

Evidence for a role for IL-5 and eotaxin in activating and recruiting eosinophils in drug-induced cutaneous eruptions

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Background: Cutaneous drug reactions may be associated with increased numbers of eosinophils in the blood and tissue. However, the factors leading to the generation of eosinophilia have not been fully delineated.

Objective: The aim of this study was to investigate the in situ expression of IL-5, eotaxin, RANTES, monocyte chemoattractant protein 3, and IL-8 together with the appearance of eosinophils in acute cutaneous drug reactions.

Methods: Skin biopsy specimens were obtained from drug-induced maculopapular exanthems (n = 9), from normal skin of control subjects (n = 9), and from the skin of patients with psoriasis (n = 8). The in situ expression of IL-5, eotaxin, RANTES, monocyte chemoattractant protein 3, and IL-8 was analyzed by using immunohistochemistry. Furthermore, the corresponding numbers of eosinophils were determined in the blood and skin sections.

Results: Compared with normal skin and psoriatic skin, a significantly higher number of eosinophils was found both in the blood and tissue of patients with a drug-induced exanthem. In comparison with normal skin, immunoreactivity for IL-5 and all the chemokines was also significantly enhanced in drug-induced exanthem, whereas significant differences in psoriatic were only observed for IL-5 and eotaxin.

Conclusion: Our data indicate that IL-5 and eotaxin may particularly contribute to the activation and recruitment of eosinophils and thereby play an important pathogenic part in the development of skin inflammation in drug-induced maculopapular exanthems. (*J Allergy Clin Immunol* 2000;106:1171-6.)

Key words: Eotaxin, monocyte chemoattractant protein 3, RANTES, IL-5, IL-8, drug allergy, psoriasis, immunohistochemistry

The precise underlying pathomechanism of most cutaneous adverse drug reactions is still incompletely understood. In recent years, increasing in vivo and in vitro data

Abbreviations used

APAAP: Alkaline phosphatase-antialkaline phosphatase
MCP: Monocyte chemoattractant protein
SI: Stimulation index
TCC: T-cell clone

indicate that cell-mediated immune mechanism are particularly involved in some of these reactions, such as drug-induced maculopapular exanthems.^{1,2} On histologic examination, these reactions show a mixed inflammatory cell infiltrate, which is mainly composed of T lymphocytes and, to a lower extent, dendritic cells, macrophages, neutrophils, and eosinophils. Recently, we postulated that cytotoxic T cells (CD4⁺ and CD8⁺ cells) may induce necrosis of keratinocytes in maculopapular exanthems, whereas other T cells without cytotoxic potential may primarily orchestrate the inflammatory skin reaction through the release of cytokines and chemokines.^{1,2} In addition to T cells, eosinophils may also contribute to the generation of tissue damage by the release of various toxic granule proteins, such as eosinophilic cationic protein, major basic protein, and eosinophil peroxidase.³ Although it is likely that an array of various cytokines and chemokines are involved in the recruitment and activation of these inflammatory cells, the precise local factors responsible for the induction of skin inflammation still remain to be clarified.

Chemokines are a superfamily of 8- to 10-kd basic heparin-binding polypeptide mediators, which play an important role in immune and inflammatory reactions.^{4,5} According to the number and position of cysteine residues in the amino-terminal region, 4 subtypes of chemokines (CXC, CC, C, and CX₃C) have been identified. Eotaxin (also called *cysteine cysteine ligand 11* [CCL11]), RANTES (CCL5), and monocyte chemoattractant protein (MCP) 3 (CCL7) represent members of the CC chemokine family and are chemoattractants for eosinophils, basophils, T cells, and monocytes, although not for neutrophils. IL-8 (CXCL8), a member of the CXC chemokine family, is a potent chemoattractant for neutrophils. To our knowledge, no data on the involvement of these chemokines are thus far available in drug-induced exanthems. The present study was therefore designed to investigate the in situ expression and distribution of eotaxin, RANTES, MCP-3,

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TABLE I. Clinical and laboratory data of patients with a drug-induced maculopapular exanthem

Culprit drug	Concurrent disorder	Time at beginning of symptoms	Blood eosinophils (10 ⁹ /L)	Scratch-patch test	Lymphocyte transformation test (SI)
Amoxicillin/clavulanic acid	Pneumonia	10 d	0.1	Negative	5.1 [§] (amoxicillin)
Amoxicillin/clavulanic acid	Skin infection	3 d	1.3	++	12.5 [§] (amoxicillin)
Amoxicillin/clavulanic acid	Skin infection, arthritis	10 d	0.5	+	4.1 [§] (amoxicillin)
Cefazoline	Antibiotic prophylaxis before surgery	7 d	1.1	Negative	2.4 [†]
Prednisolone	Eczema of the face	2 d	1.6	++	2.1 [*]
Carbamazepine	Idiopathic syncope	14 d	3.0	Not done	23.4 [†]
Indapamide	Arterial hypertension	10 d	0.2	Negative	11.5 [‡]
Metozalone	Cardiac disease	5 d	0	Not done	12.5 [*]
Vitamin B complex	Nil	15 d	1.5	Negative	7.5 [†]

Concentration of the drug in the lymphocyte transformation test: *1 µg/mL; †10 µg/mL; ‡100 µg/mL; §500 µg/mL.

+, Weak positive reaction (erythema, infiltration, and possibly papules); ++, strong positive reaction (erythema, infiltration, papules, and vesicles).

and IL-8 in patients with a drug-induced maculopapular exanthem by means of immunohistochemistry. Furthermore, we sought to examine the in situ expression of IL-5, which has previously been reported to be involved in the activation and recruitment of eosinophils in cooperation with eotaxin in allergic diseases.⁶⁻⁸

METHODS

Subjects and skin biopsy specimens

The Ethical Committee of the Medical Faculty of the University of Bern approved the study. Nine white patients (5 female and 4 male patients; mean age, 50.1 years) with a generalized maculopapular drug eruption were included in the study after obtaining their informed consent. Seven of these patients were also involved in our previous study.¹ The culprit drugs were as follows: amoxicillin/clavulanic acid (n = 3), cefazoline (n = 1), prednisolone (n = 1), carbamazepine (n = 1), indapamide (n = 1), metozalone (n = 1), and vitamin B complex (n = 1; Table I). All drugs were given by mouth, except for cefazoline, which was administered intravenously. In all patients the development of cutaneous eruptions occurred after initiation of therapy and resolved within 1 to 2 weeks after drug discontinuation. Punch biopsy specimens of 5 mm in diameter were usually taken 2 to 3 days after the onset of symptoms from an acute maculopapular skin eruption. Normal skin was obtained from 9 patients undergoing reconstructive surgery. In addition, biopsy specimens were taken from 8 patients with psoriasis vulgaris as a further control group. Before the investigation, the patients did not receive any systemic corticosteroids or topical treatment with corticosteroids at the site of the punch biopsy.

Epicutaneous testing (scratch-patch test)

Epicutaneous testing (scratch-patch test) was performed approximately 2 months after recovery with PBS (negative control), amoxicillin/clavulanic acid (375 mg/mL PBS), cefazoline (500 mg/mL PBS), prednisolone (50 mg/mL PBS), indapamide (12.5 mg/mL PBS), and vitamin B complex (1 tablet/0.5 mL PBS), as described previously.⁹ Briefly, after scarification of the epidermis, freshly prepared drug solutions were applied on the upper back by using Al-test patch test disks (IMAL Pharmaceutica AG, Zürich, Switzerland). The test reactions were read at 24 and 48 hours and scored as recommended by the International Contact Dermatitis Research Group.¹⁰ All substances were also tested at the same concentrations in at least 10 healthy control subjects without eliciting any skin reaction.

Lymphocyte transformation analysis

Lymphocyte transformation analysis was performed with the heparinized blood of the patient 2 months after recovery, as described in detail previously.¹¹ Briefly, PBMCs were isolated by using standard Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation and cultured at a cell density of 10⁶/mL. The cultures were set up in 1 or 2 mL of RPMI-1640 supplemented with either 20% AB serum or 20% autologous plasma. The same drugs (plus metozalone and carbamazepine) as used for epicutaneous testing were diluted to reach nontoxic final concentrations of mostly 1, 10, or 100 µg/mL (with amoxicillin up to 500 µg/mL) in the culture medium. After 5 to 7 days, the amount of incorporated tritiated thymidine (0.5 mCi) was determined with a β-counter (Packard, Canberra Corp, Meriden, Conn). Results were expressed as stimulation indices (SIs) as follows:

$$SI = \frac{\text{counts per minute (cpm) of cultures with drug}}{\text{cpm of culture without drug}}$$

An SI value of more than 2.0 was interpreted as a positive result. Unspecific enhancement of proliferation by the drug was excluded by evaluating the proliferation of PBMCs in unexposed individuals. The specificity and sensitivity of this assay has also been reported previously.¹¹

Hematoxylin and eosin staining

Punch biopsy specimens were equally divided into 2 pieces. For standard histology, one half was fixed in 4% formalin, routinely processed, embedded in paraffin, and stained with hematoxylin and eosin.

Immunohistochemistry

The second half of the skin biopsy specimen was snap-frozen in tissue-embedding medium by using isopentane precooled in liquid nitrogen and stored at -70°C. The following mAbs were used in the study: anti-IL-5 (clone JES1-39D10, concentration: 10 µg/mL; PharMingen/Becton Dickinson, Mountain View, Calif); anti-eotaxin (clone 10C11, concentration: 50 µg/mL; PharMingen/Becton Dickinson), anti-RANTES (clone 2D5, concentration: 10 µg/mL; PharMingen/Becton Dickinson); anti-IL-8 (clone G265-8, concentration: 10 µg/mL; PharMingen/Becton Dickinson); anti-MCP-3 (clone 9H11, concentration: 10 µg/mL; PharMingen/Becton Dickinson); and anti-CD3 (clone UCHT1; concentration: 3 µg/mL; DAKO, Glostrup, Denmark). Substitution of the primary antibody with isotype-matched IgG and omission of the primary antibody served as negative controls.

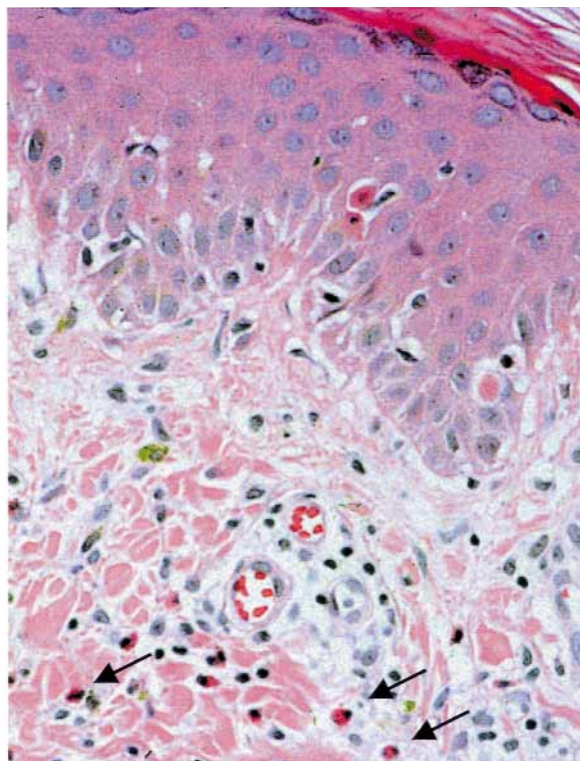


FIG 1. Representative histology of a drug-induced maculopapular exanthem showing a superficial mixed inflammatory cell infiltration with eosinophils (arrows; original magnification 250 $\times$).

Immunostaining for IL-5 and RANTES was performed by using the alkaline phosphatase–antialkaline phosphatase (APAAP) method.¹² Briefly, 6- μ m cryostat tissue sections were air-dried, fixed in 2% formaldehyde for 8 minutes, and rehydrated in TRIS-buffered saline with 0.1% saponin. Slides were then incubated for 3 hours with the primary (rat anti-IL-5 or mouse anti-RANTES) antibody, followed by a rabbit anti-rat antibody (Z-0455; DAKO) or a rabbit anti-mouse antibody (Z-259; DAKO) and the APAAP complexes (D-0488 or D-651; DAKO). To enhance the signal, incubation with the bridging antibody and the APAAP complexes was repeated once.

Immunostaining for CD3, eotaxin, MCP-3, and IL-8 was performed with the avidin-biotin complex/alkaline phosphatase method.¹ Briefly, after fixation for 8 minutes with acetone (for CD3) or 2% formaldehyde (other antibodies), slides were incubated for 1 hour (for CD3) or overnight (other antibodies) with the primary antibody, followed by a biotinylated rabbit anti-mouse IgG (E0413; DAKO) and thereafter with avidin-biotin complex/alkaline phosphatase (K0376; DAKO). Finally, all sections were developed in new fuchsin-naphthol (Sigma, St Louis, Mo) and counterstained with hematoxylin.

Evaluation of sections

The slides were coded with numbers and evaluated in a blinded fashion. The sections were assessed on 10 to 15 fields at 400 $\times$ magnification with a Leitz Dialux 20EB by 2 independent investigators. The number of eosinophils on skin sections stained with hematoxylin and eosin and the number of positive cells for IL-5, eotaxin, RANTES, MCP-3, and IL-8 were counted by using a 0.063-mm² grid; the number of positive cells (mean $\pm$ SEM) per square millimeter was calculated. Cells displaying a strong cytoplasmic staining were considered to be positive. Only positively stained cells with a nucleus were included in the counting.

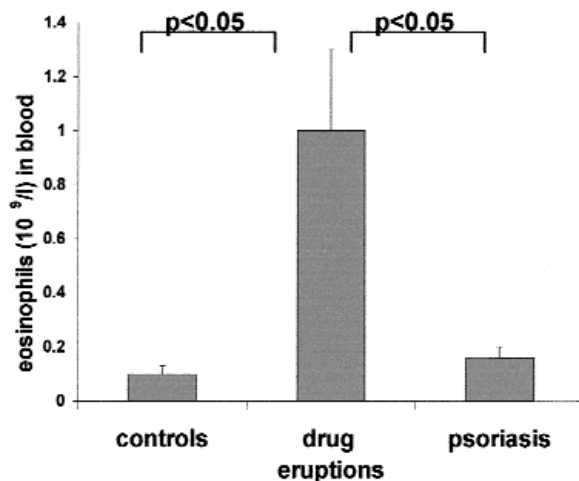
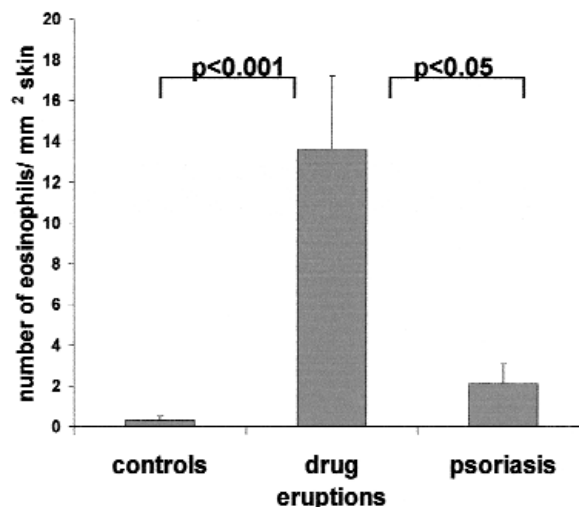


FIG 2. Numbers of eosinophils in skin and blood from normal control subjects ($n = 9$), patients with a drug-induced maculopapular exanthem ($n = 9$), and patients with psoriasis ($n = 8$).

Statistical analysis

Statistical analysis was performed by using the Kruskal-Wallis nonparametric ANOVA test and subsequently by using the Mann-Whitney U test with the Bonferroni correction. A P value of less than .05 was considered statistically significant.

RESULTS

Clinical and laboratory data

The clinical and laboratory data of the patients are summarized in Table I. In all of these patients the causative drug was clearly identified by the case history, histologic examination of biopsy specimens (see below), epicutaneous (scratch-patch) tests, and/or lymphocyte transformation tests.

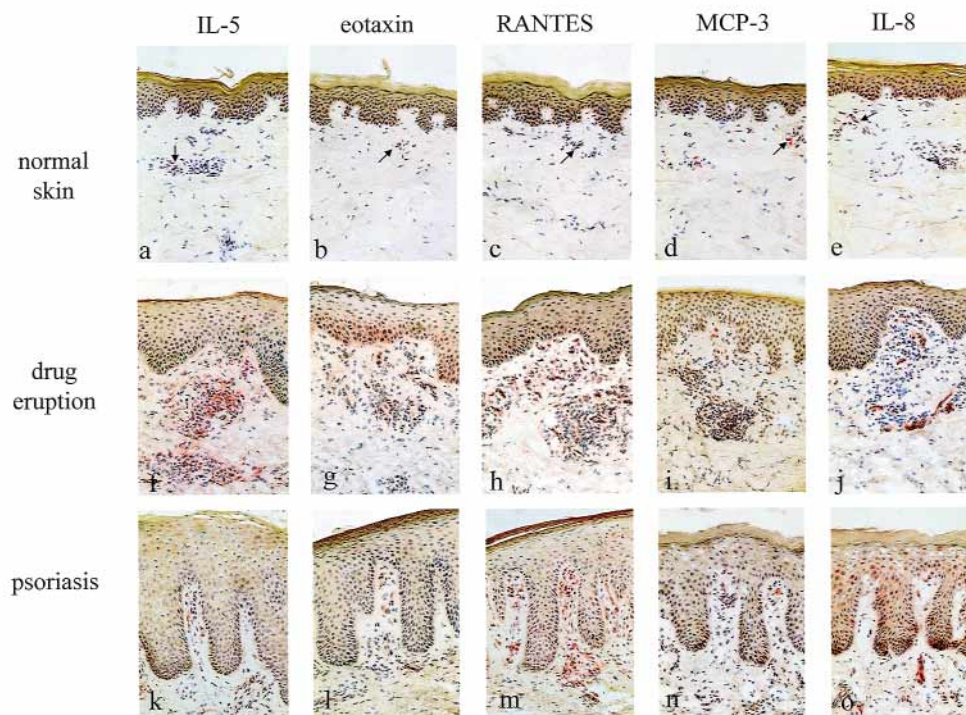


FIG 3. Localization of IL-5, eotaxin, RANTES, MCP-3, and IL-8 immunoreactivity. Representative examples from skin sections from normal skin (**a-e**), one patient with a drug-induced maculopapular exanthem (**f-j**), and from skin of a patient with psoriasis (**k-o**) are shown. Arrows indicate positively stained cells (original magnification 250 $\times$).

Numbers of eosinophils are increased in acute drug-induced maculopapular exanthems

All skin sections showed features of interface dermatitis together with a superficial mixed inflammatory cell infiltration of a varying extent. A representative skin section is shown in Fig 1. A significantly increased number of eosinophils per square millimeter was observed in these skin sections compared with those of normal control subjects and patients with psoriasis (Fig 2). In addition, a significantly enhanced number of eosinophils (normal values, 0.02-0.4 $10^9/L$) was also found in the blood of the patients with a drug-induced maculopapular exanthem (Fig 2 and Table I).

IL-5 and eotaxin are particularly enhanced in the drug-induced cutaneous lesions

The distribution of IL-5, eotaxin, RANTES, MCP-3, and IL-8 immunoreactivity and the quantification of positive cells in normal skin, drug-induced maculopapular exanthems, and skin of patients with psoriasis are shown in Figs 3 and 4. In normal skin, immunoreactivity for IL-5 and the chemokines was not detectable or was only detectable in a few cells. In contrast to normal skin, a significant enhancement of IL-5, eotaxin, and RANTES expression and, to a lesser degree, that of MCP-3 and IL-8 was observed in the drug-induced cutaneous lesions. Expression of IL-5 was mainly found in mononuclear

cells of the inflammatory cell infiltrate. The morphology of the stained cells, as well as staining of serial sections, strongly suggest that CD3⁺ T lymphocytes (Fig 5) are the major source of IL-5. Immunoreactivity for eotaxin, RANTES, IL-8, and, to a lesser extent, MCP-3 was also seen in mononuclear cells of the inflammatory cell infiltrate. In addition, resident cells (ie, endothelial cells and keratinocytes) were positively stained, particularly for eotaxin, RANTES, and IL-8. Double-immunostaining experiments to precisely characterize the cells producing IL-5, as well as the chemokines, were not feasible.

Expression of IL-5 and the chemokines were also significantly increased in the psoriatic skin lesions in comparison with normal skin. However, a significantly higher number of IL-5- and eotaxin-positive cells was observed in the drug-induced maculopapular exanthems compared with psoriatic skin specimens. The number of IL-8-positive cells in the maculopapular exanthems were markedly lower than those in psoriatic specimens, although the difference was not statistically significant ($P = .055$).

DISCUSSION

In this study we examined skin sections from patients with a characteristic, acute, drug-induced, maculopapular exanthem to determine the potential role of IL-5 and distinct chemokines in the recruitment and activation of eosinophils into the skin. Our data demonstrate that drug-

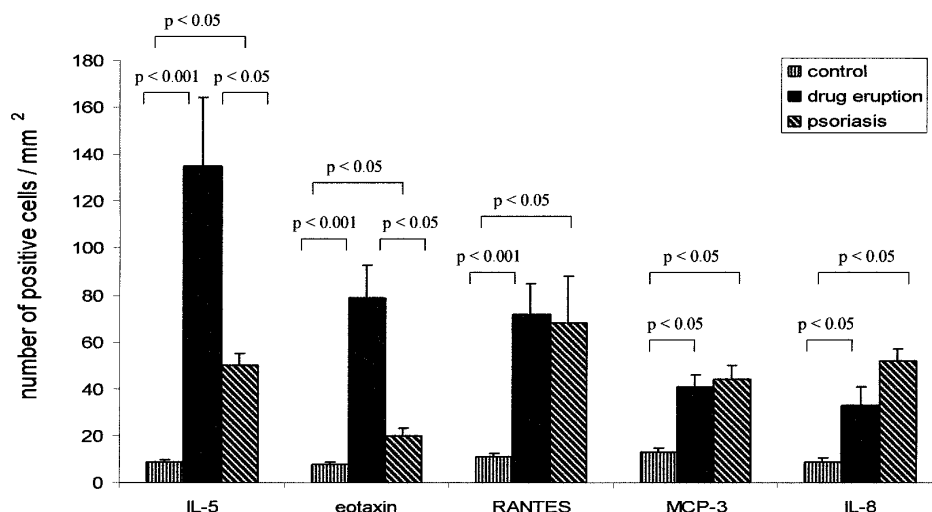


FIG 4. Quantification of IL-5-, eotaxin-, RANTES-, MCP-3-, and IL-8-positive cells in normal control subjects (n = 9), patients with a drug-induced maculopapular exanthem (n = 9), and patients with psoriasis (n = 8). Mean values $\pm$ SEM are shown, and statistical analysis is indicated.

induced maculopapular exanthems express significantly increased amounts of IL-5 and eotaxin and markedly lower amounts of IL-8 in comparison with psoriatic specimens.

Hypersensitivity reactions to drugs can cause different types of skin disorders, with the most frequent being maculopapular eruptions.^{13,14} The pathomechanism of these drug-induced skin reactions has not been fully clarified, which is in contrast to the rather well-defined pathomechanism of IgE-mediated urticarial and angioedematous skin diseases. Nevertheless, in recent years, an increasing body of evidence indicates that T cells may play a crucial part in the elicitation of drug-induced maculopapular eruptions.^{1,2} Analysis of drug-specific T-cell clones (TCCs) in vitro has shown that drug recognition by T cells may lead to activation of heterogeneous T-cell subsets with distinct phenotypes and cell functions.¹⁵⁻²⁰ Furthermore, we have recently provided evidence that perforin-containing cytotoxic T cells are present along the dermoepidermal junction zone in drug-induced maculopapular exanthems and that drug-specific CD4⁺ T cells are able to kill keratinocytes in vitro by means of a perforin-mediated mechanism.^{1,20} These data strongly suggest that drug-specific cytotoxic T cells substantially contribute to the generation of tissue damage and the initiation of skin inflammation.

In addition to their cytotoxic function, drug-specific T cells are likely to promote the inflammatory skin response in vivo through the release of various cytokines and chemokines. Analysis of various drug-specific TCCs in vitro has revealed that these cells exhibit a heterogeneous cytokine profile, including type 1 and type 2 cytokines.¹⁶⁻¹⁸ In many cases IL-5 was thereby found to be the most abundantly produced cytokine. Further support for an important role for IL-5 is derived from in vivo studies showing increased serum levels of IL-5 during acute drug eruptions.²¹ These findings are further substantiated by

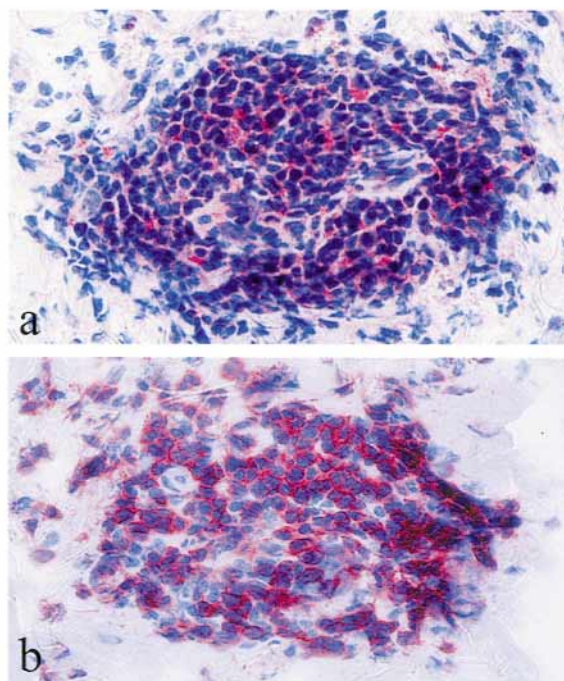


FIG 5. IL-5 is highly expressed in T lymphocytes in drug-induced maculopapular exanthem. Consecutive skin sections stained with anti-IL-5 (a) and anti-CD3 (b) are shown.

the results of this study, which demonstrates a significant enhancement of IL-5 immunoreactivity in T lymphocytes in drug-induced maculopapular exanthems. The enhanced expression of IL-5 is of particular importance because this cytokine is known to be a key factor in regulating the growth, differentiation, and activation of eosinophils.²² It is noteworthy that 7 of the patients in this study were also

investigated in our previous study, in which the expression of the cytotoxic proteins IFN- γ and IL-12 was found to be enhanced.¹ Thus in accordance with in vitro data from drug-specific TCCs, our previous and present data from cutaneous drug eruptions provide further evidence that drugs may induce a heterogeneous cytokine profile, including type 1 and type 2 cytokines in vivo.^{1,16-18}

As a further important finding, the expression of eotaxin was also found to be strongly enhanced in this study. In accordance with the distribution of eotaxin in our study, recent reports indicate that different cell populations, including CD68⁺ macrophages, CD3⁺ T cells, and keratinocytes, are capable of expressing eotaxin.^{23,24} In addition to IL-5, eotaxin has been reported to have major effects, particularly on the recruitment and activation of eosinophils. Recent reports indicate that eotaxin is involved in the homing of eosinophils into the skin and lungs after allergen challenge.^{23,25} Furthermore, eotaxin may also regulate the mobilization of eosinophils and their precursors from the bone marrow into the blood.^{26,27} Interestingly, increasing data suggest a cooperation between eotaxin and IL-5 in inducing optimal maturation, activation, and recruitment of eosinophils.⁶⁻⁸ The enhanced expression of both IL-5 and eotaxin in this study provides further evidence for a synergistic effect of these factors in generating eosinophilia in cutaneous drug allergies. The significance of this observation is further enhanced by the finding that IL-5 and eotaxin were decreased in psoriasis, which was not associated with increased blood or tissue eosinophilia. Eosinophils are well recognized as important effector cells in promoting tissue inflammation and inducing tissue damage through the release of toxic granule proteins, lipid mediators, cytokines, and chemokines in various allergic diseases.³ The increased number of eosinophils in blood and skin found in a previous study²⁸ and the present study suggests that these cells, in addition to drug-specific T cells, may also be involved in amplifying the underlying immune response in drug-induced maculopapular exanthems.

In conclusion, our results provide evidence that eotaxin and IL-5 cooperate to regulate eosinophil recruitment and activation in drug-induced maculopapular exanthems and suggest that these factors have an integral part in the pathogenesis of this skin disease.

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