

In vitro detection of specific IgE antibodies to erythromycin

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Background: *In vitro* tests for detecting drug-specific IgE would be useful in identifying patients at risk for immediate hypersensitivity reactions to therapeutic doses of a drug.

Objective: We attempted to verify that IgE-mediated reactions to erythromycin occur and to identify IgE antibodies specific for erythromycin in serum from a patient who had urticaria immediately after administration of the drug.

Methods: Skin prick testing was performed on the patient and five control subjects. Serum from the patient, pooled sera from nonatopic subjects allergic to common aeroallergens, and cord blood controls were analyzed for erythromycin-specific IgE by radioimmunoassay. Sepharose (Pharmacia, Uppsala, Sweden) was used as solid phase covalently linked to erythromycin.

Results: We were able to detect erythromycin-specific IgE antibodies in serum from the patient who had an allergic reaction to this antibiotic, but specific IgE could not be detected in control sera.

Conclusion: Immunologic IgE-mediated reactions to erythromycin do occur, and *in vitro* diagnosis of such reactions can be made by using Sepharose as a solid phase covalently linked to this drug. (*J ALLERGY CLIN IMMUNOL* 1995;95:668-71.)

Key words: IgE antibodies, erythromycin, radioimmunoassay

Allergic and pseudoallergic macrolide antibiotic reactions appear to be relatively uncommon, and with few exceptions are mild, with most being limited to the skin.¹ The few cases described with probable IgE-mediated hypersensitivity as determined by means of positive prick test responses and suggestive symptoms were all attributed to spiramycin.^{2,3} This macrolide causes occupational asthma. Five patients were recently described as having generalized skin reactions with a short period of latency (less than 3 hours); one reaction was related to erythromycin and four were related to spiramycin. None of these patients had a positive skin prick test response. Histamine release was negative, but the oral challenge reproduced the

Abbreviations used

cpm:	Counts per minute
PBS:	Phosphate-buffered saline
RIA:	Radioimmunoassay

symptoms in every case.⁴ There have been some cases of urticaria and dermatitis caused by erythromycin, but the fixed drug reaction with a positive epicutaneous test response is the most common immunologic reaction attributed to this macrolide.⁵

Intensive research efforts have been made in the last decade to develop *in vitro* diagnostic procedures for IgE-mediated drug hypersensitivity. It is well known that tests for detecting drug-specific IgE would be very useful in identifying subjects with increased risk of experiencing anaphylaxis or other, more mild immediate hypersensitivity reactions with therapeutic doses. Recently, Epoxy-activated Sepharose 6B (Pharmacia, Uppsala, Sweden) has been used as the solid phase covalently linked to the drug. This compound has shown

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efficiency in binding cephalosporins, ampicillin, amoxicillin,⁶ trimethoprim, and thiopental.⁷

In this study we performed a radioimmunoassay (RIA) with a solid-phase drug-Sepharose complex to identify IgE antibodies specific for erythromycin in serum from a patient with suspected history of immediate erythromycin hypersensitivity and a positive skin prick test response to this drug.

METHODS

A 27-year-old woman, previously diagnosed with allergic rhinitis caused by olive tree pollen sensitization, was evaluated for two episodes of acute urticaria occurring 30 minutes after the administration of a single dose of erythromycin. The first episode occurred in 1989 and the second in 1991. There were no records of other adverse reactions or occupational exposure to drugs. Skin prick testing with erythromycin lactobionate (Abbott Laboratories, Abbott Park, Ill.) in phosphate-buffered saline [PBS]-glycerin to 10 mg/ml concentration was positive (8 × 10 mm wheal with a flare of 12 × 15 mm). Saline solution and 10 mg/ml histamine solution were the negative and positive controls. A wheal 3 mm larger than the negative control was considered positive. Results of skin prick tests with the same erythromycin batch carried out in five subjects with no history of allergy were negative. All skin tests were performed on the forearm with an Osterballe needle (Dome Hollister Stier—by Needle Industries, G.L. Studley, U.K.).

Additionally, as controls for the *in vitro* test, we also used pooled sera from 10 nonatopic subjects, 10 subjects allergic to common aeroallergens with high total IgE levels (above 1000 IU/ml), 20 samples of cord blood (IgE-free control), and serum from a patient with a high level of IgE antibodies to amoxicillin.

Serum from the erythromycin-reactive patient, pooled sera from nonatopic subjects allergic to common aeroallergens, and cord blood controls were analyzed for erythromycin-specific IgE according to the method of Harle et al.⁶ with minor modifications. Briefly, erythromycin lactobionate (Abbott Laboratories) (200 mg) was dissolved in 10 ml of 0.1 mol/L carbonate buffer, pH 8. We then added 4 ml of carbonate buffer, pH 10.5, adjusted the pH to 12.5 with 1N NaOH, and mixed the buffer with 1 gm of Epoxy-activated Sepharose 6B. The mixture was gently shaken at 37°C for 18 hours, then washed repeatedly with: 0.1 mol/L carbonate buffer, pH 10.5; distilled water; 0.1 mol/L carbonate buffer, pH 8; and 0.1 mol/L acetate buffer, pH 4. The remaining free activated groups were blocked with 1 mol/L ethanolamine in 0.1 mol/L carbonate buffer, pH 11, for 4 hours at 37°C. After another series of washing steps, the gel was resuspended in 2 ml of saline solution with sodium azide (1 ppm) and stored at 4°C. Activity remained for 8 weeks. In addition, serum from a patient with a known high level of IgE antibodies to amoxicillin was tested for amoxicillin-specific IgE with the method described above. Briefly, we dissolved 200 mg amoxicillin trihy-

TABLE I. Direct RIA results comparing the IgE binding in patient and control pooled sera to the free solid phase (Epoxy-activated Sepharose 6B) and to the solid phases covalently linked to the drugs

	cpm (mean ± SEM)	Ratio	cpm (%)
Free Sepharose			
Patient serum	170 ± 9		0.2
Erythromycin-Sepharose			
Patient serum	8966 ± 199	10.5	14.0
Control sera from			
High total IgE pool	853 ± 26		1.3
Nonallergic pool	425 ± 22		0.6
Cord blood pool	170 ± 9		0.2
Erythromycin-hsa discs			
Patient serum	425 ± 5	1.3	0.6
Control sera from			
Nonallergic pool	334 ± 15		0.5
Cord blood pool	195 ± 10		0.3
Amoxicillin-Sepharose			
Amoxicillin-positive control	6476 ± 26	8.3	9.2
High total IgE control pool	772 ± 7		1.1

Each serum sample or serum pool was tested in triplicate.

drate (Antibioticos S.A., Madrid, Spain) in 10 ml of 0.1 mol/L carbonate buffer, pH 10.5, adjusted the pH to 12.5 with 5N NaOH, and added 1 gm of Epoxy-activated Sepharose 6B. The rest of the procedure was as previously described for erythromycin. Additionally, serum from the erythromycin-reactive patient, pooled sera from nonatopic subjects allergic to common aeroallergens, and cord blood controls were analyzed for erythromycin-specific IgE with erythromycin-human serum albumin linked to CNBr-activated paper disks (Bioallergy, Rome, Italy). Each serum sample or serum pool to be tested (100 µl) was incubated with 200 µl of solid-phase drug for 3 hours at room temperature, then washed three times with 0.1% PBS-Tween. We then added 100 µl of iodine 125-labeled anti-human IgE (about 64,000 counts per minute [cpm]) (Pharmacia) and incubated the mixture for 16 to 18 hours at room temperature. After the series of washing steps was repeated, the activity was counted in a Packard Multi-detector RIA System (Packard Instrument Co., Meriden, Conn.). The radioactive uptake ratio was obtained by dividing the radioactive uptake in cpm of ¹²⁵I-labeled anti-human IgE of the patient serum by the uptake of the highest control serum. A ratio greater than 2 was considered positive. The percent cpm for each serum sample was obtained by dividing the residual cpm on the solid phase by the total cpm initially added to each. An uptake of ¹²⁵I-labeled anti-human IgE greater than 10% of the total cpm added was considered indicative of a large amount of drug-specific IgE.⁶ The same amounts of serum and ¹²⁵I-labeled anti-human IgE were used

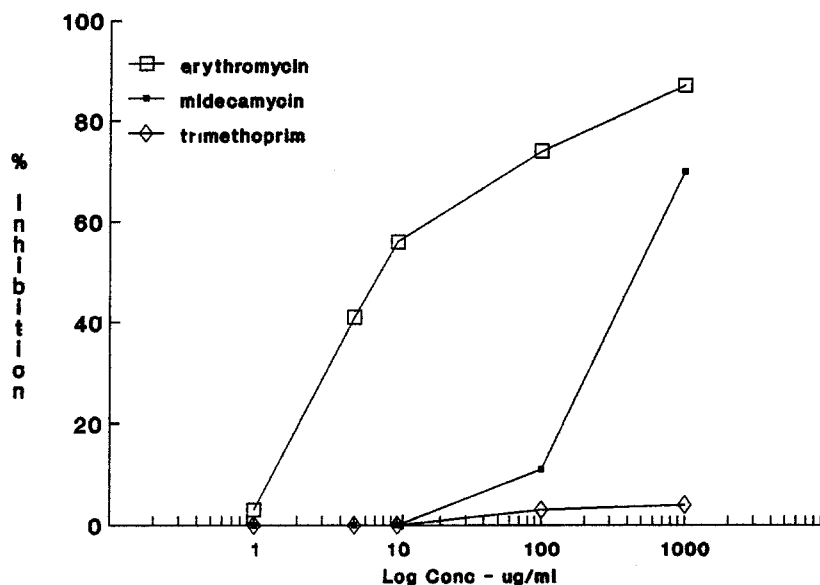


FIG. 1. Inhibition by erythromycin, diacetylmidecamycin, and trimethoprim of IgE binding to erythromycin linked to Sepharose in serum from a patient who experienced an allergic reaction to erythromycin. Mean basal cpm was 4439 ± 228 (patient serum was diluted 1:1 with PBS).

with the commercial erythromycin disks. Each serum sample or serum pool was tested in triplicate.

A competitive inhibition of IgE binding in erythromycin-reactive patient serum to the drug-Sepharose complex was performed by preincubation with erythromycin, diacetylmidecamycin (Menarini S.A., Barcelona, Spain), and trimethoprim (Almirall S.A., Barcelona, Spain). Patient serum was diluted 1:1 with various drug dilutions and was then incubated for 3 hours before centrifugation. After centrifugation, 100 μ l of the inhibited serum was used in the radioisotope assay described above. The percent inhibition was calculated by taking the erythromycin cpm value minus the incubated drug cpm value, then dividing by the erythromycin cpm value. This number is multiplied by 100 to get the percentage of inhibition. The inhibition protocol for the three drugs was repeated three times with similar results. The data shown represent the average of the three assays.

RESULTS

RIA results comparing the IgE binding to free solid phase (Epoxy-activated Sepharose 6B alone) with solid phase covalently linked to drugs are displayed in Table I. Patient serum incubated with the free solid phase, blocked with 0.1 mol/L ethanolamine, as previously described, did not show nonspecific ^{125} I uptake. Specific IgE binding to the erythromycin-Sepharose complex was evaluated in serum from the patient, the serum pool from nonallergic subjects, the serum pool from patients with high total IgE levels, and the serum pool from cord blood. Only the patient serum demonstrated significant amounts of specific IgE, as measured by

uptake of ^{125} I-labeled anti-human IgE greater than 10% of the total cpm added and by high uptake ratio compared with that of the control sera. Sera from subjects allergic to common aeroallergens with high total IgE levels showed twice as much uptake as the sera from nonallergic subjects, yet the percentage bound of the total cpm added was only 1.3%.

Drug-specific IgE was not detected in patient and control sera when the commercially available erythromycin-human serum albumin cellulose CNBr-activated paper disks were used.

Additionally, the serum of a patient with a high level of amoxicillin-specific IgE (positive control) and the serum pool from subjects with high total IgE were incubated with amoxicillin linked to Sepharose (Table I). Only the positive control showed significant uptake of ^{125} I-labeled anti-human IgE.

The RIA-inhibition results are shown in Fig. 1. Inhibition of IgE binding to erythromycin linked to Sepharose in patient serum was carried out by preincubation with erythromycin, with a structurally related compound (diacetylmidecamycin), and with a structurally unrelated compound (trimethoprim). The concentration of erythromycin required to produce 50% inhibition was 8 μ g/ml, compared with 500 μ g/ml for diacetylmidecamycin. Control experiments with trimethoprim did not show significant inhibition, demonstrating the specificity of the inhibition assay.

We tried to perform a direct RIA with diacetylmidecamycin linked to Sepharose, but the poor solubility of this compound (maximum 2 mg/ml in PBS) precluded this experiment.

DISCUSSION

The serum we obtained was from a patient who demonstrated urticaria immediately after the administration of erythromycin and whose skin prick test response to this macrolide was positive. We therefore presumed a possible immediate hypersensitivity mechanism, and in vitro demonstration of IgE antibodies to erythromycin was attempted.

Binder-ligand assays have been successfully applied to the in vitro study of some immunologic drug reactions.^{6,7} In the same way, necessary procedures and criteria for detection of drug-specific IgE antibodies have been summarized elsewhere.⁸ In the solid phase type of assay, the drug under investigation must be immobilized for reaction yet antigenically unaltered in order for it to be recognized by specific antibodies. Covalent binding to the solid phase is the best means to achieve this.⁸ The CNBr-activated paper disk coupled to a drug by means of the human serum albumin complex has been widely used for penicillin-specific IgE antibody detection. However, the commercial method used in this assay was unable to bind erythromycin-specific IgE antibodies in our patient serum.

In our study, using Sepharose as solid phase covalently linked to erythromycin, we were able to detect drug-specific IgE antibodies in serum from a patient who experienced an allergic reaction to this antibiotic, whereas specific IgE could not be demonstrated in control sera. The specificity of this assay is supported by the inhibition of binding of IgE antibodies in patient serum to erythromycin-

solid phase complex by incubation with free drug; partial inhibition with another, structurally related macrolide antibiotic (diacetylmidecamycin); and lack of inhibition with a structurally unrelated drug (trimethoprim).

In summary, we have demonstrated that immunologic IgE-mediated reactions to erythromycin occur and that in vitro diagnosis of these reactions can be successfully carried out by using Sepharose as solid phase covalently linked to this drug. In addition, we believe that this method could be valuable in the detection of IgE specific for other macrolides in patients in whom the possibility of an immediate hypersensitivity reaction is suspected.

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