy 1990;20: 245-6.
11. Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. Am J Respir Cell Mol Biol 1993;8:349-55.
12. Henocq E, Vargaftig BB. Accumulation of eosinophils in response to intracutaneous PAF-acether and allergens in man. Lancet 1986;1:1378-9.
13. Henocq E, Rihoux JP. Does reversed-type anaphylaxis in healthy subjects mimic a real allergic reaction? Clin Exp Allergy 1990;20:269-72.
14. Eriksson NE. Allergy screening with Phadiatop and CAP Phadiatop in combination with a questionnaire in adults with asthma and rhinitis. Allergy 1990;45:285-92.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.
16. Roquet A, Raud J, Halldén G, et al. Effects of loratadine on anti-IgE-induced inflammation, histamine release, and leukocyte recruitment in skin of atopics. Allergy 1995;50: 414-20.
17. Fleekop PD, Atkins PC, von Allmen C, Valenzano MC, Shalit M, Zweiman B. Cellular inflammatory responses in human allergic skin reactions. J Allergy Clin Immunol 1987;80:140-6.
18. Charlesworth EN, Hood AF, Soter NA, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Cutaneous late-phase response to allergen. Mediator release and inflammatory cell infiltration. J Clin Invest 1989;83:1519-26.
19. Thorell JI, Johansson BG. Enzymatic iodination of polypeptides to high activity. Biochim Biophys Acta 1971; 251:363-9.
20. Halldén G, Andersson U, Hed J, Johansson SGO. A new membrane permeabilization method for the detection of intracellular antigens by flow cytometry. J Immunol Methods 1989;124:103-9.
21. Halldén G, Hed J. The FOG-method—a new approach to detecting activated eosinophils. ACI News 1993;5:139-43.
22. Hed J, Halldén G. Counts of activated blood eosinophils for monitoring asthma. Allergy 1993;48(suppl 17):87-93.
23. Ihre E, Halldén G, Hed J, Zetterström O. Change in eosinophil expression of ECP and its relation to bronchial hyperresponsiveness after repeated inhalation of low doses of allergen. In: Ihre E, ed. Physiological changes and eosinophil activation in allergen induced asthma. Stockholm: Karolinska Institutet; 1993. Thesis.
24. Ohto H, Hitomi Y, Kambayashi H, Sato T, Maeda H. CD9 defined antigen (p24) is expressed on eosinophils but not on normal monocytes. In: McMichael A, ed. Leukocyte typing III. Oxford: Oxford University, 1987:731-2.
25. Rihoux J-P. Eosinophils in late cutaneous reactions [Letter]. Clin Exp Allergy 1991;21:378.
26. Zweiman B, Slott RI, Atkins PC. Factors in the tissue eosinophil responses in human immediate hypersensitivity reactions. Int Arch Allergy Appl Immunol 1976;52:48-52.
27. Tada T. Regulation of reaginic antibody formation in animals. Prog Allergy 1975;19:122-94.
28. Hanahoe TH. Mechanism of histamine release form rat isolated peritoneal mast cells by dextran: the role of immunoglobulin E. Agents Actions 1984;14:468-74.
29. Moser R, Fehr J, Bruijnzeel PL. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. J Immunol 1992;149:1432-8.
30. Warringa RA, Schweizer RC, Maikoe T, Kuijper PH, Bruijnzeel PL, Koendermann L. Modulation of eosinophil chemotaxis by interleukin-5. Am J Respir Cell Mol Biol 1992;7:631-6.
31. Bruijnzeel PL, Kuijper PH, Rihs S, Betz S, Warringa RA, Koenderman L. Eosinophil migration in atopic dermatitis. I: Increased migratory responses to N-formyl-methionyl-leucyl-phenylalanine, neutrophil-activating factor, platelet-activating factor, and platelet factor 4. J Invest Dermatol 1993;100:137-42.
32. Czarnetzki BM, Rosenbach T. From eosinophil chemotactic factor of anaphylaxis to leukotriene B_4—chemistry, biology and functional significance of eosinophil chemotactic leukotrienes in dermatology. Dermatologica 1989; 179(suppl 1):54-9.
33. Sehmi R, Cromwell O, Taylor GW, Kay AB. Identification of guinea pig eosinophil chemotactic factor of anaphylaxis as leukotriene B_4 and 8(S),15(S)-dihydroxy-5,9,11,13(Z,E,Z,E)-eicosatetraenoic acid. J Immunol 1991;147:2276-83.
34. Bray MA. Leukotrienes in inflammation. Agents Actions 1986;19:87-99.
35. Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Waltmann P, Kay AB. Interleukin-5 selectively enhances the

chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. Blood 1992;79:2952-9.
36. Bochner BS, Luscinskas FW, Gimbrone MA Jr, et al. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. J Exp Med 1991;173:1553-7.
37. Weg VB, Williams TJ, Lobb RR, Nourshargh S. A monoclonal antibody recognizing very late activation antigen-4 inhibits eosinophil accumulation in vivo. J Exp Med 1993; 177:561-6.
38. Henocq E, Vargaftig BB. Skin eosinophilia in atopic patients. J Allergy Clin Immunol 1988;81:691-5.
39. Leiferman KM, Fujisawa T, Gray BH, Gleich GJ. Extracellular deposition of eosinophil and neutrophil granule proteins in the IgE-mediated cutaneous late phase reaction. Lab Invest 1990;62:579-89.
40. Zweiman B, Atkins PC, von Allmen C, Gleich GJ. Release of eosinophil granule proteins during IgE-mediated allergic skin reactions. J Allergy Clin Immunol 1991;87:984-92.
41. Solley GO, Gleich GJ, Jordon RE, Schroeter AL. The late phase of the immediate wheal and flare skin reaction. Its dependence upon IgE antibodies. J Clin Invest 1976;58: 408-20.
42. Frew AJ, Kay AB. The relationship between infiltrating CD4+ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. J Immunol 1988;141:4158-64.
43. Charlesworth EN, Kagey-Sobotka A, Schleimer RP, Norman PS, Lichtenstein LM. Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late-phase response to allergen. J Immunol 1991;146:671-6.
44. Wedmore CV, Williams TJ. Control of vascular permeability by polymorphonuclear leukocytes in inflammation. Nature 1981;289:646-50.
45. Arfors K-E, Lundberg C, Lindbom L, Lundberg K, Beatty PG, Harlan JM. A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. Blood 1987;69:338-40.
46. Richerson HB, Rajtora DW, Penick GD, et al. Cutaneous and nasal allergic responses in ragweed hay fever: lack of clinical and histopathologic correlations with late phase reactions. J Allergy Clin Immunol 1979;64:67-77.
47. Nish WA, Charlesworth EN, Davis TL, et al. The effect of immunotherapy on the cutaneous late phase response to antigen. J Allergy Clin Immunol 1994;93:484-93.
48. Williams TJ. Interactions between prostaglandins, leukotrienes and other mediators of inflammation. Br Med Bull 1983;39:239-42.
49. Raud J, Lindbom L. Studies by intravital microscopy of basic inflammatory mechanisms and acute allergic inflammation. In: Brain S, ed. The handbook of immunopharmacology: immunopharmacology of the microcirculation. London: Academic Press, 1994:127-70.
50. Grönneberg R, Dahlén S-E. Interactions between histamine and prostanoids in IgE-dependent, late cutaneous reactions in man. J Allergy Clin Immunol 1990;85:843-52.
51. Camp RD, Coutts AA, Greaves MW, Kay AB, Walport MJ. Responses of human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. Br J Pharmacol 1983;80:497-502.
52. Dorsch W, Ring J, Weber PC, Strasser T. Detection of immunoreactive leukotrienes LTC_4/D_4 in skin-blister fluid after allergen testing in patients with late cutaneous reactions (LCR). Arch Dermatol Res 1985;277:400-1.
53. Raud J, Lindbom L, Hedqvist P. Histamine and leukotriene C_4 act synergistically via a blood flow-independent mechanism to enhance microvascular plasma leakage. Acta Physiol Scand 1992;146:545-6.
54. Atkins PC, Kaplan AP, von Allmen C, Moskovitz A, Zweiman B. Activation of the coagulation pathway during ongoing allergic cutaneous reactions in humans. J Allergy Clin Immunol 1992;89:552-9.