

Severe anaphylactic reaction to thymostimulin

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Thymostimulin is a partially purified extract of calf thymus, consisting of a group of polypeptides with a total molecular weight of approximately 12,000 D. It is used as an immunologic response modifier in primary and secondary immunodeficiencies. We report a patient who had a severe anaphylactic reaction with the first injection of thymostimulin. Type I hypersensitivity to thymostimulin was suggested by an immediate skin test response, specific IgE determination by indirect ELISA and a positive histamine release test. This patient also reacted to bovine serum albumin (BSA), fetal calf serum (FCS) and cow hair and dander. The controls did not react to any of these tests. Clinicians need to be aware that the use of medical products of xenogeneic origin may lead to severe allergic reactions.

Key words: anaphylaxis; bradycardia; cattle; drug hypersensitivity; thymus hormones.

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Thymostimulin (TP-1) is a partially purified extract of calf thymus. This extract was prepared as described by Falchetti & Berguesi at Instituto Farmacologico Sero, Rome (4). It is a group of polypeptides with a total molecular weight of approximately 12,000 D.

Thymostimulin has been shown to induce T-cell markers on lymphocytes from T-cell deficient patients and to produce clinical improvement when administered to them, as well as increased T-cell number and function (10). No effects on IgE levels have been demonstrated (11).

This report describes the evaluation of a patient who experienced a severe anaphylactic reaction to thymostimulin. Immunologic studies were performed to determine the pathogenic mechanism of the reaction.

CASE REPORT

The patient was a 38-year-old man admitted for examination of a right neck tumour. A

percutaneous biopsy was performed and showed a poorly differentiated carcinoma of epidermoid origin. As it was not possible to find the primary tumour, the patient was treated with chemotherapeutic agents. He received three courses of 5-FU and cis-platin chemotherapy, and in the third course he also received thymostimulin. Within seconds of the first injection of thymostimulin 75 mg, the patient became breathless. Physical examination revealed a lethargic, apneic and cyanotic man with a blood pressure of 60/30 mmHg, a heart rate of 40/min. There was no wheezing, urticaria or angioedema. After endotracheal intubation, the heart rate was 100/min with a blood pressure of 100/70 mmHg and he was conscious. Arterial blood gases revealed pH 7.06, pO_2 70 mmHg, pCO_2 59.4 mmHg and the ECG was normal pH. The patient was treated with bicarbonate, oxygen and volume expanders in an intensive care unit. At the time of this episode, the patient was not receiving beta-adrenergic blocking agents.

Four months later, the patient underwent surgical resection of the tumour and the result of the biopsy was benign ectopic neck thymoma.

The patient's past medical history was significant for alcohol abuse. He had not received any previous xenogeneic sera, particularly no bovine serum products. He lived opposite a cattle farm but denied any direct exposure to cattle. He had no previous history of atopy, asthma or anaphylaxis and denied any known allergic or untoward reactions to foods, especially beef or veal.

METHODS

Skin prick testing was performed with a panel of commercially available common inhalants, including pollens, mites, molds and cat and dog dander (Bencard, Worthing, UK). Cutaneous sensitivity to thymostimulin was tested by prick test at a concentration of 12.5 mg/ml (25 mg of thymostimulin diluted in 2 ml of saline solution). Skin prick tests with different bovine proteins were also performed: BSA (10% w/v, Sigma Chemical Co., St. Louis, Mo.), pure FCS (Flow Laboratories, Scotland), bovine milk extract (50% w/v, Bencard, Worthing, UK), bovine globulins (10% w/v, Sigma Chemical Co., St. Louis, Mo.) and cow hair and dander extracts (150% w/v, Bencard, Worthing, UK). Histamine phosphate 1:100 w/v and 0.9% normal saline were used for positive and negative skin test controls, respectively. Reactions were interpreted after 15 min by measuring the diameter of the wheal, and a wheal of at least 3 mm larger than the negative control was considered as positive. When skin prick tests were negative, intradermal skin tests were carried out with 1/100 and 1/10 dilutions of the extracts used in skin prick tests.

The same tests were performed on five atopic and five normal individuals who served as controls.

Indirect ELISA for determining specific IgE antibodies detects the presence of specific IgE antibodies by using antigen-coated microtiter plates (3). After coating, the sera (optimally diluted) are incubated in the wells. Thereafter,

monospecific antisera (anti-human IgE) labelled with peroxidase are added and, finally, the presence of specific antibodies is revealed by means of a chromogenic substrate (orthophenylenediamine).

Histamine release test was performed using an automated fluorometric method according to Siraganian (9) with the modifications previously described (6), with four different concentrations (1:5, 1:25, 1:125, 1:625) of the same thymostimulin extract used in skin tests. A result higher than 10% release was considered positive.

Total IgE was measured by IgE EIA technique according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden).

RESULTS

An immediate positive response (9 mm wheal with pseudopods) was obtained in the thymostimulin prick test with a concentration of 12.5 mg/ml. Skin prick tests with bovine proteins were positive for BSA 10% w/v (5 mm), pure FCS (7 mm), cow hair and dander 150% w/v (8 mm). However, prick and intradermal skin tests with bovine milk 50% w/v and bovine globulins 10% were negative. Skin tests with common inhalants were positive for *Dermatophagoides pteronyssinus*, grass pollen and cat dander, but were negative with the other inhalants and saline control. Total IgE was 489 KU/l.

Specific IgE to thymostimulin was detected in the patient's serum by indirect ELISA; readings at 492 manometres revealed a value of 5.174 OD (optical density). A value of 0.320 OD was considered positive (mean of the control sera + 2 SD). The histamine release test was also positive (16%). The control subjects did not react to any of these tests.

DISCUSSION

The clinical findings and results of the investigations carried out in our patient strongly suggest that the anaphylactic reaction was induced by thymostimulin and that the underlying mechanism was probably an IgE-mediated immunologic reaction.

Of note in this case report is the clinical manifestation of anaphylaxis. Our patient did not develop tachycardia first. Apparently vagal activity predominated during the early development of this reaction. This may have been due to the Bezold-Jarisch reflex that is an inhibitory reflex originating in sensory receptors in the left ventricle and is activated by local ischemia (8). Bradycardia associated with hypotension is usually thought to signify a vasovagal phenomenon. Evidence against this in our patient was that he was cyanotic and his arterial blood gases revealed pH 7.06, $p\text{CO}_2$ 59.4, $p\text{O}_2$ 70. Metabolic acidosis and hypoxemia, as seen in this patient, are typical of severe anaphylaxis. This case emphasizes the possibility of an initial bradycardia in association with anaphylactic hypotension, as has been previously reported (8).

This patient developed a severe anaphylactic IgE-mediated reaction after the first injection of thymostimulin. Initially, we thought that the patient could be sensitized to calf proteins, so we performed skin tests with various bovine proteins. These tests revealed that the patient was positive to BSA, FCS and bovine hair and dander. The negative results obtained in the control subjects support the specificity of these findings. It is possible that the patient was sensitized to bovine albumin, present in cow hair and dander (1,7), FCS and possibly contaminates thymostimulin. Thus, this patient could well be sensitized by ingestion of beef products or by exposure to airborne cow dander. He may not have had any symptoms when ingesting beef products because a higher threshold dose is required to provoke an IgE-mediated gastric response, as has been demonstrated (5).

This case illustrates the risk of anaphylactic reactions with the use of medical products of xenogeneic origin. The possibility of such reaction can be evaluated, prospectively or retrospectively, by *in vivo* or *in vitro* testing. It is hoped that with the improved purification

methods and the development of synthetic hormones, such reactions will be notably reduced in the future.

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