

Erythema multiforme to phenobarbital: Involvement of eosinophils and T cells expressing the skin homing receptor

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In general, adverse reactions to anticonvulsants are characterized by fever, rash, erythroderma, and hematologic complications including agranulocytosis, leukopenia, atypical lymphocytes, eosinophilia, and pseudolymphoma. Because of the variable presentation, diverse clinical features, and delayed onset (which can range from a few days to several weeks after initiation of drug therapy) this syndrome can be confused with other illnesses including viral infection, collagen vascular disease, lymphoma, Kawasaki syndrome, and porphyria.¹ The anticonvulsant hypersensitivity syndrome has an incidence of from 1 in 1000 to 1 in 10,000 exposures.² We report the case of a patient who had a severe desquamative skin reaction after the administration of phenobarbital. The involvement of different inflammatory cells and the potential mechanism causing the reaction were studied.

CASE REPORT

On February 13, 1996, a 21-year-old man was first seen in the emergency department, 2 months after he had been treated with phenobarbital for seizures. He had an exanthematic reaction affecting 70% of the skin surface including the face, arms, chest, abdomen, and legs. Fever (39° C) was present, and the total blood count revealed leukocytosis with eosinophilia. He was kept under observation in the emergency ward. Four hours after the administration of 240 mg of prednisolone, a blood sample was taken, and the patient was discharged 24 hours later with reduction in skin redness and pruritus and a normal temperature (37° C). Values for leukocyte differential counts and the rest of the parameters determined are described below. Twelve hours later, the subject was readmitted because of exacerbation of the intensity of the skin symptoms with conjunctival injection and facial angioedema plus fever (40° C) and systemic pruritus. He was given a continuous tapering dose of

Abbreviations used

CLA: Cutaneous lymphocyte-associated antigen
ECP: Eosinophil cationic protein

80 mg of prednisolone every 24 hours. When we tried to decrease the dose several days later, an exacerbation of the symptoms occurred, consisting of flares with more redness of the skin, facial angioedema, and increased temperature (39° C). To control the symptoms, it was necessary to increase and maintain the dose of corticoids, tapering it slowly. During this period, the exanthematic skin manifestations were replaced by desquamative vesicular lesions over the areas of existing erythema. At this time, the patient's temperature tended to be under 37° C. After 24 days, the patient was discharged with no fever and residual skin desquamative lesions.

Peripheral blood mononuclear cells were isolated from heparinized venous blood by density gradient centrifugation, and flow cytometry analysis was carried out as previously described.³ Cells (10^5 /test) were sequentially stained with HECA-452 (a rat IgM against cutaneous lymphocyte-associated antigen [CLA], kindly provided by Dr. Louis J. Picker), goat anti-rat conjugated to phycoerythrin (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.), CD3-fluorescein isothiocyanate and HLA-DR-PE-Cy5 (Immunotech, Marseilles, France). Five-parameter analysis was performed on an Ortho Cyturon Absolute flow cytometer, and list mode multiparameter data files were analyzed with Ortho Immunocount System software. An *in vitro* lymphocyte transforming test was done. Briefly, 1×10^6 cells were cultured in duplicate in 1 ml of culture medium in 24-well plates (Nunc, Roskilde, Denmark) in the presence of different drugs. The culture medium used was RPMI-1640 (Bio-Whittaker, Verviers, Belgium) containing 2 mmol/L L-glutamine (ICN Biomedicals Inc., Irvine, Calif.), 20 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (Seromed, Berlin, Germany), and 10% heat-inactivated autologous serum. Cells were cultured in the presence of phenobarbital (100 µg/ml; Bayer, Leverkusen, Germany), phenytoin sodium (100 µg/ml; Rubio Laboratories, Barcelona, Spain), or tetanus toxoid (10 µg/ml; Instituto Berna, Madrid, Spain) as a control antigen or in the presence of no antigen for 6 days. Tritiated thymidine (ICN Biomedicals) was then added, and 18 hours later, the incorporation of the labeled nucleotide was determined. Stimulation indexes were calculated as: Counts per minute in culture with antigen ÷ Counts per minute in culture without antigen. Measurement of IL-2, IL-4, IL-5, and interferon-γ cytokine expression was performed by means of reverse-transcriptase polymerase chain reaction.

Total eosinophils were calculated, and the serum level of

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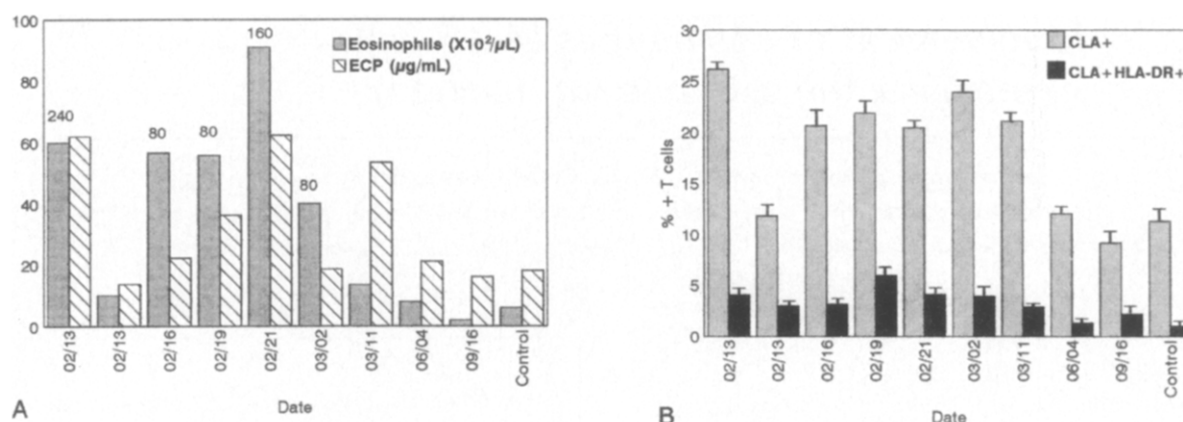


FIG. 1. A, Total eosinophil counts (in cells per microliter) and ECP laboratory values (in micrograms per milliliter), determined during follow-up of the patient. Peripheral blood mononuclear cells were isolated, and co-expression of CLA and HLA-DR in CD3⁺ cells was determined by flow cytometry. **B,** Sequential results are mean percentages of CLA⁺ and HLA-DR⁺ in CLA⁺ ($\pm$ standard deviation) for T cells in three independent experiments. Controls are mean values obtained from nine different healthy subjects (samples taken at the moment of each determination). Numbers shown at tops of bars are doses of corticoids given throughout the treatment period and after determination of values (for more details see text).

eosinophil cationic protein (ECP) was determined by FEIA (Pharmacia, Uppsala, Sweden).

The sequential determination of eosinophils and ECP and the co-expression of CD3⁺CLA⁺ lymphocytes and CD3⁺CLA⁺/CLA-HLA-DR⁺ during the period of follow-up of the patient are presented in Fig. 1. Control samples were obtained from healthy subjects; each sample was obtained at the same time as the patient's determination. Fig. 1, A, shows the sequential levels of eosinophils and ECP. After an initial level of 6000 total eosinophils per microliter and an ECP value of 62 μ g/ml, a sharp decrease to 1000 eosinophils/ μ l and an ECP value of 13.6 μ g/ml occurred 4 hours after the intravenous administration of 240 mg of prednisolone (date, 02/13 in Fig. 1, A). This corresponded with a reduction in the symptoms as stated above. The corticoid dose was reduced to 80 mg/day, and there was a worsening of symptoms with an increase in total eosinophils to 5600 eosinophils/ μ l and an ECP value of 22.2 μ g/ml. The levels in subsequent samples showed a tendency to increase, reaching 9100 eosinophils and an ECP value of 62.3 μ g/ml. The corticoids were increased to 160 mg/day, and the values of eosinophils and ECP tended to decrease. These values were decreased to 80 mg/day on March 2, and on March 11, corticoids were stopped; the values became normal 3 months after the onset of symptoms (Fig. 1, A). The expression of CD3⁺CLA⁺ and HLA-DR⁺ was elevated with respect to control values (Fig. 1, B). The initial CD3⁺CLA⁺ value showed a 54% increase, and the CD3⁺CLA⁺HLA-DR⁺ was 75% over the mean value of the negative control group. As occurred with the levels of eosinophils and ECP, a decrease in the expression of these markers was seen after the initial administration of corticoids. An increase that paralleled those of eosinophils and ECP was later observed. After 3 months of follow-up, these values were normalized and approached control values (Fig. 1, B).

A lymphocyte transforming test of peripheral blood mononuclear cells showed a stimulation index of 3.3 for phenobarbital. The expression of interleukin pattern, determined by reverse-transcriptase polymerase chain reaction, was positive for IL-4 and negative for interferon- γ , IL-2, and IL-5.

A skin biopsy specimen showed a mononuclear cell infiltrate

with eosinophils in the dermis and necrosis of the cells of the basal layer and stratum spinosum.

DISCUSSION

The clinical syndrome of anticonvulsant hypersensitivity is characterized by a skin rash and hematologic abnormalities, including eosinophilia and atypical lymphocytes.¹

It has been reported that T cells are involved in the skin manifestations of these subjects.⁴ However, sufficient studies determining the phenotype of cells involved in the skin lesion and the mechanisms of recruitment are unavailable.

The extravasation of T cells at sites of inflammation has been reported to be dependent on the activity of homing receptors. The CLA is the skin-selective homing receptor that enables T cells to localize in the skin on interaction with E-selectin.³ The number of these cells has been reported to be increased in different types of allergic skin diseases, including allergic drug reactions.⁵ Our data indicate an increase in CLA expression in peripheral blood lymphocytes (CD3⁺ cells), as well as an increase in the expression of HLA-DR, an activation marker of T cells in the CD3⁺CLA⁺ population.

We believe that the increased percentage of circulating CLA⁺ T cells in peripheral blood may play an important role in allergic reactions to anticonvulsant drugs in which the patient has clinical skin manifestations. These data, together with the specific response of lymphocytes on stimulation with phenobarbital, the presence of eosinophils and activation markers, and the detection of IL-4 in lymphocytes, indicate that an immunologic mechanism is involved in the production of the reaction.

The severity of the patient's condition and the worsening of symptoms after reduction in the dose of corticoids was accompanied by an increase in circulating

HLA-DR⁺ CLA⁺ T cells and eosinophils with a release of ECP. This indicates that a close correlation existed between the evolution of the symptoms and the abnormalities in the immunologic parameters.

The T helper 2 (Th2) cytokine pattern plays an important role in human allergic diseases, and it is characterized by the presence of IL-4 and IL-5 and the absence of interferon- γ . Thus IL-4 regulates ϵ germline transcription, whereas IL-5 is involved in eosinophil differentiation, maturation, and attraction. On activation, eosinophils release proteins, such as ECP, which are implicated in the development of eosinophil-associated inflammation and tissue injury.⁶ Although the case reported in this study was not an IgE-mediated reaction, a Th2 pattern was detected with production of IL-4 and absence of IL-2 and interferon- γ . The mechanism involved in eosinophil activation is at present unknown. Although we observed the expression of IL-4, our results were negative for IL-5 expression. IL-4 selectively up-regulates endothelial expression of the vascular cell adhesion molecule-1, the ligand of very late antigen-4 expressed on lymphocytes and eosinophils, contributing to the selective recruitment of eosinophils to sites of allergic inflammation.

To our knowledge, this is the first case in which follow-up of activated peripheral cells expressing skin homing receptor has been done in a patient with an adverse reaction to an anticonvulsant drug. The sequential values of the parameters described above support the role of an immunologic mechanism in the induction of the reaction.

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