

Lack of Cross-Reactivity Between IgE to Salmon and Protamine Sulfate

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ABSTRACT: Immediate type-generalized reactions to protamine sulfate are uncommon but may be fatal. The mechanisms of severe or fatal reactions are unknown in most cases. One theory is that contaminating fish (salmon) proteins present in protamine solutions induce anaphylaxis in salmon-sensitive subjects. A second hypothesis is that protamine interacts with anti-salmon IgE to cause anaphylaxis. We assessed these hypotheses by establishing an indirect amplified enzyme-linked immunosorbent assay (ELISA) for IgE to salmon. Sera obtained from two subjects anaphylactically sensitive to salmon demonstrated high binding to salmon that was not inhibited by preincubation of sera with 500 or 1000 μ g of protamine or *Aspergillus fumigatus*. Serum from a patient who experienced anaphylactic shock from protamine was indistinguishable from control sera in the ELISA for IgE to salmon. Anti-protamine IgE could not be demonstrated in separate experiments. The assays prove that 1) serum IgE to salmon is not inhibited by protamine and 2) serum from a patient experiencing a severe reaction to protamine did not contain IgE to salmon or protamine. The experiments do not support the notion that there is cross-reactivity between IgE to salmon and protamine sulfate in the cases evaluated. **KEY INDEXING TERMS:** Protamine; Salmon; Anaphylaxis. [Am J Med Sci 1989; 298(2): 104-108.]

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Protamines are low molecular weight, strongly cationic proteins that occur in sperm or testis of salmon and some other species of fish. The strongly basic property of protamine accounts for its antiheparin effect.¹ Intravenous injections of protamine may cause a sudden decline in blood pressure, dyspnea, flushing,¹ and even cardiac arrest.²⁻⁵ There have been no satisfactory explanations as to the mechanism of such reactions. Some theories have included 1) IgE-mediated anaphylaxis,² 2) direct mast cell activation,⁶⁻⁸ 3) classical complement pathway activation by heparin-protamine complexes,^{9,10} 4) inhibition of C1 esterase inhibitor,¹¹ and 5) presence of contaminating salmon or fish proteins in protamine solutions.^{12,13} Several reports have suggested that fish hypersensitivity¹³ or exposure to insulin-containing protamine¹⁴⁻¹⁶ may have sensitized subjects for subsequent protamine reactions.

The goals of this study were to determine whether there is cross-reactivity between anti-salmon IgE and protamine sulfate and to establish whether sera from a patient who developed a severe immediate type-generalized reaction to protamine contained IgE antibodies to salmon or protamine. We could not inhibit IgE binding to salmon with protamine sulfate or demonstrate presence of serum IgE to protamine in enzyme-linked immunosorbent assay (ELISA) experiments.

Materials and Methods

Sera. Sera from two patients who had experienced anaphylactic reactions upon ingestion of salmon were obtained and stored at -15° C. Symptoms began within 15 minutes in both patients, who developed dyspnea, wheezing, urticaria, and generalized pruritus necessitating emergency therapy. Both patients demonstrated 4+ immediate cutaneous reactions upon prick skin testing with salmon extract (1:10 w/v, Hollister-Stier, Spokane, WA). Additional sera were obtained from a patient who survived a life-threatening immediate reaction manifested by hypotension and wheezing following a 50-mg protamine infusion.

This third patient had no known food hypersensitivity. Sera from three nonatopic subjects were used for control samples.

Detection of Serum IgE to Salmon. We established an indirect amplified double antibody ELISA system to detect serum IgE to salmon. Immulon microELISA plate wells (Greiner & Sons, Nürtingen, West Germany) were incubated overnight at 4° C with salmon extract (Hollister-Stier, Lot #CO71838301) and diluted in carbonate buffer (pH, 9.6) at a final concentration of 100 µg/ml. The wells were then washed for 3 minutes, three times with phosphate-buffered saline 0.5% Tween 20 (PBS-Tween) (Sigma Chemical Co., St. Louis, MO). This washing procedure was done after each step in this ELISA method except after the substrate addition step. Also, each microtiter well was filled with 0.2 ml of the appropriate reagents or serum dilutions at each step. All incubations were at 37° C unless otherwise stated. Serum dilutions (1:2, 1:5, and 1:20) made in PBS-Tween were added and incubated for 30 minutes. Then, affinity chromatography-purified rabbit IgG anti-human IgE (Behring Diagnostics, LaJolla, CA) diluted 1:1000 in PBS-Tween was added and incubated 30 minutes. Next, goat anti-rabbit IgG conjugated to alkaline phosphatase (Sigma) and diluted 1:1000 in PBS-Tween was added and incubated for 30 minutes. Finally, the substrate *p*-nitrophenyl phosphate (Sigma) at a 1-mg/ml concentration in diethanolamine buffer (pH, 9.8) was added and incubated for approximately 55 minutes, at which time the optical density was read at 405 nm on an Automated ELISA Reader (Artek Systems Corporation, Farmingdale, NY).

Inhibition experiments were similar to the salmon ELISA described above except inhibition dilutions (sera preincubated with increasing quantities of inhibitor) were used instead of regular serum dilutions. In test tubes, sera (kept constant at a 1:2 dilution) and increasing amounts of inhibitor (0, 500, and 1000 µg/ml of whole serum) were incubated for 30 minutes at 37° C prior to being transferred to microELISA wells that were previously sensitized with salmon. The plate was incubated for 30 minutes at 37° C. The inhibition ELISA was completed by proceeding with the rabbit IgG anti-human IgE step in the salmon ELISA method described above. The inhibitors used were salmon extract (Hollister-Stier), protamine sulfate (Quad Pharmaceuticals, Indianapolis, IN, Lot #605F178B) and *Aspergillus fumigatus* (a gift from V. Kurup, Milwaukee, WI). An ELISA method to detect serum IgE to protamine sulfate was carried out according to a method described previously,² with varying protamine concentrations (20 µg/ml, 50 µg/ml, and 150 µg/ml). A second method to detect IgE to protamine sulfate was tried using an initial protamine-coating concentration of 100 µg/ml and serum dilutions of 1:2, 1:10, 1:100, and 1:1000. The double

antibody reagents were identical to the salmon ELISA method described in this article.

Results

Figure 1 illustrates ELISA results for detection of serum IgE to salmon. Sera from both subjects who were anaphylactically sensitive to salmon (#1 and #2) demonstrated elevated optical densities as expected. Neither sera from three nonatopic subjects nor the patient (#3) who experienced the severe immediate reaction to protamine had detectable IgE to salmon in these experiments. Inhibition experiments with salmon, protamine, and an unrelated antigen, *A. fumigatus*, are presented in Figure 2. Increasing quantities of salmon extract preincubated with sera in the ELISA experiments inhibited binding of IgE to salmon. However, neither protamine sulfate nor *A. fumigatus* in doses to 1 mg/ml inhibited IgE binding to salmon. We were unable to detect serum IgE to protamine using the sample from the patient who experienced a severe immediate type-generalized reaction to protamine.

Discussion

The current experiments do not support the notion that there is immunologic cross-reactivity between IgE to salmon and protamine sulfate. Although it is theoretically possible that subjects who are hypersensitive to fish are at increased risk of protamine-induced immediate generalized reactions, such an hypothesis has never been verified. Studies of a patient who was reported to be allergic to codfish, mackerel, and herring and who experienced a life-threatening reaction to protamine revealed a total serum IgE of 1145 IU/ml.¹² A radioallergosorbent test for codfish was described as positive, and a titer of 1:1024 for serum IgE to protamine (methods not described) was reported.¹² Caplan and Beckman¹³ noted that four of 140 healthy leukopheresis donors who received protamine sulfate experienced immediate type reactions. Urticarial reactions developed in two patients and angioedema occurred in a third. The fourth donor was described as having "occipital pain and numbness on two occasions." Two of the four donors and an un-stated number of the nonreactors were said to be allergic to fish.¹³ The authors proposed that contaminating salmon or other fish proteins present in protamine solutions could induce immediate reactions in fish-allergic subjects. The present experiments do not support such a hypothesis in the patients studied who were known to be allergic to salmon.

In 1949, protamine was considered nonimmunogenic in that studies did not produce immediate cutaneous reactivity or precipitating antibodies.¹⁷ Nevertheless, some infertile and vasectomized men have complement-fixing antibodies to human protamine.¹⁸ In a case of a fatal immediate type reaction following

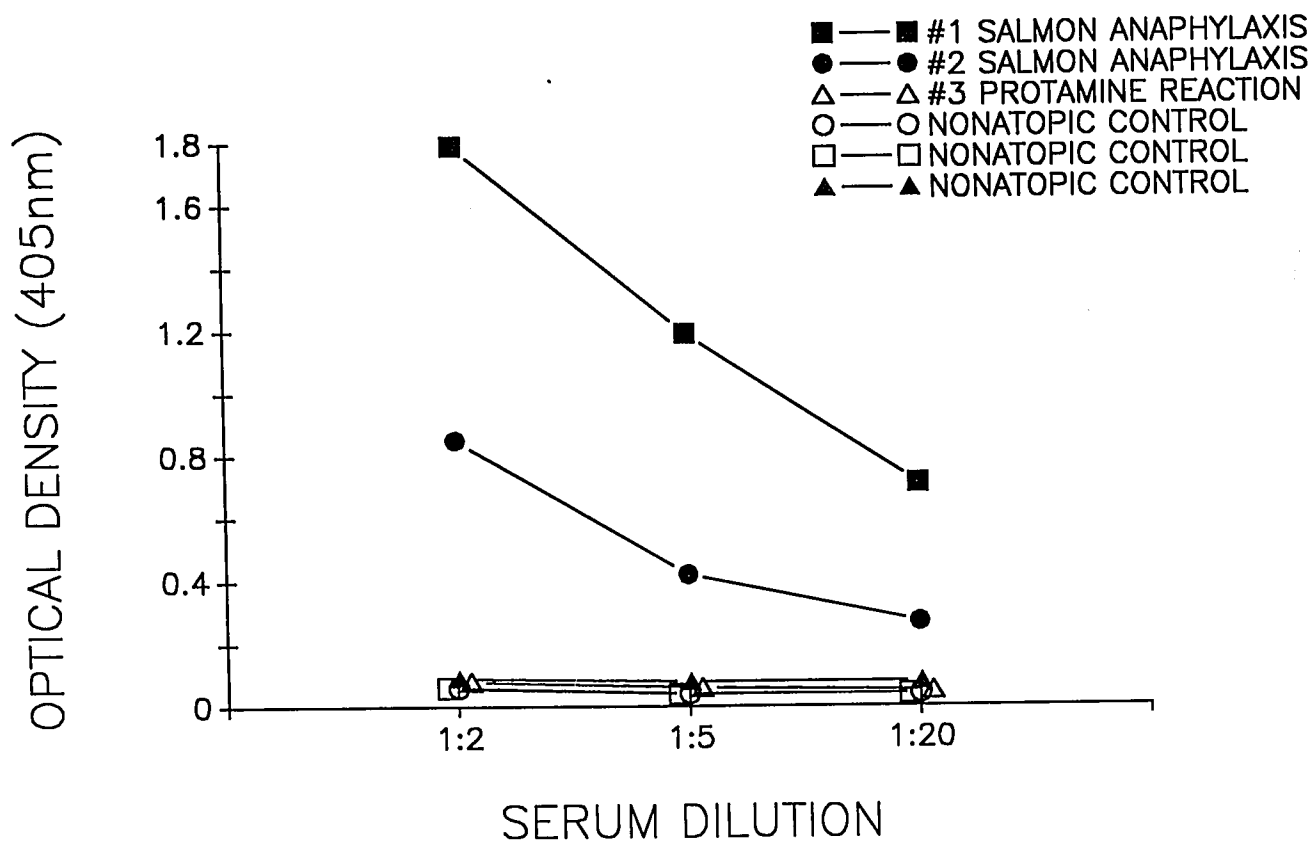


Figure 1. Serum IgE to salmon is measured by an indirect amplified ELISA.

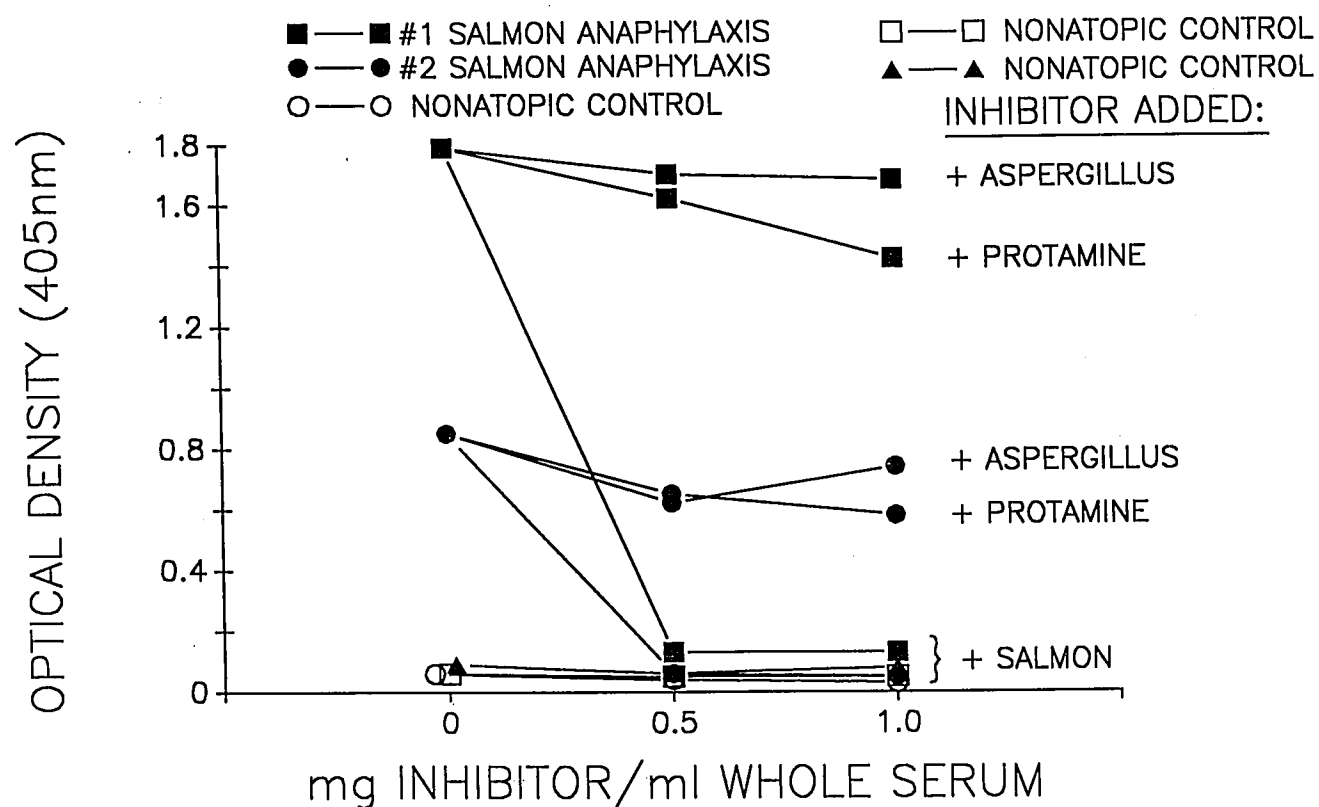


Figure 2. Inhibition experiments using salmon, protamine, and *Aspergillus fumigatus* to define specificity of anti-salmon IgE. Preincubation of sera (1:2) with salmon, but not protamine or *A. fumigatus*, resulted in inhibition of IgE binding to salmon.

infusion of protamine, complement levels were normal.² Although activation of the classical complement pathway has been noted during immediate type reactions to protamine,^{19,20} the effects of cardiopulmonary bypass itself are important.²¹ When measurements of C₃ and C₄ were undertaken on a serial basis prior to bypass, after bypass, during cold potassium cardioplegia, and after a slow infusion of protamine, evidence for complement activation occurred during bypass but not during cardioplegia or protamine infusion.²¹ Future investigations using assays of complement activation products¹⁹ should help resolve the issue of whether protamine or cardiopulmonary bypass itself can activate complement *in vivo*.

Serum IgE antibodies to protamine have been detected by an amplified ELISA in 10 of 19 patients with diabetes mellitus who used insulin containing protamine and in sera from a patient who experienced fatal protamine anaphylaxis.² A retrospective,¹⁶ but not a prospective,²² study has suggested an increased risk of protamine reactions in patients who use NPH insulin. Lakin et al²³ reported the occurrence of a skin-sensitizing IgG antibody in a patient who experienced urticaria, wheezing, cyanosis, and hypotension after receiving 6.5 mg of protamine sulfate. Immediate cutaneous reactivity during prick skin testing was present at a concentration of 1.0 ng/ml, whereas three of four normal control subjects displayed just 1-2+ prick reactions at 1 mg/ml of protamine sulfate.²³ Thus, data suggest that protamine sulfate can induce immune responses that have been associated with isolated cases of life-threatening or fatal immediate generalized reactions.

Additional proposed mechanisms for immediate generalized protamine reactions include the following: 1) direct mast cell activation by polycations⁶⁻⁸; 2) classical complement pathway activation via interaction of protamine with C-reactive protein,¹⁰ a potent stimulus for C1 activation, or by heparin-protamine immune complexes⁹; and 3) idiosyncratic, perhaps analogous to radiocontrast material or iron dextran. It appears that although *in vitro* complement activation can be demonstrated with protamine sulfate, *in vivo* complement activation has not yet been proven as an explanation for the immediate generalized reactions that may be life-threatening or fatal.

In summary, the mechanism(s) for protamine-associated immediate generalized reactions is unknown except for a limited number of cases. The literature suggests that prior exposure to protamine may well predispose certain subjects to subsequent immediate reactions. The current experiments do not support the notion that there is cross-reactivity between IgE antibodies to salmon and protamine sulfate. We cannot exclude the possibility that IgE antibodies to

salmon react with different salmon proteins in different persons; however, in the two cases studied, protamine sulfate did not inhibit IgE binding to salmon. Further, sera from the patient who was not allergic to fish and who experienced the severe protamine reaction did not demonstrate increased binding to protamine or salmon.

Some suggestions as to management of the patient who potentially is at increased risk of a protamine-induced immediate type reaction have been published.²⁴ Obviously, the literature is limited by lack of estimates of the incidence of protamine reactions in various populations. Such studies are unlikely in light of the apparent rarity of serious reactions. A history of "seafood allergy" alone has not been associated convincingly with increased risk of immediate reactions to protamine sulfate. Should patients with salmon hypersensitivity be considered at increased risk for reactions to protamine sulfate? The current experimental data do not support immunologic cross-reactivity. We performed prick skin tests with protamine sulfate (10 mg/ml and 1 mg/ml) in three patients who were allergic or intolerant to seafood other than salmon, and no patient demonstrated immediate cutaneous reactivity. All of the patients tolerated protamine without reaction. Each patient had been pretreated with corticosteroids and antihistamines analogous to radiocontrast media prophylaxis.²⁵ If a salmon-allergic patient absolutely requires protamine, such an approach would appear logical based on the doubtfulness of immunologic cross-reactivity between IgE antibodies to salmon and protamine sulfate. Incremental test-dosing²⁴ with protamine also would be recommended if its use is essential. Should patients with a past history of using NPH insulin be considered at increased risk if exposed to protamine sulfate? Although it has been reported that such diabetic subjects synthesize IgE to protamine sulfate *in vitro*,² a prospective study did not define an increased risk of protamine reactions in such subjects.²² These findings emphasize the limitations of some *in vitro* assays in that clinical correlation is necessary to confirm a proposed effect or mechanism for immediate generalized reactions.

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