

Short communication

Molecular features determining lymphocyte reactivity in allergic contact dermatitis to chloramphenicol and azidamphenicol

Background: We report on two cases of allergic contact dermatitis to chloramphenicol and azidamphenicol respectively, with *in vivo* and *in vitro* lymphocyte reactivity to both compounds. The molecular features determining lymphocyte reactivity were explored because chloramphenicol, azidamphenicol, and thiamphenicol exhibit almost identical chemical structures.

Methods: With chloramphenicol, azidamphenicol, and the chemically related thiamphenicol, we performed patch tests and lymphocyte transformation tests with both patients. Furthermore, the interleukin-5 and interferon- γ concentrations in the cultures of peripheral blood mononuclear cells of one patient were determined.

Results: Patch tests showed delayed hypersensitivity reactions to chloramphenicol and azidamphenicol, but not to thiamphenicol. These results were confirmed by lymphocyte transformation tests with peripheral blood mononuclear cells of the patients, showing a proliferative T-cell response to azidamphenicol and chloramphenicol. Moreover, lymphocytes from one patient secreted large amounts of interleukin-5, but not of interferon- γ upon coculture with azidamphenicol.

Conclusions: Since lymphocyte reactivity was observed to chloramphenicol and azidamphenicol, but not to thiamphenicol, the epitope(s) recognized by the allergen-reactive T cells may be formed by the nitro-group of the benzene ring shared by chloramphenicol and azidamphenicol.

**B. Sachs, S. Erdmann,
T. al Masaoudi, H. F. Merk**

Department of Dermatology, University hospitals,
Rheinisch-Westfälische Technische Hochschule
Aachen, Aachen, Germany

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Dr med. Bernhardt Sachs
Department of Dermatology
University hospitals
Rheinisch-Westfälische Technische Hochschule
Aachen
Pauwelsstraße 30
52074 Aachen
Germany

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Chloramphenicol and azidamphenicol are chemically closely related antibiotics (Fig. 1). The administration of chloramphenicol has decreased due to the occurrence of life-threatening aplastic anemia, which may also follow resorption after external application (1). Allergic contact dermatitis to topical chloramphenicol or azidamphenicol in eye-drops or antibiotic ointments, respectively, has been described (2, 3). The sensitizing potential of topically applied chloramphenicol is considered to be low according to animal studies (1). Cross-reactivities in patch tests between chloramphenicol and azidamphenicol and between chloramphenicol and thiamphenicol have been reported (2, 4). The possibility of cosensitization, however, has not definitely been excluded. Chloramphenicol is also used in veterinary medicine, possibly leading to the presence of residues in the meat of treated animals (5). Whether these residues, in addition to the promotion of resistance to antibiotics, suffice for the sensitization of man or

may, on the contrary, lead to chloramphenicol tolerance considering the oral ingestion of the residues remains speculative. Since T-cell recognition of the antigen is the initial step in the immunologic cascade of allergy, sensitized T cells are present in both immediate and delayed-type hypersensitivity reactions. The lymphocyte transformation test (LTT) detects sensitized T cells and has thus proven useful as an *in vitro* test for the detection of drug allergy at the cellular level irrespective of the clinical phenotype (6). In the present cases, lymphocyte reactivity to chloramphenicol and azidamphenicol, but not to thiamphenicol, could be demonstrated in patch tests and with the LTT. In one case, the culture supernatants in the LTT revealed large amounts of interleukin-5 (IL-5) in the cultures incubated with azidamphenicol, corresponding to the strong proliferation observed in these wells, whereas no relevant amounts of interferon- γ (IFN- γ) could be determined.

Material and methods

LTT

The LTT was performed according to a standard protocol, as described elsewhere (6, 7). Briefly, peripheral blood mononuclear cells (PBMC) from the patients and two healthy, sex-matched, untreated controls with no history of allergies to the tested compounds were isolated from heparinized whole blood by gradient centrifugation and cultured for 6 days in 96-well, round-bottom plates as triplicate or sixfold cultures with various concentrations of the indicated drugs dissolved in phosphate-buffered saline (PBS) or with PBS alone. Incubations of the PBMC with the mitogen phytohemagglutinin and tetanus toxoid served as positive controls (data not shown). For the last 18 h, ^3H -labeled thymidine was added. Cells were then harvested, and incorporated radioactivity reflecting T-cell proliferation was measured in a scintillation counter as counts per minute (cpm). The stimulation index (SI) represents the ratio of average cpm in culture with and without (PBS) antigen. SIs exceeding 2 were considered positive results, as indicated by the cutoff strip in Fig. 2a), suggesting specific T-cell proliferation (7).

Cytokine determination

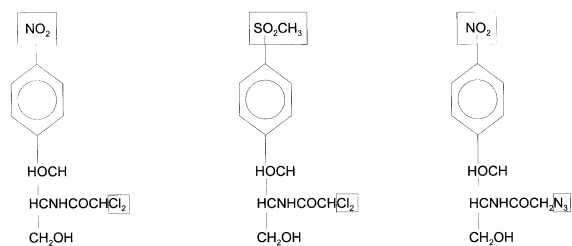
Volumes of 100 μl culture supernatant of three of the sixfold cultures with PBMC of patient 1) were collected after 48 and 120 h. Supernatants were pooled and diluted two- or threefold when high IL-5 or IFN- γ concentrations were assumed. IL-5 and IFN- γ concentrations were determined by commercially available ELISA kits (CoulterImmunotech) according to the manufacturer's recommendations. Cytokine concentrations in the culture supernatants with antigen exceeding five times the cytokine concentrations in the culture supernatants without antigen were regarded as relevant amounts.

Results

A 56-year-old man (patient 1) was seen in our dermatology department with periorbital contact dermatitis after applying chloramphenicol-containing eye-drops for the treatment of conjunctivitis. The second patient was an 80-year-old man (patient 2) who developed periorbital contact dermatitis after application of an azidamphenicol-containing ointment. No previous history of allergic contact dermatitis was reported in either patient.

Allergy testing

Patch tests. Extensive epicutaneous testing was carried out with, among others, the German and European standard series, chloramphenicol, azidamphenicol, and thiamphenicol (5% in petrolatum), using Finn Chambers on Scanpor[®], and was evaluated by a standard reading scale (8). Twofold positive patch test reactions were observed after 48, 72, and 168 h of both patients to chloramphenicol and azidamphenicol, but no reaction to thiamphenicol was observed. Patient one also exhibited a onefold positive reaction to perubalsam after 48 and 72 h.



Chloramphenicol

Thiamphenicol

Azidamphenicol

Figure 1. Chemical structure of chloramphenicol, thiamphenicol, and azidamphenicol. Relevant molecular features are framed by rectangles. Chloramphenicol and azidamphenicol share common nitro-group. Like chloramphenicol, thiamphenicol has same p-side chain on opposite side of benzene ring containing two chloride ions, whereas azidamphenicol exhibits N_3H_2 group at this position.

LTT

Patient 1. Incubation of the patient's PBMC with chloramphenicol resulted in SIs of 2.8 and 3.2, at concentrations of 50 and 100 $\mu\text{g/ml}$, respectively. Incubation with azidamphenicol resulted in SIs of 2.9, 3.6, 9.4, and 13.8 at concentrations of 12.5, 25, 50, and 100 $\mu\text{g/ml}$, respectively (Fig. 2a). There was no significant T-cell response to thiamphenicol. PBMC of a healthy control did not proliferate in response to any of the three antibiotics.

Patient 2. PBMC of this patient proliferated strongly in response to chloramphenicol and azidamphenicol, but not to thiamphenicol, at 20 $\mu\text{g/ml}$ (Fig. 2b), whereas PBMC of a control showed no lymphocyte reactivity to any of the three antibiotics.

Cytokine determination

No relevant IL-5 or IFN- γ concentrations were detected in the culture supernatants taken after 48 h with the PBMC of patient 1) or a healthy control incubated with the three compounds. In contrast, large amounts of IL-5 (169, 202, and 585 pg/ml), but not of IFN- γ (0.43, 0.58, and 0.68 IU/ml) compared to the cultures without antigen (IL-5: 14.9 pg/ml ; IFN- γ : 0.36 IU/ml), were determined in the supernatants taken after 120 h in those cultures which had shown a strong proliferation in the LTT, namely, azidamphenicol at concentrations of 25, 50, and 100 $\mu\text{g/ml}$. No relevant amounts of IL-5 or IFN- γ could be detected either in the culture supernatants taken after 120 h with PBMC from patient 1 incubated with chloramphenicol or thiamphenicol, or in the cultures with PBMC from a control person compared to the cultures without antigen.

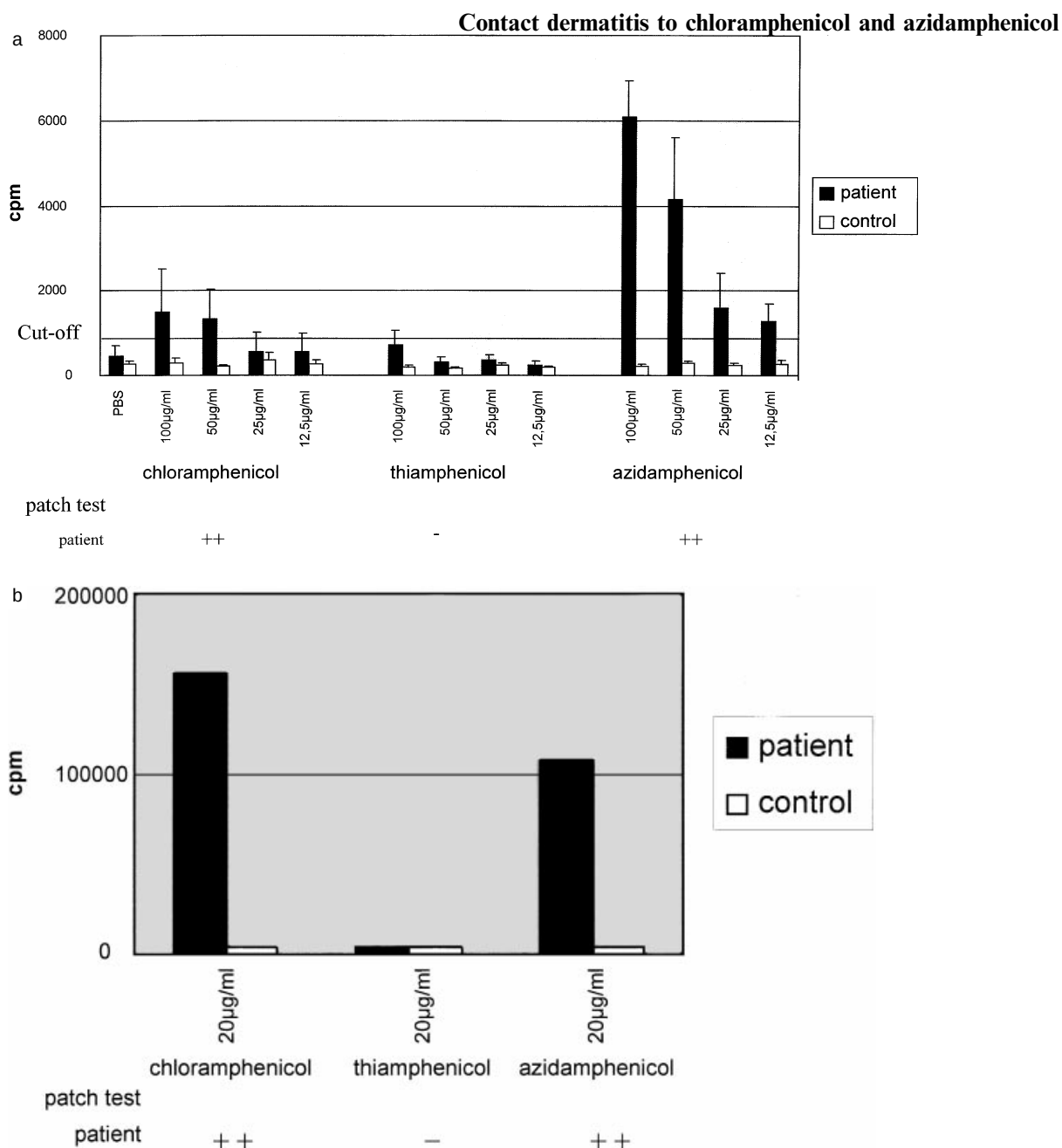


Figure 2a and b. Lymphocyte transformation tests performed with peripheral blood mononuclear cells (PBMC) of two patients and healthy controls. PBMC were incubated with chloramphenicol, azidamphenicol or thiamphenicol, or with solvent phosphate-buffered saline (PBS) alone (media control) for 6 days as triplicate or sixfold cultures. For last 18 h, ^3H -labeled thymidine was added. Cells were then harvested, and incorporated radioactivity reflecting T-cell proliferation was measured as counts per minute (cpm) and expressed as average of values. Cpm in cultures with antigen exceeding by twofold cpm in cultures without antigen (PBS) were regarded as positive results, as indicated by cutoff strip in panel a. In panel a, proliferation of PBMC of patient 1 to various concentrations of antibiotics is shown, while panel b depicts strong proliferation of PBMC from patient 2 in response to chloramphenicol and azidamphenicol at 20 µg/ml.

Discussion

In the presented case reports, allergic contact dermatitis to chloramphenicol and azidamphenicol was demonstrated both *in vivo* by epicutaneous testing and *in vitro* by the LTT. The molecular features determining lymphocyte reactivity could be explored since chloramphenicol, azidamphenicol, and thiamphenicol share an identical chemical backbone

structure differing only in distinct side chains (Fig. 1). Chloramphenicol and azidamphenicol are structurally identical except for the p-side chain opposite the nitro-group. In this position, chloramphenicol displays two chloride ions and azidamphenicol three nitrogen ions and a hydrogen ion. Thiamphenicol however, differs from chloramphenicol and azidamphenicol by having an SO_2CH_3 group in-stead of the

nitro-group, and from azidamphenicol in addition by the chloride ions contained in the opposite side chain. Thus, the greatest structural difference can be observed between thiamphenicol and azidamphenicol, making cross-reactivity between these two compounds more unlikely than between chloramphenicol and azidamphenicol or thiamphenicol. If we assume that only one side chain of the molecules is recognized by T cells in connection with the MHC molecule, the observed lymphocyte reactivity to chloramphenicol and azidamphenicol, and the lack of reactivity to thiamphenicol suggest

- 1) involvement of the nitro-group in the formation of the nominal T-cell epitope(s)
- 2) a true T-cell cross-reactivity on the cellular level.

Since the present investigations were carried out with PBMC, the existence of different T-cell clones circulating in the peripheral blood specific for chloramphenicol and azidamphenicol, respectively, cannot be ruled out completely. Cross-reactivity between chloramphenicol and thiamphenicol has also been described (2), suggesting a role for the $-CO-CHCl_2$ side chain present in both compounds in the formation of the nominal T-cell epitope(s), or the existence of T-cell clones recognizing chloramphenicol and thiamphenicol, respectively. Interestingly, it has been postulated that the nitro-group of chloramphenicol is the structural feature causing chloramphenicol-induced aplastic anemia, probably by undergoing chloramphenicol reduction to the nitroso-chloramphenicol intermediate (9). It has been reported that

nitroso-chloramphenicol has a damaging effect on DNA, whereas even large concentrations of chloramphenicol and thiamphenicol lacking the nitro-group were without effect (9). Since reactive metabolites play a crucial role in carcinogenesis and immunologic sensitization, either by forming DNA-adducts or by binding to proteins (10), formation of a nitroso derivative may lead to T-cell reactivity to the nitro-group of chloramphenicol. In the present investigation, no microsomes were added to the PBMC cultures. However, oxidating and reducing capacities have also been attributed to leukocytes *in vitro* (11), suggesting the possibility of reduction of the nitro-group by the PBMC in the cultures.

In addition to the significant lymphocyte proliferation, PBMC from patient 1 upon coculture with azidamphenicol secreted relevant amounts of IL-5, but not of IFN- γ . A Th2-skewed cytokine profile of human drug-specific T cells has also been reported in systemic drug allergies (12, 13). Thus, it is tempting to speculate that the molecular mechanisms leading to systemic exanthems, as well as to allergic contact dermatitis upon challenge with distinct drugs, such as the chloramphenicol derivatives, may be similar.

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