Acute generalized exanthematous pustulosis (AGEP) is an uncommon eruption most often provoked by drugs, by acute infections with enteroviruses, or by mercury. It is characterized by acute, extensive formation of nonfollicular sterile pustules on erythematous background, fever, and peripheral blood leukocytosis. We present clinical and immunological data on four patients with this disease, which is caused by different drugs. An involvement of T cells could be implied by positive skin patch tests and lymphocyte transformation tests. Immunohistochemistry revealed a massive cell infiltrate consisting of neutrophils in pustules and T cells in the dermis and epidermis. Expression of the potent neutrophil-attracting chemokine IL-8 was elevated in keratinocytes and infiltrating mononuclear cells. Drug-specific T cells were generated from the blood and skin of three patients, and phenotypic characterization showed a heterogeneous distribution of CD4/CD8 phenotype and of T-cell receptor Vβ-expression. Analysis of cytokine/chemokine profiles revealed that IL-8 is produced significantly more by drug-specific T cells from patients with AGEP compared with drug-specific T cells from patients that had non-AGEP exanthemas. In conclusion, our data demonstrate the involvement of drug-specific T cells in the pathomechanism of this rather rare and peculiar form of drug allergy. In addition, they indicate that even in some neutrophil-rich inflammatory responses specific T cells are engaged and might orchestrate the immune reaction.
T-cell involvement in drug-induced acute generalized exanthematous pustulosis

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Introduction

Acute generalized exanthematous pustulosis (AGEP) is a rare disease that has been classified as pustular psoriasis von Zumbusch type for years. In 1968 Baker and Ryan were the first to assume that AGEP represents its own entity (1). Subsequently, this disorder was better characterized by Beylot et al. (2) and Roujeau et al. (3), who clarified its relationship to pustular psoriasis and assessed the place of drugs in its etiology.

The following features were found to be characteristic for AGEP: numerous, mostly nonfollicular intraepidermal or subcorneal pustules (<5 mm) arising on a widespread edematous erythema, fever above 38°C, and blood neutrophils above 7 × 10³/µl. The onset of the disease is acute; resolution of pustules occurs spontaneously within fewer than 15 days (4).

Fever, elevated leukocyte counts, and appearance of multiple pustules tend to be associated with an acute bacterial infection, but AGEP is not due to bacterial pathogens as the content of the pustules is sterile. In a few cases the etiology of AGEP appears to be a viral infection (enterovirus or parvovirus B19) or a hypersensitivity reaction to mercury, but most cases of AGEP (90%) have been described in association with the intake of drugs, in particular antibacterials such as aminopenicillins (4).

Adverse reactions to drugs manifest themselves in a variety of diseases, and the pathomechanism of some cutaneous adverse drug reactions may be of immunological origin (5, 6). Immune responses due to drug-specific T cells seem to be more relevant in certain cutaneous adverse drug reactions (7, 8). Such T cell–mediated sensitization to certain drugs has been detected by epicutaneous testing, lymphocyte transformation tests (LTTs), and generation of T-cell lines (TCLs) and clones (TCCs) (9–13). They are due to drugs such as aminopenicillins, which represent typical haptens able to covalently modify peptides and proteins (13, 14). However, cutaneous T-cell reactions might also be elicited by a direct recognition of drugs that bind to the MHC-peptide complex in a noncovalent way (15–17).

A neutrophilic inflammation as a manifestation of a drug allergic reaction — as in AGEP — is unusual, since eosinophilia, but not neutrophilia, is the typical hallmark of drug allergies (12, 18, 19). Indeed, various studies have documented a high IL-5 production by tissue-infiltrating T cells in exanthema as well as drug-specific...
T cells from the circulation (19, 20) and an enhanced eotaxin production by endothelial cells and infiltrating T cells (19). Interestingly, previous studies in patients with AGEP have revealed a high rate of strongly positive patch tests to drugs compared with patients with other drug eruptions (21–24). This suggests that T cells are involved in AGEP as well. They may contribute to the accumulation of polymorphonuclear neutrophils (PMN) at the site of the lesions by the preferential release of PMN-attractive chemokines, mainly CXC chemokines such as IL-8 (25, 26).

To understand the function of T cells in AGEP patients we generated drug-specific TCLs and TCCs from the circulation and skin biopsy specimens and analyzed their phenotypes, specificities, and cytokine and chemokine profiles in vitro. Immunohistochemical analysis of the acute and patch-test lesions ex vivo was performed to determine the nature of the inflammatory cell infiltrate in vivo and to supplement the in vitro studies. The data point to an important role of drug-specific T cells in AGEP, which clearly exert distinct functions compared with other cutaneous drug eruptions.

Methods

Patients. A summary of the patients’ clinical characteristics is given in Table 1. Patient AP developed a generalized erythema after oral administration of Augmentin (amoxicillin and clavulanic acid; SmithKline Beecham Pharmaceuticals, Irvine, United Kingdom), which was given to treat an otitis media. She had fever (40°C) and developed pustules on the back, arms, and face. She was then treated with antihistamines and local steroids. A similar pustulous reaction to the same drug had already occurred 5 years previously.

Patient JS developed generalized exanthema, pustules, and fever (41°C) after intake of Clamoxyl (amoxicillin; Smith-Kline Beecham Pharmaceuticals) to treat a urinary tract infection. The patient required hospitalization in the intensive care unit. He had an adverse drug reaction (exanthema, no AGEP) to an unknown antibiotic 1 year before and once an angioedema of the tongue after administration of acetylsalicylic acid.

Patient AF received Cotrim (sulfamethoxazole and trimethoprim; Spirig, Basel, Switzerland) and later Klacid (clarithromycin; Abbott AG, Baar, Switzerland) to treat an upper respiratory tract infection. She developed generalized erythema, pustules, and fever (41°C) and was hospitalized for 7 days in the unit for burn victims. No other drug allergies are known.

Patient EB was treated for the first time with Celebrex (celecoxib; Pfizer, Zürich, Switzerland) for periarthritis of the left shoulder. At the same time she was suffering from a viral infection (rhinitis). She developed a generalized erythema followed by pustules (face, shoulder) and fever (38°C). She was treated with topical steroids. No other drug allergies are known.

Epicutaneous testing. Epicutaneous testing (patch test) was performed at least 2 months after recovery with PBS (negative control), Augmentin (half a pill of 375 mg/0.5 ml PBS), Clamoxyl (375 mg/0.5 ml PBS), or Celebrex (one capsule containing 100 mg celecoxib/0.5 ml PBS), respectively, as described in detail previously (27). Briefly, the freshly prepared drug/PBS mixtures were applied on the upper back using Al patch-test disks (Imal Pharmaceutica AG, Glarus, Switzerland). The test reactions were read at 48 hours and scored as recommended by the International Contact Dermatitis Research Group (28). No positive reactions to Augmentin and celecoxib were found in 19 controls, confirming the specificity of the patch test (data not shown).

Staining of biopsy specimens. Punch-biopsy specimens (5 mm) were obtained from the acute lesions of patient AF, fixed in 4% formalin, and routinely stained with hematoxylin and eosin (HE) for standard histology. In addition, immunohistochemistry was performed of skin biopsy specimens from the acute lesions as well as from the positive epicutaneous test reactions at 48 hours (patients AP and JS). The biopsy specimens were snap-frozen in tissue-embedding medium using isopentane precooled in liquid nitrogen and stored at −80°C.

Immunostaining for IL-5 and RANTES was performed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method (D0651; Diagnostics AG, Zug, Switzerland) and for CD4, CD8, CD25, human leukocyte antigen-DR (HLA-DR), neutrophil elastase, EG2, eotaxin, and IL-8 using the avidin-biotin complex/alkaline phosphatase method (K0376; Diagnostics AG), as described previously in detail (8, 19). Finally, all sections were developed in fuchsin substrate-chromogen (K0624; Diagnostics AG) and counterstained with hematoxylin (Haemalaun; Dr. Grogg Chemie AG, Stettlen, Switzerland). Substitution of the primary Ab with isotype-matched IgG and omission of the primary Ab served as negative controls.

Culture media. Culture medium (CM) consisted of RPMI-1640 (Sigma Chemical Co., St. Louis, Missouri, USA) supplemented with 10% pooled heat-inactivated human AB serum (Swiss Red Cross, Bern, Switzerland), 25 mM HEPES buffer (Biochrom KG, Berlin, Germany), 2 mM L-glutamine (no. 663.710; Biotest AG, Dreieich, Germany), 10 µg/ml streptomycin, and 100 U/ml penicillin (no. 4-01F00-H; Amimed Products AG, Allschwil, Switzerland). For the culture of TCLs or TCCs the CM was enriched (CM+) with 30 U/ml human recombinant IL-2 (rIL-2).

Epstein-Barr virus–transformed (EBV-transformed) B-lymphoblastoid cell lines (B-LCLs) were generated as described (29) and cultured in RPMI-1640 supplemented with 10% FCS, 25 mM HEPES buffer, but without L-glutamine and without antibiotics.

Drugs and antigens. The following drugs were used for T-cell culture and proliferation assays: amoxicillin (Sigma Chemical Co.), clavulanic acid (Smith-Kline Beecham Pharmaceuticals), Celebrex capsules containing 200 mg celecoxib (Pfizer), and sulfamethoxazole (SMX; Hoffmann-La Roche Ltd., Basel, Switzerland). Tetanus toxoid (TT; Serum and Vaccine Institute, Bern, Switzerland) was used as a control antigen. Stock solu-
tions of each drug were always freshly prepared in CM just before use. Celecoxib and SMX were dissolved in RPMI-1640 with 0.05 M NaOH.

Lymphocyte proliferation and generation of drug-specific T cells. Some 2 × 10⁶ freshly isolated PBMCs from allergic patients were cultured for 6 days in 1 ml CM at 37°C in upright 12 ml-culture tubes (no. 3033; Falcon BD, Switzerland) in the presence of antigen. The following antigens and concentrations were used: amoxicillin, 200 and 500 µg/ml; celecoxib, 0.1, 1, 10, and 50 µg/ml; SMX, 100 and 200 µg/ml; TT, 10 µg/ml. Then 0.5 µCi ³H-thymidine was added to the culture for 8–14 hours and radioactivity was measured by a β-counter (Packard Instrument Co., Meriden, Connecticut, USA) (12). Stimulation indices (SI) were calculated as cpm in culture with antigen divided by cpm in culture without antigen.

For generation of TCLs from PBMCs of patient AP, JS, and EB, 2 × 10⁶ cells were cultured in 1 ml CM in 24-well plates with 500 µg/ml amoxicillin, 50 µg/ml celecoxib, or 200 µg/ml SMX, respectively. At day 7, rIL-2 was added, and after 14 days, bulk cultures were restimulated with autologous irradiated (45 Gy) PBMCs and the respective drug as antigen. Drug-specific TCLs were cloned by limiting dilution as described previously (20). Growing T cells were expanded in CM+ and restimulated every 14 days with allogeneic PBMCs and phytohemagglutinin (PHA). In addition, TT-specific TCLs from patients AP and JS were generated to compare their functional activity with drug-specific TCCs.

To test the specificity of drug-specific TCLs and TCCs, 5 × 10⁴ T cells were incubated at day 10–16 after restimulation with either 5 × 10⁴ of the respective irradiated PBMCs or with 10⁴ autologous irradiated (60 Gy) B-LCLs as antigen-presenting cells (APCs) in the presence or absence of the indicated drug as antigen in 200 µl CM in U-bottom 96-well microplates. After 48 hours, ³H-thymidine incorporation of cultures was measured as indicated above. Cell cultures with SI greater than or equal to 2 were further expanded. Determination of HLA restriction of TCCs was done as described elsewhere (30).

Isolation of drug-specific T cells from epicutaneous test reactions. Some 5-mm punch-biopsy specimens were obtained after 48 hours from the positive epicutaneous test reactions of patients AP, JS, and EB (Table 1). Drug-specific T cells were isolated and cloned as described previously in detail (31). The isolated cells were cultured in U-bottom 96-well plates in a final volume of 100 µl/well with 2.5 × 10⁴ allogeneic irradiated PBMCs in CM enriched with 1 µg/ml PHA and 20 U/ml rIL-2 and expanded as described above.

Flow-cytometry analysis. Aliquots containing 10⁶ cells were stained with fluorochrome-conjugated Ab's in 50 µl buffer (PBS/1% FCS/0.02% NaN₃) for 15–30 minutes at 4°C. After two washings with buffer, cells were fixed (buffer plus 0.5% paraformaldehyde) and then analyzed on a Coulter EPICS XL-MCL Flow Cytometer (Beckman Coulter Inc., Hialeah, Florida, USA). Monoclonality of generated TCCs was shown by T-cell receptor (TCR) Vβ-chain staining using fluorochrome-labeled anti-CD3 mAb (PharMingen/BD, Basel, Switzerland) and a panel of 22 mAb's recognizing different Vβ gene products detecting about 75% of all Vβ families as described (20). Phenotype characterization and expres-

Table 1
Patients’ characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>AP</th>
<th>JS</th>
<th>AF</th>
<th>EB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/sex</td>
<td>30 years, f</td>
<td>60 years, m</td>
<td>29 years, f</td>
<td>55 years, f</td>
</tr>
<tr>
<td>Recent drug intake</td>
<td>Amoxicillin and clavulanic acid</td>
<td>Amoxicillin</td>
<td>Sulfamethoxazole</td>
<td>Celecoxib</td>
</tr>
<tr>
<td>Interval between drug intake and:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- erythema</td>
<td>4 hours</td>
<td>24 hours</td>
<td>Unknown</td>
<td>10 days</td>
</tr>
<tr>
<td>- formation of pustules</td>
<td>3 days</td>
<td>3 days</td>
<td>30 days</td>
<td>11 days</td>
</tr>
<tr>
<td>Previous drug reaction</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Duration of rash</td>
<td>14 days</td>
<td>14 days</td>
<td>19 days</td>
<td>8 days</td>
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<tr>
<td>Fever (&gt;38°C)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Liver enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASAT (11–36 U/l)</td>
<td>Not done</td>
<td>24</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>ALAT (10–37 U/l)</td>
<td>Not done</td>
<td>40</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>Blood cell count</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leukocytes (3.2–9.0)</td>
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<td>25.5</td>
<td>15.2</td>
</tr>
<tr>
<td>Neutrophils (10–7.2)</td>
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<td>24.1</td>
<td>23.2</td>
<td>13.5</td>
</tr>
<tr>
<td>Eosinophils (0.02–0.4)</td>
<td>Not done</td>
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<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>CRP (&lt;5 mg/l)</td>
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<td>197</td>
<td>133</td>
<td>91</td>
</tr>
<tr>
<td>Psoriasis in personal and family history</td>
<td>No</td>
<td>No</td>
<td>Not done</td>
<td>No</td>
</tr>
<tr>
<td>Patch test</td>
<td>Positive</td>
<td>Positive</td>
<td>Not done</td>
<td>Positive</td>
</tr>
</tbody>
</table>

 Characteristics of patients AP, JS, AF, and EB with AGEP are shown. ASAT, aspartic acid aminotransferase; ALAT, alanine aminotransferase; CRP, C-reactive protein. For liver enzymes, blood cell counts, and CRP, standard values are given.
The AGEP and non-AGEP patients are given. A median for IL-8 production of AGEP patient–derived TCCs is 813.5 pg/ml, for non-AGEP patient–derived TCCs is 32.5 pg/ml. HLA-DR typing of SUS non-AGEP patients. The median for GM-CSF production of AGEP patient-derived TCCs is 11,432 pg/ml, for non-AGEP patient–derived TCCs is 4,448 pg/ml.

### Results

**Patients’ characteristics.** Table 1 summarizes the clinical characteristics of patients AP, JS, AF, and EB. They were classified as having AGEP in accordance with the criteria of Roujeau (4).

Table 1 also shows the results of HLA typing. One of the patients had the phenotype HLA-B51, which is thought to be more frequent in patients with AGEP (32). Interestingly, three of the patients had HLA-DR7, which has been associated with psoriasis (33). However, patient AB from the control group also has HLA-DR7, implying that HLA-DR7 alone is not responsible for AGEP development (Table 2).

**Histology and immunophenotyping of acute lesions.** A representative histology of an acute AGEP skin lesion is shown in Figure 1, a and b (patient AF). The main histopathological findings were subcorneal pustules, papillary edema, and a subepidermal lympho-histiocytic perivascular infiltrate with some polymorphonuclear neutrophils (PMNs) and eosinophils. Furthermore, necrosis of some keratinocytes was observed in the epidermis.

Phenotypic staining patterns of the inflammatory infiltrate are shown in Figure 1, c–f. Almost all cells within the intraepidermal pustules expressed neutrophil elastase. The perivascular infiltrate was composed mainly of T cells, of which 60–70% were CD4+ and 30–40% were CD8+. EG2+ eosinophils were found at about 5–10% of the cell infiltrate, while patient JS, AP S9 AGEP 07/09 82.2 (399) 2,180 3,454 17,660 4,622 6,920 451

### Table 1

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<th>IL-5 (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
<th>GM-CSF (pg/ml)</th>
<th>IL-8 (pg/ml)</th>
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### Table 2

Cytokine and chemokine secretion pattern of drug-specific blood- and skin-derived TCCs

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who had also eosinophilia, had approximately 15–20% eosinophils. Furthermore, the lympho-histocytic infiltrate showed positive staining for the activation marker HLA-DR (80–90%) and CD25 (10–15%) (Figure 1, g and h). Interestingly, keratinocytes almost did not stain positive with the anti–HLA-DR Ab. Figure 1, i–l, shows a clear positive staining for IL-8 as well as a moderate positive staining for IL-5 in the epidermis, while RANTES and eotaxin were not clearly present in the epidermis. Staining for IL-8, IL-5, and to a lesser degree for RANTES and eotaxin was detected in the dermal cell infiltrate.

**Drug-specific proliferation of patients’ PBMCs.** Dose-dependent proliferative responses of PBMCs to the respective drug were observed in all four patients (Figure 2). These results confirm a strong T-cell sensitization for the three patients AP, JS, and AF to amoxicillin and SMX, respectively (SI between 3.4 to 8.3) and a weak T-cell sensitization for EB to celecoxib (SI: 2.3).

**T cells isolated from PBMCs are drug-specific.** In total, 1,244 TCLs and TCCs were generated from PBMCs of patients AP, JS, and EB. Some 27 amoxicillin-specific TCLs and TCCs were obtained from patients AP and JS (SI: 2.0–13.9) and 30 celecoxib-specific from patient EB (SI: 2.0–56.5). We analyzed extensively for a potential cross-reactivity between the two sulfonamides celecoxib and SMX. PBMCs and more than 200 SMX-specific TCCs from a SMX-allergic patient and PBMCs of EB did not reveal any cross-reactivity (data not shown). This suggests that the sulfonamide structure per se does not account for T cell–mediated cross-reactivity. Figure 3, a–e, shows the specificity and the phenotype of some selected blood-derived TCLs and TCCs, and Figure 3, f–g, illustrates the dose-dependent proliferation of three representative TCCs. The TCLs and TCCs were CD4+ or CD8+, and all expressed the αβ TCR. Immunofluorescence staining revealed a distinct Vβ-chain usage of the TCCs. This heterogeneity in the TCR Vβ of TCCs suggests the lack of oligoclonality of the drug-specific T-cell response.

**T cells isolated from positive epicutaneous test reactions are drug-specific.** To further prove the involvement of drugs in eliciting AGEP, we performed patch tests in patients AP, JS, and EB. In agreement with previous reports (7, 21–23), application of the drug resulted in erythema, induration, and vesicle formation (Figure 4, a–f, h) in all three patients.

Forty-eight hours after drug application, biopsies from positive epicutaneous test reactions were obtained and divided into two equal parts. One part served to elute drug-specific T cells (Figure 3, d and e), and the second part was used to analyze the cell infiltration by immunohistochemistry (Figure 4, a–n).

A total of 94 TCLs and TCCs were generated from skin-biopsy specimens of patient AP, of which 27 were specific for amoxicillin (SI: 2.0–13.8) (Figure 3d). All skin-derived T cells of patient AP reacted to the drug in the presence of autologous PBMCs, but not B-LCLs. Phenotype characterization revealed that TCLs of patient AP were mainly CD4+; however, some CD8+ TCLs were found as well. All TCCs were CD4+.

We also gained 283 TCLs from the positive epicutaneous skin test of patient JS. Some 22 of them were amoxicillin-specific (SI: 2.0–43.4) (Figure 3e). Ten were CD8+, four CD4+, and eight TCCs consisted of a CD4+/CD8+ T-cell mixture. TCLs and TCCs from patient AP and JS expressed the αβ TCR.

**Figure 1**
Representative immunohistochemistry of acute skin-biopsy specimens of patient AF. (a and b) Staining of acute AGEP skin lesion with HE is shown. (a) ×100. (b) ×400. For immunophenotyping of acute lesions stainings of (c) CD4, (d) CD8, (e) neutrophil elastase, and (f) eosinophil marker EG2 are demonstrated. Activation of infiltrating cells and tissue is shown by (g) HLA-DR and by (h) CD25 staining. (i–l) Cytokine and chemokine production is shown for IL-5, IL-8, eotaxin, or RANTES. (c–l) ×250.
Patient EB also showed positive epicutaneous test reaction to celecoxib. Some 30 TCLs (all CD4+) could be generated from the skin biopsy but were not specific to celecoxib. The TCR was not further characterized.

Cytokine/chemokine production and IL-8 receptor expression of drug-specific TCCs. Cytokine and chemokine expression were measured in the supernatant of stimulated TCCs by a sandwich ELISA 48 hours after stimulation. We analyzed ten TCCs of patient AP (seven skin derived and three blood derived), one TCC of patient JS (blood derived), and one TCC of patient EB (blood derived). Table 2 shows that drug-specific TCCs from patients AP, JS, and EB (AGEP patients) produce variable amounts of cytokines/chemokines.

This cytokine/chemokine profile (IL-4, IL-5, IFN-γ, RANTES, and GM-CSF) was similar to the one obtained with TCCs from patients with other drug allergies (Table 2, non-AGEP patients). However, TCCs from AGEP patients showed a tendency to produce higher amounts of GM-CSF, but it was not significant ($P > 0.05$; Mann-Whitney U test). Some 25% of TCCs of AGEP patients produced low amounts of IL-8 (S15 and S39 of patient AP and B7 of patient JS; 69-121 pg/ml). However, 75% of TCCs produced relatively high levels of IL-8 (307–4,846 pg/ml). A comparison of the IL-8 production of the 12 AGEP (mean 1,204 pg/ml) with the ten non-AGEP TCCs (mean 34 pg/ml) gave a significantly higher IL-8 production by AGEP TCCs ($P < 0.025$, Mann-Whitney U test). This highly
elevated IL-8 production seems to be related to the stimulatory antigen. TT-specific TCLs from AGEP patients AP and JS and TT-specific TCCs from non-AGEP patients did not produce detectable IL-8 levels after specific (TT) or nonspecific (anti-CD3/anti-CD28 mAb) stimulation, although they were DR4 or DR7 restricted, respectively (data not shown).

We also analyzed for the expression of CXCR1, one of the two IL-8 receptors. CXCR1 was found to be highly expressed (51–71%) by drug-specific TCCs from AGEP patients that also produce high amounts of IL-8, but is expressed to a lower extent (20–37%) by TCCs and TCLs that produce low or no IL-8 ($P < 0.05$, Mann-Whitney U test).

**Immunohistochemistry of biopsy specimens from positive epicutaneous test reactions.** Immunohistochemical analysis from biopsy specimens of positive epicutaneous test reactions at 48 hours are shown in Figure 4, a–n (patient JS). The mononuclear cell infiltrate was composed mainly of T cells (50–70%). About two thirds of the T cells expressed CD4 (Figure 4a), whereas approximately one third were CD8+ (Figure 4b). PMNs as well as EG2+ eosinophils were scattered throughout the skin sections (Figure 4, c and d), representing about 10–15% and 5–10% of the cell infiltrate, respectively. Interestingly, at the time of the biopsy the vesicles were not filled with PMNs but contained mainly CD4+ T cells (Figure 4, a and b). Ninety to one hundred percent of the cell infiltrate strongly expressed HLA-DR, while the keratinocytes were practically not positively stained for HLA-DR (Figure 4e). About 10–15% of the infiltrating cells expressed CD25 (Figure 4f).

In immunohistochemical studies with anti–IL-5, anti–IL-8, anti-eotaxin, and anti-RANTES, only a few positive cells were detectable in healthy skin (Figure 4, g, i, k, and m). In contrast, in the patch-test samples of patient JS strong expression of IL-8, RANTES, and, to a lesser degree, eotaxin was detected in the cell infiltrate (Figure 4, h, j, l, and n).

Similar staining patterns with a high expression of IL-8 and lower expression of eotaxin and RANTES, together with lower numbers of eosinophils, were also observed in biopsy specimens of patient AP (data not shown).

**Discussion**

In this study we present clinical and immunological data for four patients with AGEP, which address the immunological background of this drug-allergic disease and clarify the involvement of T cells in AGEP. All four patients fulfilled the criteria of AGEP (3), and the histology of the acute lesion was typical: subcorneally localized neutrophilic pustules and accumulation of T cells perivascularly and around the pustules.

The crucial role of T cells in AGEP could be confirmed by distinct means: all four patients showed a positive in vitro proliferative response of T cells to the incriminated drug. Other individuals exposed to these drugs without side effects were negative in this assay.

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**Figure 4**

Immunohistochemical analysis of skin biopsy obtained from positive epicutaneous test reactions of patient JS at 48 hours. For phenotype characterization and localization of infiltrating cells, sections were stained with mAb specific for CD4 (a), CD8 (b), neutrophil elastase (c), or eosinophil marker EG2 (d). Activation of infiltrating cells and tissue was demonstrated by staining for HLA-DR (e) or CD25 (f). To compare the expression and localization of cytokines and chemokines in the skin of AGEP patients with that in healthy individuals, respective sections were stained for IL-5 (g and h), IL-8 (i and j), eotaxin (k and l), or RANTES (m and n). ×250.
tion, CD8+ T cells also appear to be involved in this pecu-
lar way (data not shown) (30). All drug-reactive T cells had
activated CD4+ T cells with distinct functions (cytokine
or CD8 cell involvement, as well as the degree of acti-
vation). This is in agreement with previous observations on
drug-specific TCLs and could be observed in the
immunohistology of the acute as well as of the patch-test
lesions (up to 30% activated CD8+ T cells). However, until
now we were unsuccessful in cloning these CD8+ T cells.

The TCCs recognized the drug in an HLA-DR-restrict-
ed way, although not all reacted in an allele-restricted
way (data not shown) (30). All drug-reactive T cells had
the αβ TCR phenotype and were mainly CD4+. In addi-
tion, CD8+ T cells also appear to be involved in this pecu-
lar drug reaction because they were present within the
drug-specific TCLs and could be observed in the
immunohistology with eosinophils. The somewhat stronger T-cell
response in patch-test reactions, compared with the
acute drug allergy, might be due to the higher local
drug concentration in the skin test.

The TCCs recognized the drug in an HLA-DR-restrict-
ed way, although all of the patients tested (AP, JS, EB).
Drug-specific proliferation and skin infiltration
by T cells could be proven by generating drug-specific
TCLs and TCCs from primary cell cultures and from
biopsy specimens of positive epicutaneous test reac-
tions at 48 hours. In addition, histology revealed activ-
ated (HLA-DR+ or CD25+) CD4 and CD8 cell infiltr-
atation in the dermis and epidermis, vesicle formation,
and some eosinophilia. The somewhat stronger T-cell
response in patch-test reactions, compared with the
acute drug allergy, might be due to the higher local
drug concentration in the skin test.

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drug-specific TCLs and could be observed in the
immunohistology of the acute as well as of the patch-test
lesions (up to 30% activated CD8+ T cells). However, until
now we were unsuccessful in cloning these CD8+ T cells.

The TCR Vβ analysis after 7 and 10 days of primary
culture did not indicate an oligoclonality (data not
shown), and the few TCCs obtained indeed had a dis-
tinct usage of TCR Vβ. This is in agreement with previ-
ous observations on drug-specific T-cell reactions (34),
although occasionally an oligoclonal outgrowth has
been observed in some patients (20, 35, 36). This sug-
gests that the drug can be presented in various ways,
giving rise to distinct antigenic determinants, or that
different TCRs can recognize the same antigenic deter-
ninant formed by the MHC-drug complex.

The function of the drug-specific T cells from
patients with AGEP seem to be heterogeneous: As in
other drug-induced reactions, the degree of either CD4
or CD8 cell involvement, as well as the degree of acti-
vating CD4+ T cells with distinct functions (cytokine
production), might determine the clinical picture (37, 38).
In AGEP, immunohistology as well as the charac-
terization of TCCs revealed evidence for production of
the potent PMN-attractive chemokine IL-8 by ker-
atinocytes and drug-specific CD4+ T cells. Neither
the IL-8 production by T cells nor the PMN infiltration is
seen to such an extent in other forms of drug allergy,
e.g., maculopapular drug eruptions (19). Thus, IL-
8–producing T cells seem to be rather typical for AGEP,
and they could — together with their relatively strong
production of GM-CSF — contribute to the recruit-
ment of PMN into the epidermis. Moreover, the local
IL-8 production might contribute to the recruitment of
further IL-8–producing T cells, because they express
high levels of CXCR1.

Although some infiltrating T cells synthesize IFN-γ —
a finding confirmed by ELISA measurements of drug-
activated TCLs derived from the skin or the circulation
(data not shown) — keratinocytes did not or only moder-
ately express MHC class II. Most likely, the IFN-γ pro-
duction by the infiltrating T cells was insufficient to
induce MHC class II expression on tissue cells, or other
factors necessary for MHC class II upregulation by ker-
atinocytes were missing. This is clearly in contrast to
the high MHC class II expression in patients with mac-
ulopapular drug eruption (8).

The eosinophilia in one particular patient (JS) was
probably due to the high IL-5 expression by infiltrating
T cells, as well as to the rather strong eotaxin and
RANTES expression by perivascular cells, while in the
other patients practically no eotaxin-positive cells were
found. This is in accordance with observations of Rou-
jeau et al., where eosinophilia is seen in about 30% of
patients with AGEP (3).

Epicutaneous drug application by patch test also
induced some PMN infiltration into the epidermis and
a rather strong IL-8 expression by keratinocytes, but
the vessels contained — in contrast to the acute reac-
tion — only few PMN, but mainly CD4+ and some
CD8+ T cells. As another report on patch-test reaction
of AGEP patients describes pustule formation after
longer skin test application (96 hours) (22), the biopsy
that was taken at 48 hours might reflect the early
events. This would suggest that the drug-specific CD4+
—and probably also some CD8+ T cells are reacting first,
and they may be responsible for vesicle formation.
PMNs immigrate later to fill the vesicles and transform
them to pustules. The patch-test reaction appears to
nicely mirror the events of an acute AGEP reaction.
This shows that the once-generated immune response
to a drug in AGEP patients is persistent and does not
change on subsequent drug exposures, which can occur
via the skin or by the oral route. We believe that this is
due to a particular priming of drug-specific T cells in
AGEP, which react in the same way to drug re-exposure.
The reproducibility of the rather rare systemic drug
allergy in a skin-test reaction opens the possibility of
better analyzing these allergic diseases, even in vivo,
which is particularly important because animal mod-
els do not exist.

What is priming T cells in favor of more IL-8 produc-
tion in patients with AGEP is unclear at present.
Indeed, it is a puzzling aspect of drug allergy that the
same drug (i.e., amoxicillin or SMX) can elicit quite dis-
tinct immunological reactions leading to different dis-
ases, such as anaphylaxis, maculopapular drug erup-
tion, bullous skin disease, interstitial lung disease, etc.
Drugs are peculiar antigens because they can bind to
and modify soluble or cell-bound molecules such as
MHC class II or I molecules and, in particular, their
embedded peptides (39). The clinical and immunolog-
ic heterogeneity of drug allergy may be the result of
drugs binding to distinct immunogenic peptides, or to
distinct MHC molecules, or to other molecules
involved in the immune response (cytokine receptors,
adhesion molecules, etc.). In this context, the rather fre-
quent presence in the affected population of the HLA-
DB1*07 allele — which is, interestingly enough, also associated with psoriasis (33) — must be explored.

Another reason for the enhanced IL-8 production might be a peculiar drug presentation, which could lead to distinct signals and T-cell functions. Drugs can be presented covalently bound to the peptide/MHC complex (i.e., amoxicillin) or in a rather labile, noncovalent way (i.e., SMX) (15, 16). However, both presentations appear to be able to elicit an AGEP-related T-cell response because celecoxib was presented in a labile way, without requirement of drug processing or metabolism (data not shown) and two patients reacted to the covalently binding drug amoxicillin.

In conclusion, AGEP appears to be a disease where a cell-bound drug presentation elicits a drug-specific CD4 and CD8 immune reaction, which results in a preferential IL-8 production by T cells. Other immune reactions, i.e., IL-5 production by T cells, can occur simultaneously as well and contribute to the slightly different clinical picture in each patient. Further work is required to better understand the interplay of T cells with other cells, in particular keratinocytes, which seem to have an important role in AGEP by expressing PMN- and eosinophil-attracting chemokines. It is tempting to speculate that the elucidation of this new pathogenic mechanism involving T cells and neutrophils may not only help avoid such side effects of drug therapy, but may also provide a clue to further our understanding of similar diseases (i.e., pustular psoriasis).

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