Fenoterol-induced Erythema Exudativum Multiforme-like Exanthem: Demonstration of Drug-specific Lymphocyte Reactivity *In Vivo* and *In Vitro*

Bernhardt Sachs, Claudia Renn, Tames al Masaoudi and Hans Friedrich Merk

Department of Dermatology and Allergology, Rheinisch-Westfälische Technische Hochschule Aachen, Pauwelsstraße 30, 52074 Aachen, Germany. E-mail: Bernhardt.Sachs@post.rwth-aachen.de

Accepted August 27, 2001.

Sir,

Adverse drug reactions common in daily clinical practice (1) include allergic drug reactions characterized by underlying immune reactions, and account for about one-seventh of all adverse drug reactions (2). Their pathophysiology is not fully understood, but T cells have been found to play a crucial role (3).

Conclusive diagnosis of drug allergy remains a major problem. Currently, the lymphocyte transformation test (LTT) is the only available *in vitro* test for detecting drug sensitization at the cellular level, irrespective of the effector mechanisms and the clinical phenotype of the reaction (3, 4). A common clinical feature of drug allergy is eosinophilia (5), which is promoted by interleukin (IL)-5 secretion from antigen-specific activated T cells (6).

We report on a female who developed an erythema exudativum multiforme-like exanthem after receiving fenoterol for tocolysis due to preterm labour. Lymphocyte sensitization to fenoterol could be confirmed *in vivo* by a patch test and *in vitro* by a LTT additionally employing the chemically related terbutaline and propranolol, which did not induce lymphocyte proliferation. Determination of IL-5 concentrations in the culture supernatants of the LTT revealed – in addition to fenoterol – lymphocyte reactivity to terbutaline, which could be confirmed in a second patch test.

MATERIALS AND METHODS

The LTT was performed according to a standard protocol as described elsewhere (4). Briefly, peripheral blood mononuclear cells (PMBC) from the patient and healthy control were isolated and cultured for 6 days in 96-well round-bottom plates (Becton Dickinson, Le Pont de Claix, France) with various concentrations of the indicated drugs dissolved in phosphate-buffered saline (PBS) or with PBS alone. For the last 18 h, 0.6 μCi ^{3}H-thymidine (NEN, Vilvoorde, Belgium) was added to each well. Cells were then harvested and incorporated radioactivity was measured as counts per minute (cpm). Stimulation index (SI) represents the ratio of average cpm in cultures with and without antigen. SIs exceeding 2.5 were considered as positive results, suggesting drug-specific T-cell proliferation (4).

Culture supernatant (100 μl) of each well with PBMC from the patient and the control were collected after 5 days. IL-5 and interferon-γ concentrations were determined using commercially available cytokine specific sandwich ELISAs (Immunotech, Marseille, France) in accordance with the manufacturer's recommendations.

RESULTS

The patient showed twofold positive (+ +) reactions in the patch test to fenoterol after 48, 72 and 168 h. In the second patch test following detection of drug-specific IL-5 secretion to terbutaline the patient gave positive reactions to terbutaline (+, + +, +) on all three reading days, but not to propranolol.

Incubation of the patient's PBMC with fenoterol in the LTT resulted in SIs of 2.7, 4.7 and 4.7 at concentrations of 0.1, 1 and 10 μg/ml, respectively (Fig. 1). No significant proliferative T-cell response to terbutaline or propranolol could be detected (data not shown). PBMC from a healthy control did not proliferate in response to any of the three substances. Incubation of the patient's PBMC with fenoterol at concentrations of 0.01, 0.1, 1 and 10 μg/ml resulted in secretion of considerable amounts of IL-5, whereas in the culture wells incubated with PBS the IL-5 concentration was below the detection limit (1.5 pg/ml) of the ELISA (Fig. 2). Although with regard to the SI no drug-specific proliferation of the patient's PBMC following incubation with terbutaline was observed, IL-5 could be detected in the supernatants of these cultures, namely 33.5, 7.1 and 3.7 pg/ml IL-5 at concentrations of 0.01, 0.1 and 1 μg/ml terbutaline. The IL-5 concentrations determined in the culture supernatants of the patient's PBMC incubated with propranolol were below the detection limit of the ELISA. PBMC of the healthy control did not release significant amounts of IL-5 upon incubation with fenoterol, terbutaline or propranolol. Further on, neither PBMC of the patient nor of the control secreted significant amounts of interferon-γ when incubated with the three drugs.

DISCUSSION

The β_2-adrenoceptor agonists fenoterol and terbutaline belong to the group of direct sympathomimetic drugs and are routinely

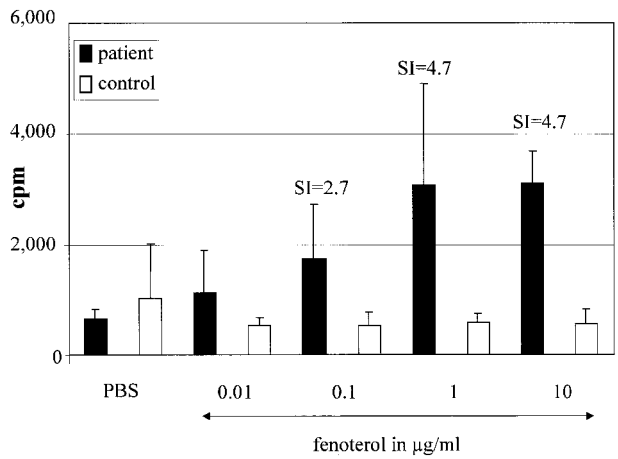

Fig. 1. Lymphocyte transformation tests with peripheral blood mononuclear cells from the patient and a healthy control incubated with fenoterol or with the solvent phosphate-buffered saline (PBS). SI: Stimulation index (see Methods for explanation).

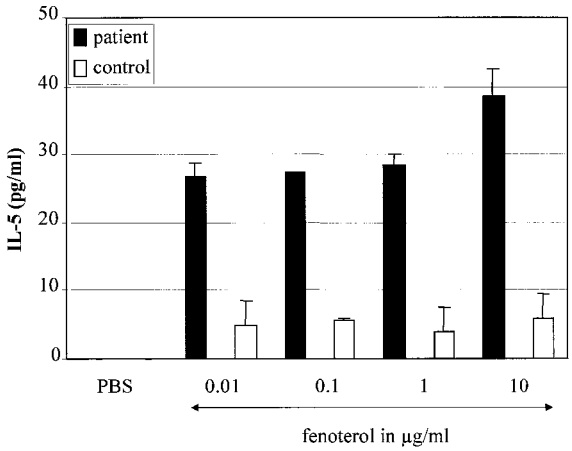

Fig. 2. IL-5 concentration in the culture supernatants of the lymphocyte transformation test.

applied in the treatment of preterm labour and asthmatic disorders (7, 8). The direct sympathomimetic drugs share a common chemical backbone structure similar to the catecholamines and depending on the substitutes preferentially activate α- or β-adrenoceptors.

In the present investigation, drug-specific proliferative lymphocyte reactivity of the patient's PBMC to fenoterol but not to terbutaline or propranolol was observed in the LTT. Since drug-specific IL-5 secretion of antigen-specific activated T cells has been described previously (9–11), we determined the IL-5 concentrations in the culture supernatants of the LTT in order to increase the sensitivity of our *in vitro* test system for the detection of drug-specific lymphocyte sensitization. Considerable amounts of IL-5 were detected in the cultures incubated with fenoterol and terbutaline but not with propranolol, whereas no significant amounts of interferon-γ could be measured following incubation of PBMC from the patient and control with the three drugs. The additional sensitization of the patient's lymphocytes to terbutaline detected by *in vitro* drug-specific IL-5 secretion was subsequently confirmed *in vivo* by a second patch test, which gave negative results with propranolol. Since the patient was non-atopic, and developed a delayed-type immune reaction, the observed drug-specific IL-5 secretion could not be attributed to a disposition of the patient to Th2-skewed immune responses. Hence, these data in conjunction with recently published observations (9–11) suggest that *in vitro* determination of drug-specific IL-5 secretion may serve as an additional parameter for the *in vitro* detection of drug-sensitization in the LTT.

ACKNOWLEDGEMENTS

This work was supported by Deutsche Forschungsgemeinschaft grant (SA 866/1-1), by the START-program of the Rheinisch-Westfälische Technische Hochschule Aachen and by the Interdisciplinary Center for Clinical Research in Biomaterials and Tissue-Material-Interaction in Implants (BMBF project no. 01 KS 9503/9).

REFERENCES

1. Zürcher K, Krebs A. Cutaneous drug reactions. Basel: Karger, 1992.
2. Hoigné R, Schlumberger HU, Vervleot D, Zoppi M. Epidemiology of allergic drug reactions. Monogr Allergy 1993; 31:140–170.
3. Pichler WJ, Schnyder B, Zanni MP, Hari Y, von Greyerz S. Role of T cells in drug allergy. Allergy 1998; 53: 225–232.
4. Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. Clin Exp Allergy 1997; 27: 175–181.
5. de Shazo R, Kemp SF. Allergic reactions to drugs and biologic agents. JAMA 1997; 278: 1895–1906.
6. Choquet-Kastylevsky G, Intrator L, Chenal C, Bocquet H, Revuz J, Roujeau JC. Increased levels of interleukin 5 are associated with the generation of eosinophilia in drug-induced hypersensitivity syndrome. Br J Dermatol 1998; 139: 1026–1032.
7. Herzog S, Cunze T, Martin M, Osmers R, Gleiter C, Kuhn W. Pulsatile vs. continuous parenteral tocolysis: comparison of side effects. Eur J Obstet Gynecol Reprod Biol 1999; 85: 199–204.
8. Coqueret O, Dugas B, Mencia-Huerta JM, Braquet P. Regulation of IgE production from human mononuclear cells by beta 2-adrenoceptor agonists. Clin Exp Allergy 1995; 25: 304–311.
9. Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ. Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin and carbamazepine. J Immunology 1995; 155: 462–472.
10. Pichler WJ, Zanni M, Greyerz vS, Schnyder B, Mauri-Hellweg D, Wendland T. High IL-5 production by human drug-specific T cell clones. Int Arch Allergy Immunol 1997; 113: 177–180.
11. Yawalkar N, Reimers A, Hari Y, Hunziker T, Gerber H, Muller U, Pichler W. Drug-induced linear IgA bullous dermatosis associated with ceftriaxone- and metronidazole-specific T cells. Dermatology 1999; 199: 25–30.