# Anaphylaxis to infusion of autologous bone marrow: An apparent reaction to self, mediated by IgE antibody to bovine serum albumin

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*A case of anaphylaxis during autologous bone marrow infusion is reported. The patient was demonstrated to be skin test positive to fetal calf serum used in the cryopreservation of his bone marrow cells. The patient's serum was demonstrated to contain IgE antibody directed against bovine serum albumin. A second aliquot of the patient's bone marrow preparation was depleted of contaminating bovine proteins, and the patient successfully received a transplant and was engrafted. Clinicians need to be aware that the increasing use of biologic response modifiers, both as whole cells and effector molecules manipulated or produced in vitro, may lead to cryptic reactions to xenogeneic proteins. (J ALLERGY CLIN IMMUNOL 1989;83:871-5.)*

Although anaphylactic reactions to an extraordinary variety of materials has been observed, apparent anaphylactic reactions to autologous constituents is decidedly unusual.[1] We investigated a patient who had an anaphylactic reaction on infusion of his own bone marrow during autologous bone marrow transplantation. In fact, the reaction was directed against a xenogeneic protein, BSA, contained in the FCS that was used in the cryopreservation of the patient's bone marrow cells some 20 months earlier. We surveyed the bone marrow transplant centers in the United States to investigate the potential magnitude of this problem. Although it appears that direct exposure to FCS proteins during bone marrow transplantation is unlikely to be a significant problem in the future, the expanding use of biologic-response modifiers, both as whole cells and effector molecules manipulated or produced in vitro, may lead to more cryptic reactions to xenogeneic proteins.

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Supported in part by United States Public Health Service Grants Al-15251, Al-15332, Al-07126, and CA-23175.
Received for publication July 15, 1988.
Accepted for publication Oct. 24, 1988.

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*Abbreviations used*

| | |
|---|---|
| BSA: | Bovine serum albumin |
| FCS: | Fetal calf serum |
| Alk phos: | Alkaline phosphatase |
| PBS: | Phosphate-buffered saline |
| OVA: | Ovalbumin |
| OD: | Optical density |

## MATERIAL AND METHODS

### Skin testing

Immediate hypersensitivity skin testing was performed with a modification of standard prick and intradermal technique on the patient's right forearm.[2] Informed consent was obtained before skin testing. Histamine phosphate (Eli Lilly & Co., Indianapolis, Ind.) was used at a concentration of 2.75 mg/ml. For intradermal testing, 0.05 ml of each reagent was used because the patient required this amount of histamine to produce a positive response. Results were reported as millimeters of wheal and erythema, and a wheal of ≥5 mm with surrounding erythema was interpreted as a positive result.

### ELISA for IgE to FCS and BSA

A modification of an enhanced Alk phos ELISA with a highly specific and sensitive Fab' Alk phos-coupled anti-IgE was used, as described in detail elsewhere.[3] Briefly, polystyrene flat-bottom microtiter plates were coated overnight with (1) 1% FCS from the same lot used to cryopreserve the patient's bone marrow, (2) crystalized BSA (2

**TABLE I.** Skin test results of the patient and control subject

| | Patient | Control subject |
|---|---|---|
| | Wheal/erythema | Wheal/erythema |
| Histamine | 10/20 | 6/12* |
| FCS | 10/15 | 0/0 |
| Media | 0/0 | 0/0 |
| Media plus FCS | 8/10 | 0/0 |
| Ficoll | 0/0 | 0/0 |
| DMSO | 0/15 | 0/10 |

*DMSO,* Dimethylsulfoxide.

*All the tests presented represent results of intradermal testing with 0.05 ml of the indicated materials except the histamine in the control subject, which was prick testing only.

μg/ml, Sigma Chemical Co., St. Louis, Mo.), or (3) a 50:50 mixture (2 μg/ml) of anti-IgE monoclonal antibodies, CIA-E7.12 and 4.15, produced in our laboratory.[4] After plates were washed with PBS/Tween three times, they were blocked for ½ hour with 1% OVA. The patient's serum, control sera, and a known IgE-deficient serum (kindly provided by Dr. G. Gleich, Mayo Clinic, Rochester, Minn.) were diluted in 1% OVA and added as indicated. Blocking of IgE anti-BSA was accomplished by adding BSA (5 mg/ml) into wells after the patient's serum had been added. The plates were incubated at room temperature for 2 hours, washed three times with PBS/Tween, and incubated for 2 hours with an antihuman IgE affinity-purified rabbit Fab' coupled to Alk phos. The plates were then given their final washes, first three times with PBS/Tween, then three times with NaCl/Tris, and then developed with the ELISA Amplification System (BRL, Gaithersburg, Md.), according to the manufacturer's instructions. The Fab' Alk phos-coupled anti-IgE (a generous gift from Dr. Michael Kemeny, Guy's Hospital, London, England) was used at a 1:3000 dilution in 1% OVA. Our assay had a sensitivity of <10 pg of total IgE with our monoclonal angi-IgE antibodies as the coating solution. The absolute amounts of BSA-specific IgE were estimated, based on equivalent ODs of an IgE standard curve performed on a portion of each plate coated with BSA. The Student's *t* test was used to test the differences of the means of the ODs obtained in the ELISAs.

## Case presentation

The patient was a 31-year-old man admitted for autologous bone marrow transplantation for stage IV Hodgkin's disease. He was first observed in July 1985 with left cervical and supraclavicular adenopathy, a 92 pound weight loss, fevers, and night sweats. A biopsy specimen revealed nodular-sclerosing Hodgkin's disease. He underwent six courses of combination chemotherapy, completed in December 1985. In March 1986, he received 33 Gy of radiation to his mediastinum as treatment for a relapse. In June 1986, 1 L of the patient's bone marrow was harvested, the bone marrow mononuclear cells separated by buoyant density centrifugation, and then cryopreserved in McCoy's medium with 10% FCS and 10% dimethylsulfoxide.[5] After additional chemotherapy, the patient was in remission until September, 1987, when he complained of a 10 pound weight loss. A computed tomography scan revealed pelvic adenopathy. He was treated with four courses of doxorubricin, vinblastine, and procarbazine chemotherapy.

He was admitted in February 1988 for autologous bone marrow transplantation. The patient received chemotherapy with cyclophosphamide (60 mg/kg/day for 2 days), carmustine (300 mg/M$^2$), and etoposide (15 mg/kg/day for 3 days). An aliquot totaling 140 ml of the cryopreserved marrow was thawed and diluted to 400 ml with McCoy's medium. This material, containing $3.1 \times 10^9$ viable cells and approximately 3.5% FCS, was infused intravenously into the patient. After infusion of <5 ml of his cryopreserved bone marrow, the patient noted warmth in his hands and feet followed shortly by the complaint of tightness in his chest. This amount of cryopreserved bone marrow in retrospect was estimated to contain <15 mg of BSA. The infusion was immediately stopped. Nevertheless, the patient became hypotensive, apneic, and required cardiopulmonary resuscitation.

Twenty-four hours after the reaction, the patient was skin tested (see Results). The patient's remaining 140 ml of cryopreserved marrow was then thawed and adjusted to 450 ml with Hanks' balanced salt solution without serum. This was gently centrifuged, decanted, and resuspended in 450 ml of Hanks' balanced salt solution. The cells were centrifuged a second time, and the remaining $0.45 \times 10^9$ viable cells were resuspended in 200 ml of McCoy's medium with 10% autologous serum. The estimated depletion of the FCS was 10,800-fold with a total of approximately 50 μg of BSA remaining. The patient was premedicated with diphenhydramine, 50 mg intravenously, 30 minutes before, and hydrocortisone, 100 mg intravenously, 1 and 4 hours before infusion of the bone marrow. The FCS-depleted cell preparation was infused during a 1-hour period with no adverse effects.

The patient's past medical history was significant for alcohol and drug abuse. He denied intravenous drug abuse. He denied any occupational exposure to cattle or beef products. The patient had not received any previous xenogeneic sera, specifically no previous FCS or beef or horse serum products and had not received insulin or any experimental biologic-response modifier treatment. The patient had no previous history of atopy, asthma, or anaphylaxis. The patient denied any known allergic or untoward reactions to foods, specifically beef or veal, despite frequent consumption.

## RESULTS

The patient and a normal control subject underwent immediate hypersensitivity skin testing with undiluted FCS from the same lot originally used in storage of the patient's bone marrow, McCoy's medium combined with 10% FCS, dimethylsulfoxide, McCoy's medium alone, and Ficoll-Hypaque. The patient had

been treated with a total of 200 mg of diphenhydramine in the 24 hours after the anaphylactic episode before skin testing. All the prick tests were negative in the patient, including the histamine control, whereas the normal individual did respond to histamine on prick testing. Intradermal skin tests were then performed with 0.05 ml of each reagent, since the patient required this amount of histamine before a positive response was obtained (Table I). Given this amount of material, the patient reacted to histamine, FCS, and medium plus 10% FCS, whereas he was nonreactive to medium alone, dimethylsulfoxide, and Ficoll-Hypaque. A normal nonatopic individual skin tested simultaneously as a control subject was reactive only to the prick test for histamine and completely nonreactive to 0.05 ml of FCS, medium plus FCS, medium alone, or Ficoll-Hypaque. These results reduced the chances of a nonspecific irritant effect from FCS causing a positive response in the patient and directed our efforts to identify IgE antibodies against FCS or its primary constituent, BSA, in the patient.

In vitro testing was used to confirm the presence of IgE against FCS in the patient and to determine a specific protein, BSA, that the IgE antibody would bind. ELISAs were established with FCS and BSA as the primary coating material. Two serum samples were obtained from the patient. The first sample ("pre") was taken just before skin testing, although some 24 hours after the anaphylactic reaction. The second sample ("post") was obtained 4 weeks later, since it was possible that the "pre" sample might have low levels of the IgE antibody of interest because of the massive antigen exposure the patient had likely endured. Additionally, the "post" sample might reflect the booster effect of that exposure, as well as the enhancement of IgE production that may occur after bone marrow transplantation.[6]

Initial experiments were performed with plates in which one half was coated with FCS and the other half coated with BSA (data not presented). These revealed equivalent IgE binding of dilutions of the patient's serum to FCS and BSA. The patient's binding was >100 times above binding by sera from both a normal control subject and an IgE-deficient individual. Since virtually all the patient's anti-FCS IgE could be accounted for by its reactivity to BSA, IgE reactivity to other FCS components, such as immunoglobulin, transferrin, fetuin, ferritin, and insulin, were not specifically evaluated.

The IgE reactivity of the patient to BSA is displayed in Fig. 1. The patient's "pre" and "post" sera are contrasted to the lack of binding observed in this assay by the control serum, derived from the control individual used in the skin testing, and by a known IgE-deficient serum, (IgE-). The "post" serum did reveal a significant increase (45% for the 1:50 dilution, $t = 6.133$ and $p < .01$) in IgE anti-BSA compared to the "pre" sample. When the patient's "post" serum was added to the ELISA followed by BSA (5 mg/ml), there was 83% blocking of IgE binding at the 1:50 dilution. An OD of 0.1 in this assay was equivalent to approximately 0.5 ng/ml of total IgE (calculated from an IgE standard curve run on the same plate as Fig. 1) (data not presented). Based on the 1:50 dilution, which was within the linear part of the standard curve, the patient's serum elicited a signal for IgE anti-BSA equivalent to 25 ng/ml of IgE.

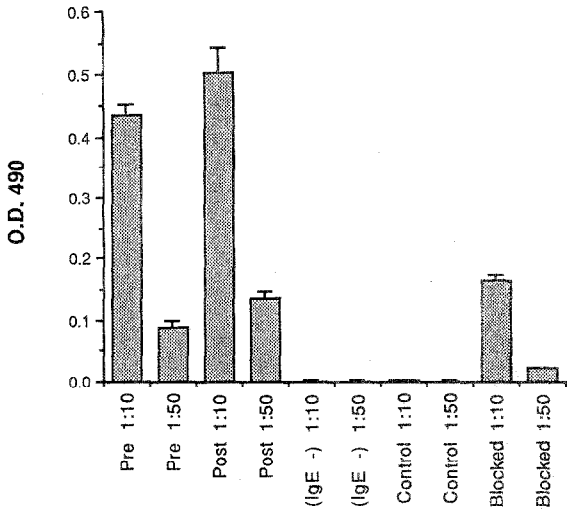


**FIG. 1.** Determination of IgE antibody against BSA. The patient and control sera were analyzed for IgE anti-BSA by ELISA. *Pre* is the patient's serum the day after his reaction and just before skin testing. *Post* is the patient's serum 4 weeks later. (IgE-) is a known IgE-deficient serum. *Control* is serum from the negative skin test control. *Blocked* is the patient's *post* serum mixed with 5 mg/ml of BSA after addition to the ELISA plate. An OD of 0.1 is approximately equal to an IgE concentration of 0.5 ng/ml calculated from an IgE standard on the same plate. *Error bars* represent 1 SD of quadruplicate determinations run on the same plate.

In view of these findings, we sent a letter regarding this potential problem and a questionnaire to 70 other centers in North America who are known to undertake bone marrow transplantation. We received 42 replies. Of the responding centers, 37 had or were performing autologous bone marrow transplantation. None of the responding centers, including our own program, were presently using FCS or other xenogeneic material during cryopreservation. Most centers, 27 of 37, used autologous plasma or serum. Of the remaining centers, five used human albumin, two used heterologous plasma, and three centers used other materials. Three centers had used FCS in the past in a minimum of 90

patients. None of the bone marrow transplantation programs reported any adverse reactions believed caused by anaphylaxis to BSA or other materials, but we were informed of four other undocumented cases of sudden death during bone marrow infusion containing xenogeneic protein (personal communication).

## DISCUSSION

In the patient in this study, FCS and, more specifically, BSA, was a significant antigen involved in inducing a life-threatening immediate hypersensitivity reaction. This case illustrates the inherent risks of intravenous infusion of xenogeneic proteins. FCS is frequently used in tissue culture to improve cell viability. In the initial descriptions of bone marrow cryopreservation FCS was used for this purpose, although more recently nearly all centers, including our own center, use autologous human serum.[7] Our survey suggests that the likelihood of anaphylaxis to infusion of autologous bone marrow occurring in bone marrow transplantation again is remote, since no center reported using xenogeneic serum, although this complication probably has been historically underreported.

There was no evidence that our patient had sustained any unusual form of exposure to FCS or other animal products. It is possible that the patient had been sensitized by ingestion of beef products, since most normal subjects have detectable IgG anti-BSA. Perhaps our patient's IgE anti-BSA response was enhanced by his Hodgkin's disease and chemotherapy before bone marrow transplantation, both of which have been demonstrated to be associated with elevated IgE levels.[6, 8] Individuals frequently are skin test positive to foods and yet have no symptoms when they are ingesting the same foods. This has been demonstrated to be caused by a higher threshold dose required to provoke an IgE-mediated gastric response.[9]

There is an extensive literature on IgE- and non-IgE-mediated reactions to xenogeneic proteins, mainly from the era of serotherapy, although equine antithymocyte globulin and several equine antitoxins are currently used.[10-13] With improved purification methods, the incidence of antibody reactions to xenogeneic proteins, such as insulin, were dramatically decreased before the advent of human insulin therapy. Anaphylaxis has been reported with a number of modern purified xenogeneic therapeutic proteins, such as porcine factor VIII concentrates.[14] Murine monoclonal antibodies have recently gained popularity as immune modulators, antineoplastic, and/or imaging agents. Human IgG antimouse immunoglobulin and anti-idiotypic responses have limited the usefulness of these products in certain settings. Specific IgE-mediated reactions to murine monoclonal antibodies have only been rarely reported.[15] It is possible that some of the apparent reactions to these products may have been directed against equine, bovine, or mouse albumin that contaminate the preparations.

Our patient's reaction was induced by <15 mg of BSA in the first bone marrow transfusion before it was stopped. He subsequently tolerated skin testing with about 1.5 mg of BSA in the 0.05 ml of FCS used, but demonstrated a local reaction. By comparison a bee sting contains only about 50 μg of total protein and is well recognized to induce anaphylaxis.[16] The second bone marrow preparation infused contained a total of about 50 μg of BSA. This amount of BSA was tolerated intravenously during a 1-hour period with premedication, despite a relatively large amount of specific IgE anti-BSA present in the patient's serum.

With the increasing use of biologic response modifiers, monoclonal antibodies, and in vitro-manipulated, reinfused autologous human cells, we believe physicians embarking on such maneuvers should be cognizant of the possibility of immediate hypersensitivity reactions, especially when the material is administered intravenously. The possibility of such reactions can be evaluated, prospectively or retrospectively, by in vivo or in vitro testing.

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# Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits

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*In recent years the morbidity and mortality of asthma has increased, although the etiology is still poorly understood. Most patients with asthma suffer acute attacks that are commonly treated in hospital emergency rooms (ER). In the present study, asthma in adults was studied with acute attacks as a marker for the disease; 102 patients first observed at a university hospital ER with acute airway obstruction were compared to 118 patients observed at the same ER with any diagnosis other than shortness of breath to evaluate allergy as a risk factor for asthma in adults. Sera were assayed for IgE antibody (Ab) to dust mites, cockroach, cat dander, and grass and ragweed pollen. The results demonstrate that in adults younger than 50 years of age, the prevalence of IgE Abs was fourfold greater among subjects with asthma than among control subjects (46/67 versus 12/81; odds ratio, 10.1; 95% confidence interval, 4.9 to 20.7). The population attributable risk for the presence of IgE Ab to one of the five allergens was >50%. Among individuals older than 50 years of age, the prevalence of serum IgE Abs was not significantly increased among patients with acute airway obstruction. In the whole group, the prevalence of IgE Abs to different allergens demonstrated significant seasonal and socioeconomic differences, suggesting that the associated risk is related to exposure to those allergens. The results establish that, with acute attacks of asthma as a marker for adult asthma, the presence of serum IgE Abs to common inhalant allergens is a major risk factor. The results imply that analysis of risk factors, both immediate hypersensitivity and exposure to allergens, should be a routine part of the management of adult asthma. (J ALLERGY CLIN IMMUNOL 1989;83:875-82.)*

Although the mortality rate for asthma is generally low, there has been a definite increase during the last few years, particularly among minority groups.[1] Acute attacks of asthma account for approximately 2 million visits to ERs per year in the United States and are responsible for much of the morbidity and mortality of this disease.[2] For some groups of the population,

*Abbreviations used*
ER: Emergency room
PAR: Population attributable risk
Ab: Antibody
CI: Confidence interval

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Supported by National Institutes of Health Grant A120565, and by a grant from the American Lung Association of Virginia.
*Recipient of Public Health Service National Research Service Award A107255.
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