

Infiltration of cytotoxic T cells in drug-induced cutaneous eruptions

N. YAWALKAR*†, F. EGLI*, Y. HARI*, H. NIEVERGELT†, L. R. BRAATHEN† and W. J. PICHLER*

*Clinic for Rheumatology and Clinical Immunology/Allergology and †Department of Dermatology, University of Bern, 3010 Bern, Switzerland

Summary

Background Previous *in vitro* data indicate that perforin containing drug-specific cytotoxic T cells are involved in cutaneous drug reactions.

Objective The aim of this study was to investigate the *in situ* expression of perforin and granzyme B together with the nature of the inflammatory infiltrate in acute drug-induced exanthem. Furthermore, expression of interleukin (IL)-12 and interferon (IFN)- γ , which are known to stimulate cytotoxic T cells, was investigated.

Methods Skin biopsy specimens were obtained from 10 patients with a generalized maculopapular exanthem and from nine controls with normal skin. Expression of CD3, CD4, CD8, CD56, CD1a, CD68, CD25, HLA-DR, CD54, perforin, granzyme B, IL-12 and IFN γ was analysed using immunohistochemistry.

Results In contrast to the controls, the skin of patients with an exanthem was mainly infiltrated by T cells (CD4 > CD8) and showed a marked enhancement of perforin and granzyme B immunostaining. Double immunostaining revealed that perforin and granzyme B were expressed in both CD4⁺ and CD8⁺ cells, which were partly located at the dermoepidermal junction and in the epidermis. In addition, strong immunoreactivity for IL-12 and IFN γ was observed in the mononuclear cells infiltrate, indicating that these cytokines may be important in activation of these cytotoxic T cells.

Conclusion The increased numbers of perforin and granzyme B containing T cells infiltrating the dermoepidermal junction may contribute to the damage of epidermal cells, which is frequently observed as a typical feature of interface dermatitis in drug-induced exanthem. Our data provide further evidence that cytotoxic T cells play an essential role in cutaneous drug reactions.

Keywords: drug allergy, granzyme B, IFN γ , IL-12, immunohistochemistry, perforin

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Introduction

Cutaneous reactions are among the most frequently observed adverse reactions to drugs [1]. Although the pathomechanism of most cutaneous adverse drug reactions remain largely unknown, some may be of immunological origin [2]. In certain reactions such as maculopapular rashes a delayed cell-mediated immune mechanism involving

drug-specific T cells is thought to be important [3,4]. Thereby, drug recognition by T cells may lead to activation of heterogeneous T-cell subsets with distinct phenotypes and cell functions [5,6]. Recently, we were able to demonstrate that sulphamethoxazole-specific T-cell lines and T-cell-clones of both CD4⁺ and CD8⁺ phenotype showed a drug-specific and MHC-restricted cytotoxicity against autologous B lymphoblasts [7]. Furthermore, autologous keratinocytes were also specifically killed predominantly by CD4⁺ T-cell clones [8]. These *in vitro* findings indicate that cytotoxicity mediated by drug-specific T lymphocytes

Correspondence: N. Yawalkar, Clinic for Rheumatology and Clinical Immunology/Allergology, Inselspital, 3010 Bern, Switzerland.

may represent an important pathway in certain forms of cutaneous adverse drug reactions. The relevance of these *in vitro* findings, however, remain to be confirmed in the skin *in vivo* and to our knowledge no data on the *in situ* expression of perforin and granzyme B in acute drug-induced maculopapular exanthem are available so far.

The present study was therefore designed to investigate the nature of the inflammatory infiltrate along with the *in situ* expression and distribution of perforin and granzyme B in patients with an acute, drug-induced, maculopapular exanthem by immunohistochemistry. Furthermore, we sought to examine the expression of IL-12 and IFN γ , which have previously been reported to be involved in the activation of cytotoxic cells [9–11].

Materials and Methods

Subjects and skin biopsy specimens

The Ethical Committee of the Medical Faculty of the University of Bern approved the study. Ten caucasian patients (six females, four males; mean age: 48.6; range 31–79) with a typical, acute, generalized, maculopapular drug eruption were included in the study after obtaining their informed consent. The drugs involved were: amoxicillin ($n=3$) and one each of prednisolone, cefazoline, dorzolamide, carbamazepine, indapamide, metozalone and vitamin B complex. In all patients the development of cutaneous eruptions occurred after initiation of therapy

and resolved upon drug discontinuation. Furthermore, the causative drug was identified by epicutaneous (scratch-patch) and/or lymphocyte transformation assay as described previously [12,13]. Punch biopsy specimens of 5 mm diameter were taken from an acute, maculopapular skin eruption. Normal skin was obtained from nine patients undergoing plastic reconstructive surgery. Prior to the investigation the patients did not receive any systemic corticosteroids or topical treatment with corticosteroids at the site of the punch biopsy.

Haematoxylin and eosin staining

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

Immunohistochemistry

The second half of the skin biopsy specimen was snap-frozen in tissue-embedding medium using isopentane pre-cooled in liquid nitrogen and stored at -70°C . The monoclonal antibodies used in the study and their specificity are shown in Table 1. Substitution of the primary antibody with isotype-matched IgG, and omission of the primary antibody served as negative controls.

Immunostaining for granzyme B, IL-12 and IFN γ was performed using the alkaline phosphatase antialkaline

Antibody against [Clone]	Specificity	Concentration ($\mu\text{g/mL}$)
CD3 [UCHT1]*	T cells	3
CD4 [MT310]*	Helper/inducer T cells	2.0
CD4 [YNB46.1.8]†	Helper/inducer T cells	10.0
CD8 [DK25]*	Cytotoxic/suppressor T cells	1.5
CD8 [YTC 141.1HL]†	Cytotoxic/suppressor T cells	10.0
CD56 [MY31]‡	NK cells	1.0
CD1a [NA1/34]*	Langerhans cells	2.9
CD68 [PGM1]*	Monocytes, macrophages	7.2
CD25 [ACT-1]*	IL-2 receptor	3.8
HLA-DR [DK22]*	MHC class II antigen	4.0
CD54 [6.5B5]*	Adhesion molecule ICAM-1	7.6
perforin [δG9]§	Cytotoxic protein	33
granzyme B [GrB-11]¶	Cytotoxic protein	30
IL-12 [B-P24]**	Interleukin 12	50
IFN γ [45.15]§	Interferon-gamma/Th 1 cytokine	50

*DAKO, Glostrup, Denmark; †Serotec, Oxford, England; ‡Beckon Dickinson, Mountain View, CA, USA; §Ancell Corporation, Bayport, MN, USA; ¶Hölzel Diagnostik, Köln, Germany **Diaclone Research, Besançon, France

Table 1. Monoclonal antibodies used in the study

phosphatase (APAAP) method [14,15]. Briefly, 6 μ m cryostat tissue sections were air-dried, fixed in 2% formaldehyde for 8 min and rehydrated in Tris-buffered saline (TBS) with 0.1% saponin (in order to enable permeabilization). Slides were then incubated for 3 h with the primary mouse antibody, followed by a rabbit antimouse antibody (dilution 1 : 30, Z-259; DAKO, Glostrup, Denmark) and the APAAP complexes (dilution 1 : 30 for granzyme B and 1 : 50 for IL-12 and IFN γ ; D-651; DAKO). To enhance the signal, incubation with the bridging antibody and the APAAP complexes were repeated once.

Immunostaining for perforin, CD3, CD4, CD8, CD56, CD1a, CD68, CD25, HLA-DR and CD54 was performed using the avidin-biotin-complex/alkaline phosphatase (ABC/AP) method. Briefly, after fixation for 8 min with 2% formaldehyde (for perforin) or acetone (for the remaining antibodies) slides were incubated with the primary mouse antibody for 3 h at room temperature, followed by a biotinylated rabbit-antimouse IgG (dilution 1 : 50 (for perforin), 1 : 100 (for the remaining antibodies), E0413; DAKO) and thereafter with avidin-biotin-complex/alkaline phosphatase (dilution: 1 : 200; K0376; DAKO). Finally, all sections were developed in new fuchsin-naphtol (Sigma, St. Louis, MO, USA) and counterstained with haematoxylin.

Double immunostaining was performed by combining two indirect staining methods with two unlabelled primary antibodies of mouse (antiperforin or antigranzyme B) and rat (anti-CD4 [YNB46.1.8] or anti-CD8 [YTC 141.1HL]) origin. Briefly, slides were initially incubated with the primary antibodies, followed by alkaline phosphatase-conjugated goat antimouse (DAKO, D0486) and peroxidase-conjugated goat antirat immunoglobulins (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). Sections were then developed using new fuchsin-naphtol and diaminobenzidine, which revealed a red and brown staining colour, respectively, and finally counterstained with haematoxylin. The specificity of the reaction was confirmed by omitting the primary antibodies and using appropriate isotype-matched antibodies as negative controls.

Evaluation of sections

The slides were analysed with a Leitz Dialux 20EB microscope by two independent investigators. In each section, staining was assessed on 10–15 adjacent fields at 400 $\times$ magnification. Cells displaying a strong cytoplasmic staining were considered to be positive. The positive cells were counted using a 0.09 mm² grid and the number of positive cells (mean $\pm$ SEM) per mm² was calculated and the percentage of positively stained cells determined. Only positively stained cells with a nucleus were included in the counting. Statistical analysis was performed using the Mann–Whitney U-test.

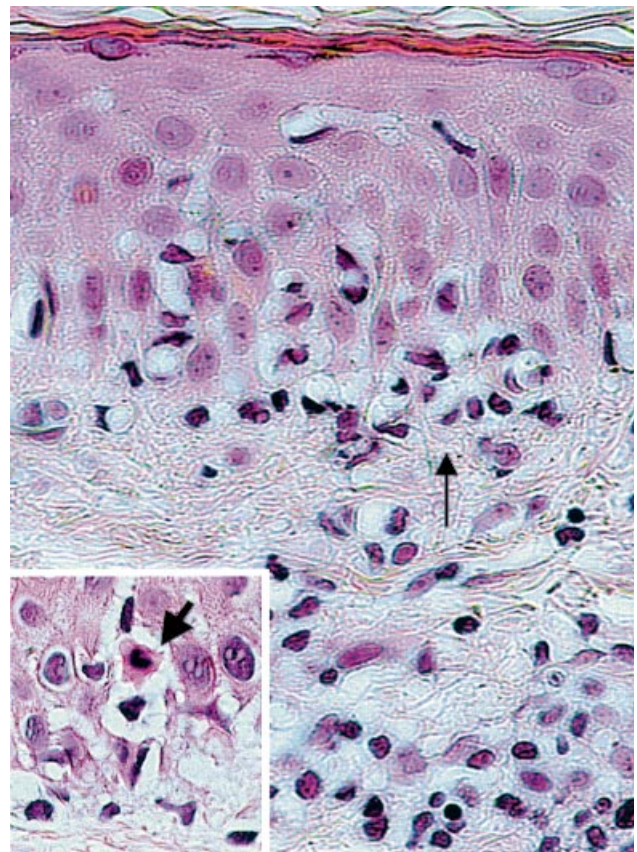


Fig. 1. Representative histology of a drug-induced maculopapular exanthem showing a mononuclear cell infiltration and an interface dermatitis (thin arrow). Vacuolar alteration in the basal cell layer with a dyskeratotic keratinocytes (thick arrow) is demonstrated in the inset. Original magnification $\times$ 250.

Results

Histological features of acute drug-induced maculopapular exanthem

Skin sections from 10 patients with a characteristic, acute, drug-induced, maculopapular exanthem were analysed by light microscopy. All of them showed a superficial, mononuclear cell infiltrate with a varying number of eosinophils and occasionally some neutrophils. Lymphocytic cells were predominantly located around the vessels in the papillary dermis and partly at the dermoepidermal junction and in the epidermis. Furthermore, focal interface changes with vacuolar alteration in the basal cell layer, some scattered individual dyskeratotic and necrotic keratinocytes were observed (Fig. 1 and inset). In addition, a mild spongiosis was occasionally present in the epidermis.

Immunophenotyping of the inflammatory infiltrate

To determine the nature of the inflammatory infiltrate skin

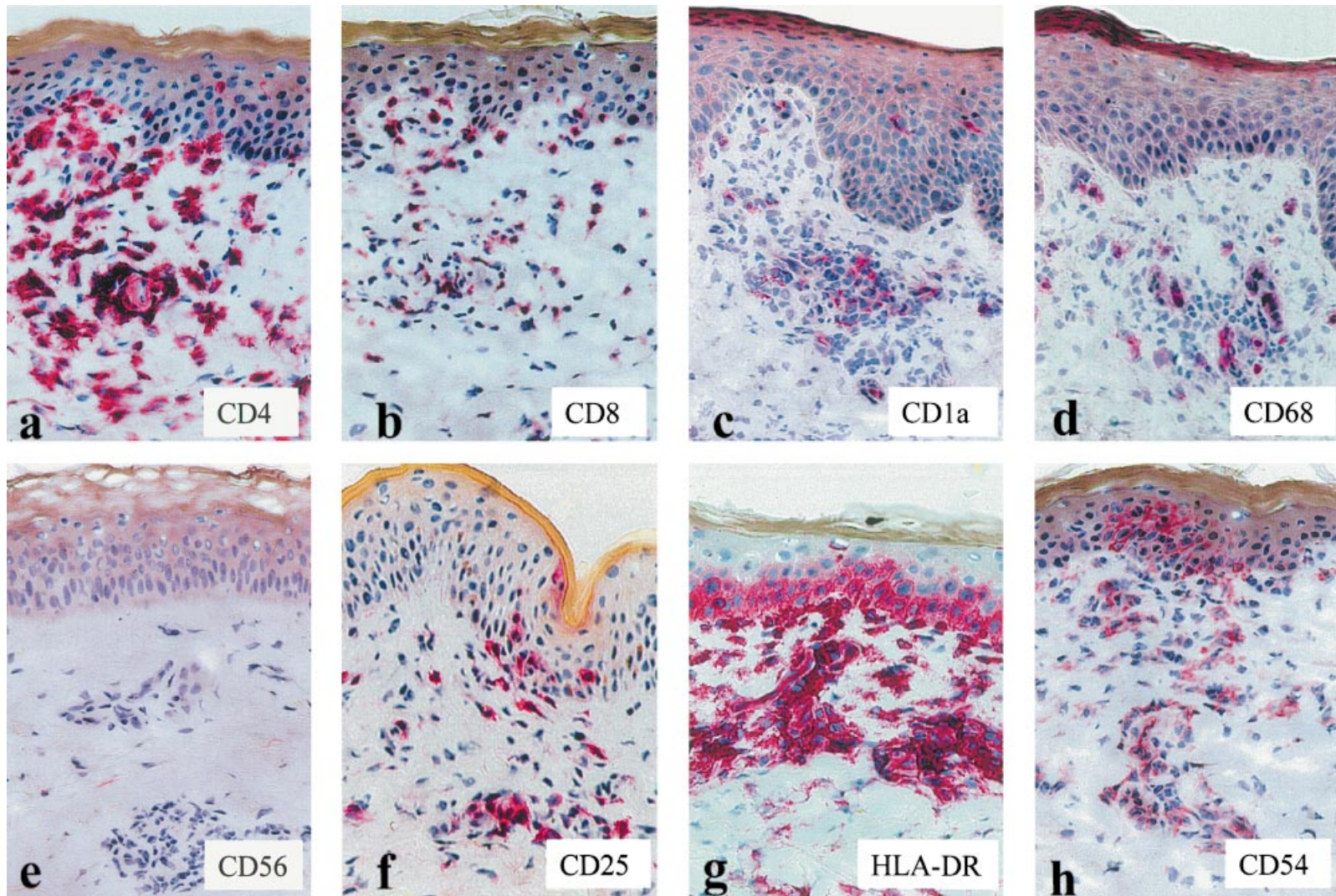


Fig. 2. Immunophenotyping of the inflammatory infiltrate. Representative stainings of CD4 (a), CD8 (b), CD1a (c), CD68 (d), CD56 (e), CD25 (f) HLA-DR (e) and CD54 (ICAM-1) (f) immunoreactivity are demonstrated. Original magnification $\times 250$.

sections were stained with a panel of monoclonal antibodies. Representative stainings are shown in Fig. 2(a-h). Immunohistochemical studies showed that the cell infiltrate was mainly composed of CD3 + T cells (40–70%) with a predominance of CD4 + cells. Whereas CD4 + cells were mainly located in the perivascular dermis, both CD4 + and CD8 + cells were found at the dermoepidermal junction and in the epidermis. CD1a + dendritic cells and CD68 + macrophages were scattered throughout the skin sections and were both found on 10–20% of the cell infiltrate. CD56 + NK cells were not or only present on up to 6% of the cell infiltrate. CD25 (IL-2 receptor) was detected on up to 15% of the cell infiltrate, especially on those located at the dermoepidermal junction. Ninety to hundred per cent of the mononuclear cell infiltrate strongly expressed HLA-DR. Interestingly, the majority of the basal keratinocytes also

stained strongly for HLA-DR and focally for CD54 (intercellular adhesion molecule-1; ICAM-1), which in places extended to the granular cell layer.

Perforin and granzyme B immunoreactivity is enhanced in drug-induced cutaneous lesions

Using previously well-characterized antibodies [16,17], perforin and granzyme B immunoreactivity was not or only detectable on a few mononuclear cells in normal skin (Fig. 3a,b). The mean numbers of perforin and granzyme B positive cells per mm² were 1.9 ± 1.3 and 5.8 ± 1.7 , respectively. In contrast to normal skin, a pronounced enhancement of perforin and granzyme B expression was observed in drug-induced cutaneous lesions (Fig. 3c,d), where the mean numbers of perforin and granzyme B positive cells per mm² were

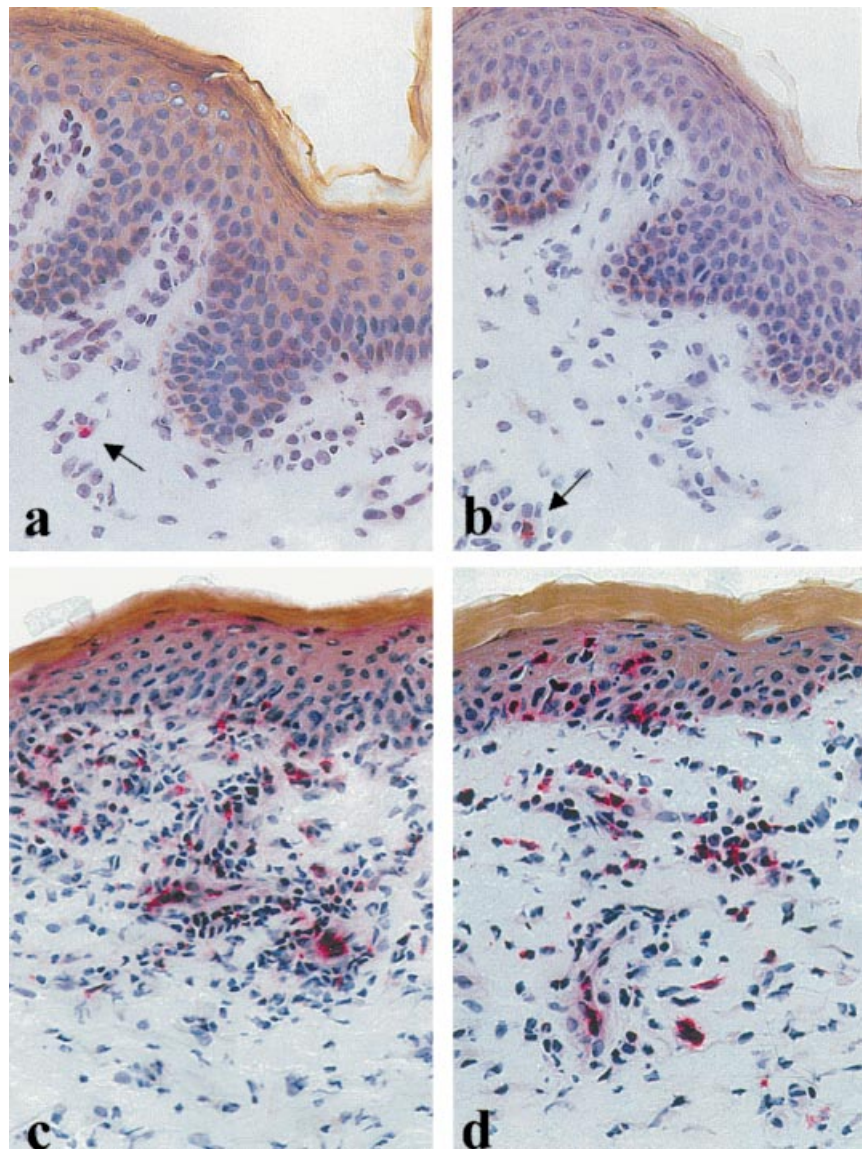


Fig. 3. Perforin and granzyme B expression in normal skin and a drug-induced maculopapular exanthem. Single immunostaining was performed as described in Material and Methods. Serial skin sections from normal skin (a,b) and one patient with a drug-induced maculopapular exanthem (c,d) are shown as representative examples. Original magnification $\times 250$.

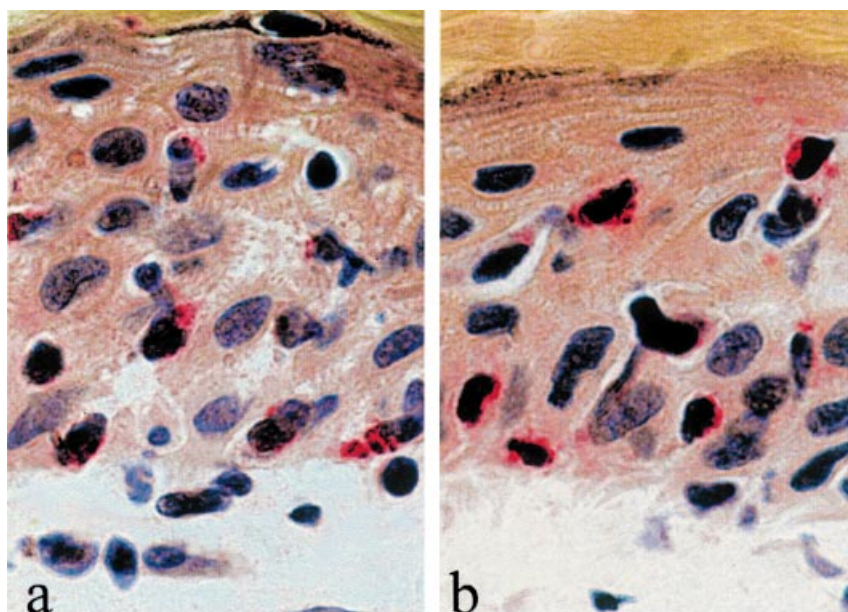


Fig. 4. Perforin (a) and granzyme B (b) immunostaining was expressed in lymphocytic cells and partly located at the dermoepidermal junction and in the epidermis. Original magnification $\times 250$.

93.0 ± 18.4 and 51.3 ± 11.4 , respectively. Statistical analysis revealed a significant difference in the number of perforin ($P < 0.002$) and granzyme B ($P < 0.05$) positive cells as compared to normal skin. No positive staining was observed when the primary antibody was substituted with an irrelevant isotype-matched IgG (not shown).

Perforin and granzyme B immunoreactivity was observed in the cytoplasm of mononuclear cells scattered throughout the dermis, the dermoepidermal junction and in the epidermis (Fig. 4a,b). The morphology of these positive cells and staining of consecutive serial sections (as shown in Figs 2a,b

and 3c,d) suggested that lymphocytes are the major source of these proteins. In addition, double immunostaining experiments confirmed that both CD4+ and CD8+ cells express perforin (Fig. 5a,b) and granzyme B (not shown). Expression of perforin was observed on 9–16% and 15–20% of the CD4+ and CD8+ T cells, whereas granzyme B was found on 4–12% and 10–13% of the CD4+ and CD8+ T cells, respectively. Double immunostaining experiments with CD56 were not feasible so that the *in situ* expression of these cytotoxic proteins on NK cells remains unknown at present.

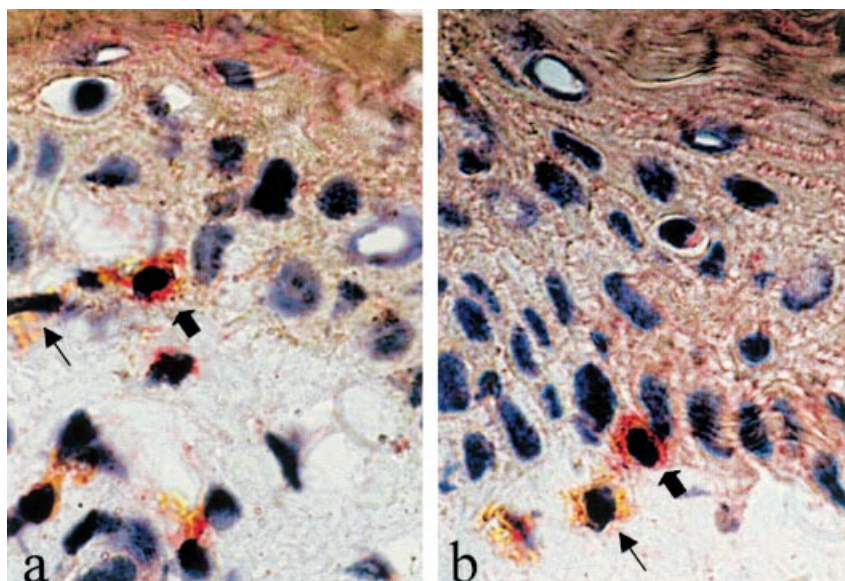


Fig. 5. Perforin is expressed in CD4+ and CD8+ cells. Double immunostaining was performed as described in Material and Methods. CD4 (a) and CD8 (b) positive cells were determined with diaminobenzidine (brown), perforin with Fast Red (red). Thin arrow (single staining), thick arrow (double staining). Original magnification $\times 400$.

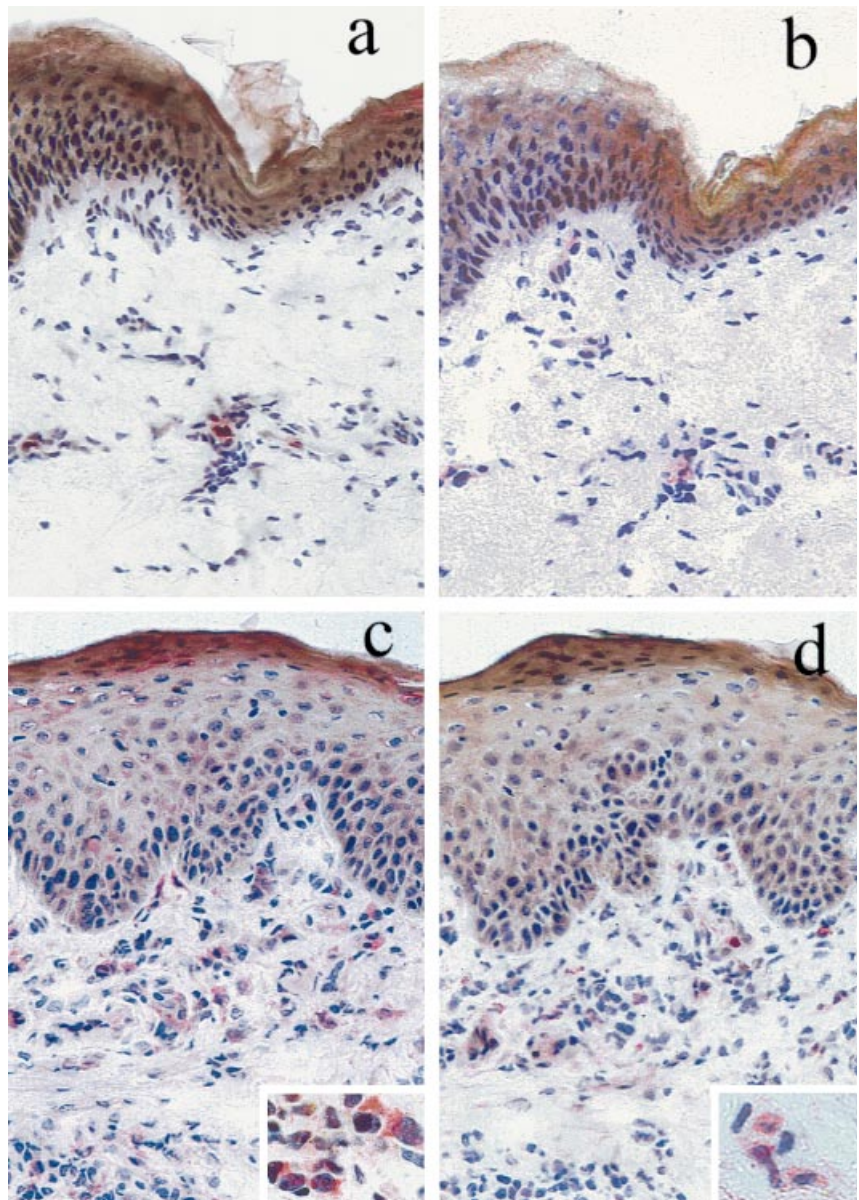


Fig. 6. IL-12 (a,c) and IFN γ (b,d) expression in normal skin and a drug-induced maculopapular exanthem. Representative examples using single immunostaining as described in Material and Methods are shown. Original magnification $\times 250$.

Perforin and granzyme B immunoreactivity is associated with an increased infiltration of IL-12 and IFN γ positive cells

In normal skin IL-12 and IFN γ positive cells were not or only detectable on a few mononuclear cells (Fig. 6a,b). The mean numbers of IL-12 and IFN γ positive cells per mm² were 12.8 ± 1.6 and 22.6 ± 2.5 , respectively. In the drug-induced cutaneous lesions IL-12 immunoreactivity was abundantly found in dendritic cells and macrophages in the dermis (Fig. 6c) and occasionally in dendritic cells in the epidermis (not shown). The mean number of IL-12 positive cells per mm² was 127.4 ± 22.1 . An accumulation

of IL-12 positive cells besides lymphocytes were predominantly found near the dermoepidermal junction. IFN γ immunoreactivity was mainly observed on lymphocytic cells throughout the cell infiltrate with a mean number of 114.6 ± 14.8 per mm² (Fig. 6d). Statistical analysis revealed a significant difference ($P < 0.05$) in the number of IL-12 and IFN γ positive cells as compared to normal skin.

Discussion

In the present study we examined skin sections from 10 patients with a characteristic, acute, drug-induced, maculo-

popular exanthem to determine the nature of the inflammatory infiltrate and the expression of cytotoxic granule proteins. Our data demonstrate that the inflammatory infiltrate is mainly composed of activated T cells. Up to 20% of the T cells expressed perforin and granzyme B and were often located at the dermoepidermal junction or in the epidermis along with signs of keratinocyte cell damage.

Cutaneous reactions to systemic drugs can produce a wide variety of clinical patterns. The most frequently encountered clinical manifestation is the maculopapular type of drug eruption, which accounts for approximately 40% of all reactions [18,19]. Clinically, these drug eruptions are characterized by erythematous macules and papules, which may coalesce into patches and plaques. Histopathological changes often show an interface dermatitis together with a mixed inflammatory cell infiltrate [20,21]. An interface dermatitis with accumulation mainly of lymphocytic cells was also typically seen at the dermoepidermal junction in the skin sections of our patients. Furthermore, a predominantly perivascular, mononuclear cell infiltrate accompanied by increased numbers of eosinophils was observed in the papillary dermis. Immunohistochemical analysis confirmed that activated T cells represent the major cell population of the inflammatory cell infiltrate, which was composed in approximately two-thirds of CD4+ and in one-third of CD8+ cells. These observations together with previous *in vitro* data on drug-specific T cells strongly support a T-cell-mediated pathogenesis in drug-induced maculopapular skin eruptions [4].

The soluble proteins perforin and granzyme B have previously been reported to play an essential role in cell-mediated cytotoxicity reactions [22–26]. These proteins are released during granule exocytosis and trigger cell death by forming pores in the target cell membrane and inducing degradation of their DNA to fragments. Perforin and granzyme B are stored together in secretory lysosomes of cytotoxic cells such as T cells and NK cells. Although initial reports indicated that CD8+ cells were mainly involved in cytotoxic responses, we and others have recently shown that CD4+ cells may contain perforin and also elicit cytotoxicity [8,27–29]. In accordance with these reports, perforin as well as granzyme B immunoreactivity was found in both CD4+ and CD8+ cells in the skin, thus providing further evidence for an involvement of cytotoxic CD4+ cells in the pathogenesis of cutaneous drug eruptions *in vivo*.

Our previous investigations with sulphamethoxazole-specific T cell clones revealed a perforin mediated killing of autologous keratinocytes, if pretreated with IFN γ , by drug-specific CD4+ lymphocytes [8]. A well known function of IFN γ is the upregulation of MHC II molecules on keratinocytes. Interestingly, IFN γ was readily detected on infiltrating lymphocytic cells, which thus may have

contributed to the enhanced expression of MHC II molecules on keratinocytes. As a particularly striking feature in this study, perforin and granzyme B positive cells, of both CD4+ and CD8+ phenotype, were also frequently observed in and adjacent to keratinocytes, which were positively stained for HLA-DR and ICAM-1. The expression of these molecules on the cell surface of keratinocytes may subsequently facilitate binding and activation of cytotoxic T cells, rendering keratinocytes more susceptible to T-cell-mediated cytotoxicity. Together with our previous *in vitro* data it is thus conceivable, that drug binding to MHC class I or II peptide complexes might occur on keratinocytes, which may lead to drug-induced cytotoxicity mediated through CD8+ as well as CD4+ cells lymphocytes [7,8,30]. Consequently, such reactions may contribute to some of the characteristic features of interface dermatitis such as vacuolar alteration and necrosis of keratinocytes, which were typically observed in our cases.

As a further notable finding an enhanced *in situ* expression of IL-12 was observed in the present study. IL-12, which is mainly produced by macrophages and dendritic cells, has been demonstrated to be a potent stimulatory and maturational factor for cytotoxic T cells and NK cells [10,11]. Furthermore, IL-12 is an important inducer of IFN γ production. The marked presence of IL-12 together with IFN γ in the mononuclear cell infiltrate may thus represent an additional important factor responsible for cell-mediated cytotoxicity in cutaneous drug eruptions.

In conclusion, the strong *in situ* expression of perforin and granzyme B together with IL-12 and IFN γ suggest the involvement of these proteins in the pathomechanism of cutaneous drug eruptions. In addition, our present findings indicate that both CD4+ and CD8+ cytotoxic cells may be responsible for causing tissue damage especially at the dermoepidermal junction and further support previous *in vitro* data on the role of cytotoxic T cells in drug-induced exanthem.

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References

- 1 Breathnach SM. Mechanisms of drug eruptions. Part I. *Australas J Dermatol* 1995; 36:121–7.
- 2 Griem P, Wulferink M, Sachs B, Gonzalez JB, Gleichmann E. Allergic and autoimmune reactions to xenobiotics: how do they arise? *Immunol Today* 1998; 19:133–41.
- 3 Hertl M, Merk HF. Lymphocyte activation in cutaneous drug reactions. *J Invest Dermatol* 1995; 105:95S–98S.
- 4 Pichler WJ, Schnyder B, Zanni MP, Hari Y, von Greysen S. Role of T cells in drug allergies. *Allergy* 1998; 53:225–32.

- 5 Brander C, Mauri-Hellweg D, Bettens F, Rolli H, Goldman M, Pichler WJ. Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals. *J Immunol* 1995; 55:2670–8.
- 6 Zanni MP, Mauri-Hellweg D, Brander C, Wendland T, Schnyder B, Frei E, von Greyerz S, Bircher A, Pichler WJ. Characterization of lidocaine-specific T cells. *J Immunol* 1997; 158:1139–48.
- 7 Schnyder B, Mauri-Hellweg D, Zanni M, Bettens F, Pichler WJ. Direct. MHC-dependent presentation of the drug sulfamethoxazole to human alphabeta T cell clones. *J Clin Invest* 1997; 100:136–41.
- 8 Schnyder B, Frutig K, Yawalkar N, Limat A, Pichler WJ. T cell cytotoxicity in sulphamethoxazole induced skin reaction. *Clin Exp Allergy* 1998; 28:1412–7.
- 9 Fitzpatrick L, Makrigiannis AP, Kaiser M, Hoskin DW. Anti-CD3-activated killer T cells. interferon-gamma and interleukin-10 cross-regulate granzyme B expression and the induction of major histocompatibility complex-unrestricted cytotoxicity. *J Interferon Cytokine Res* 1996; 16:537–46.
- 10 Ma X, Aste-Amezaga M, Gri G, Gerosa F, Trinchieri G. Immunomodulatory functions and molecular regulation of IL-12. In: Adorini L. eds. *IL-12 Chem Immunol*, Vol 68, Basel: Karger, 1997: 1–22.
- 11 Salcedo TW, Azzoni L, Wolf SF, Perussia B. Modulation of perforin and granzyme messenger RNA expression in human natural killer cells. *J Immunol* 1993; 151:2511–20.
- 12 Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* 1997; 27:175–81.
- 13 Yawalkar N, Reimers A, Hari Y, Hunziker TH, Gerber H, Müller U, Pichler WJ. Drug-induced linear IgA bullous dermatosis associated with ceftriaxone- and metronidazole-specific T cells. *Dermatology* 1999; 199:25–30.
- 14 Yawalkar N, Egli F, Brand CU, Pichler WJ, Braathen LR. Antigen-presenting cells and keratinocytes express Interleukin 12 in allergic contact dermatitis. *Contact Dermatitis* 2000; 42:18–22.
- 15 Ulfgren AK, Lindblad S, Klareskog L, Andresson J, Andresson U. Detection of cytokine producing cells in the synovial membrane from patients with rheumatoid arthritis. *Ann Rheumatic Diseases* 1995; 54:654–61.
- 16 Hameed A, Olsen KJ, Cheng L, Fox WM, Hruban RH, Podack ER. Immunohistochemical identification of cytotoxic lymphocytes using human perforin monoclonal antibody. *Am J Pathol* 1992; 140:1025–30.
- 17 Kummer JA, Kamp AM, van Katwijk M, Brakenhoff JP, Radosevic K, van Leeuwen AM, Borst J, Verweij CL, Hack CE. Production and characterization of monoclonal antibodies raised against recombinant human granzymes A and B and showing cross reactions with the natural proteins. *J Immunol Methods* 1993; 163:77–83.
- 18 Kauppinen K, Stubb S. Drug eruptions, causative agents and clinical types. A series of in-patients during a 10-year period. *Acta Derm Venereol* 1984; 64:320–4.
- 19 Swanbeck G, Dahlberg E. Cutaneous drug reactions. An attempt to quantitative estimation. *Arch Dermatol Res* 1992; 284:215–8.
- 20 Ackerman AB, Chongchitnant N, Sanchez J, Ying G, Bennin B, Reichel M, Randall MB. Inflammatory diseases. In: Stamatidis G. eds. *Histologic Diagnosis of Inflammatory Skin Diseases* 2nd Edn. Baltimore: Williams & Wilkins, 1997:317–28.
- 21 Chaffins ML, Cockerell CJ. Histopathologic characteristics of common inflammatory skin disorders. In: *Current Problems in Dermatology* St Louis: Mosby-Year Book 1996:189–236.
- 22 Griffiths GM. The cell biology of CTL killing. *Curr Opin Immunol* 1995; 7:343–8.
- 23 Liu CC, Walsh CM, Young JD. Perforin: structure and function. *Immunol Today* 1995; 16:194–201.
- 24 Masson D, Tschopp JA. family of serine esterases in lytic granules of cytolytic T lymphocytes. *Cell* 1987; 49:679–85.
- 25 Squier MK, Cohen JJ. Cell-mediated cytotoxic mechanisms. *Curr Opin Immunol* 1994; 6:447–52.
- 26 Trapani JA. Target cell apoptosis induced by cytotoxic T cells and natural killer cells involves synergy between the pore-forming protein, perforin, and the serine protease, granzyme B. *Aust N Z J Med* 1995; 25:793–9.
- 27 Liu CC, Rafii S, Granelli-Piperno A, Trapani JA, Young JD. Perforin and serine esterase gene expression in stimulated human T cells. Kinetics, mitogen requirements, and effects of cyclosporin A. *J Exp Med* 1989; 170:2105–18.
- 28 Susskind B, Shornick MD, Iannotti MR, Duffy B, Mehrotra PT, Siegel JP, Mohanakumar T. Cytolytic effector mechanisms of human CD4+ cytotoxic T lymphocytes. *Hum Immunol* 1996; 45:64–75.
- 29 Vergelli M, Hemmer B, Muraro PA, Tranquill L, Biddison WE, Sarin A, McFarland HF, Martin R. Human autoreactive CD4+ T cell clones use perforin- or Fas/Fas ligand-mediated pathways for target cell lysis. *J Immunol* 1997; 158:2756–61.
- 30 Zanni MP, von Greyerz S, Schnyder B, Brander KA, Frutig K, Hari Y, Valitutti S, Pichler WJ. HLA-restricted, processing- and metabolism-independent pathway of drug recognition by human alpha beta T lymphocytes. *J Clin Invest* 1998; 102:1591–8.