

Proteins: Chymopapain and insulin

Leslie C. Grammer, M.D., and Roy Patterson, M.D. Chicago, Ill.

We studied clinical and immunologic aspects of the reactions to two newly introduced drugs, chymopapain and human recombinant deoxyribonucleic acid insulin (HI), in patients demonstrating allergies to one of these two drugs. We then used this information to improve our ability to diagnose and prevent chymopapain allergy and to further our understanding of systemic insulin allergy and its management. Of the patients who were sensitive to chymopapain, one had severe anaphylaxis to intradisc injection while the other had rhinitis, asthma, and urticaria with occupational exposure. The latter demonstrated cutaneous reactivity to papain; the former refused skin testing. Both demonstrated immunoglobulin (Ig) E and IgG to chymopapain as measured by enzyme-linked immunosorbent assay. We have prospectively skin tested 61 patients with chymopapain. Sixty-one patients have had negative skin tests and have tolerated the intradisc injection of chymopapain without incident. We are continuing our prospective skin test study in order to identify a population at risk for allergy to chymopapain. Two patients with systemic allergic reactions to animal insulin have at least as much cutaneous reactivity and IgE and IgG antibodies to HI as to porcine insulin. A large local reaction occurred during an attempt to desensitize one of them to HI; the patient was subsequently desensitized without difficulty to porcine insulin, to which she was less skin reactive. We conclude that HI will not eliminate insulin allergy in patients with systemic allergy to animal insulin and that such patients will continue to require the usual therapeutic measures for insulin allergy. (J ALLERGY CLIN IMMUNOL 74:635, 1984.)

The immunology of proteins has been extensively studied and forms the basis for our approaches to the clinical problems of allergies to chymopapain and insulin. It is well known that foreign proteins are potent immunogens. The specificity of an antigenic site on a protein may result from a primary amino acid sequence or it may derive from a secondary conformational structure.¹ Proteins are complete antigens and thus can be used for cutaneous testing or in vitro assays without need for haptization. With regard to cutaneous testing, it should be noted that proteins possessing proteolytic enzyme activity such as chymopapain must be used cautiously in appropriate dilutions.

The first clinical problem we addressed was that of anaphylaxis to chymopapain, recently licensed for intradisc injection in patients with documented herniated

Abbreviations used

HI:	Human recombinant deoxyribonucleic acid insulin
PBS:	Phosphate-buffered saline
ELISA:	Enzyme-linked immunosorbent assay
Ig:	Immunoglobulin
OD:	Optical density

lumbar intervertebral discs who are unresponsive to conservative therapy.² In initial trials the overall incidence of anaphylaxis was 1%, while the incidence of anaphylaxis resulting in death was 0.14%.² The anaphylactic problem will probably be of some magnitude, as estimates of the number of patients likely to seek chymopapain injection are upward of 100,000 annually.³ The current package insert recommendations for prophylaxis are not clearly protective, as is demonstrated by our index case. Thus we wished to develop a cutaneous test or an in vitro assay to identify the population of patients at risk for chymopapain allergy. We suspect that cutaneous tests would probably be preferable because of their ready availability and ease of administration by physician or nurse. In

From the Section of Allergy-Immunology, Department of Medicine, Northwestern University Medical School, Chicago, Ill.

Supported by United States Public Health Service Allergy Diseases Center Grant No. AI 11403 and Ernest S. Bazley Grant.

Reprint requests: Leslie C. Grammer, M.D., Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611.

TABLE I. Insulin cutaneous test results

Patient	Insulin		
	Bovine	Porcine	Human (recombinant DNA)
<i>Prick test results*</i>			
X	4+	0	0
Y	3+	0	0
<i>Intradermal positive endpoint†</i>			
X	10^{-7}	10^{-10}	10^{-5}
Y	10^{-9}	10^{-5}	10^{-8}

DNA = Deoxyribonucleic acid.

*Prick tests performed with stock solution of 100 U/ml.

†Most dilute solution to result in 5 mm wheal, 10 mm flare; stock solution is 100 U/ml.

vitro tests require more time and good positive and negative control serum samples, but have the advantage that there is no risk associated with the diagnostic procedure.

Another clinical problem involving allergy to a protein drug is that of the diabetic who has anaphylaxis to animal insulins. With the introduction of HI,⁴ we wished to ascertain what role it would play in the treatment of diabetics with systemic allergic reactions to animal insulins. Specifically, we wondered if such patients could be switched to HI with no possibility of allergic reactions.

MATERIAL AND METHODS

Chymopapain

Case reports. Patient A, a 25-year-old white woman, had been working in the purification of chymopapain from the crude papain fraction.^{5,6} She developed asthma and rhinitis on inhalation of papain or chymopapain. Wearing a mask and gloves ameliorated but did not eliminate the symptoms. She had a 4+ reaction to a prick test of papain (Sigma Chemical Co., St. Louis, Mo.) at 1 µg/ml.

Patient B, a 59-year-old white man, had sciatica and a myelogram demonstrating a herniated nucleus pulposus at L4-5 and compression of L5 and S1. When he was admitted for chemonucleolysis, he was pretreated according to the recommendations of the package insert: cimetidine, 300 mg every 6 hours for four doses, and diphenhydramine (Benadryl), 50 mg every 6 hours for four doses. The patient complained of severe dyspnea within 30 minutes of the chemonucleolysis procedure. Poor air movement and wheezing were noted on physical examination. The patient received several doses of epinephrine before improvement occurred.

Cutaneous testing. Three nonatopic controls and 61 patients scheduled for intradisc chymopapain injection were tested by the prick method with chymopapain (Smith Laboratories Inc., Rosemont, Ill.) in the following concentrations: 10 µg/ml, 1 µg/ml, 100 ng/ml, 10 ng/ml, and 1 ng/ml. A negative control, PBS in 0.4% phenol, and a positive control, 1 mg/ml histamine, were also tested.

ELISA. The ELISA procedure was done according to methods previously described.^{7,8} Briefly, Immulon microELISA plates (Greiner und Sohne, Nurtigen, West Germany) were coated with chymopapain (0.4 mg/ml), 200 µl/well, in carbonate buffer at pH 9.6 and were incubated overnight at 4° C. Plates were then washed three times with PBS-Tween (0.05%; Sigma Chemical Co.). Samples (200 µl) of dilutions of the patient's serum were added to individual wells and plates were incubated for 1 hour at 37° C. Plates were washed three times with PBS-Tween and 200 µl of a predetermined dilution of rabbit antihuman IgG or IgE (Calbiochem-Behring, La Jolla, Calif.) was added to each well. Plates were then incubated at 37° C for 1 hour. After the plates were washed again, 200 µl of alkaline phosphatase-conjugated goat antirabbit gamma globulin (Sigma Chemical Co.) was added to each well in a predetermined optimal concentration and the plates were incubated 1½ hours at 37° C. A final washing was done to remove the unbound conjugate and the enzyme substrate, *p*-nitrophenyl phosphate (Sigma 104 phosphate, 1 mg/ml) in 10% diethanolamine buffer at pH 9.8 was added and incubation was carried out for 30 minutes at room temperature for IgG antibodies and 60 minutes for IgE antibodies. The enzyme reaction was stopped with 50 µl 3M NaOH and ODs of each well were then read at 410 nm on a Dynatech microELISA Mini Reader MR 590 (Dynatech, Santa Monica, Calif.). ELISA titer endpoints were read as the last dilution at which the test antiserum had an OD reading at least twice that of the control serum.

Insulin

Case reports. Patient X, a 73-year-old woman, had fasting hyperglycemia in spite of diet and oral hypoglycemic therapy. In 1973 the patient developed generalized urticaria and angioedema with mixed bovine-porcine insulin injections. Two attempts at desensitization had failed.

The patient was tested by the prick method to pure bovine, porcine, and human insulins at 100 U/ml. Skin test results were 4+ to bovine but she showed no reaction to porcine or human insulin. We used a previously published desensitization schedule⁹ in an attempt to desensitize her to HI. However, on subcutaneous injection of 0.01 U HI, she

developed a local 15 mm wheal/35 mm flare reaction. We then performed cutaneous endpoint titration and found that the patient was more reactive to human than to porcine insulin. She was desensitized to porcine insulin without difficulty.

Patient Y, a 26-year-old woman, was 8 weeks pregnant with her second child. During her first pregnancy, she had gestational diabetes, which was treated with insulin. Her physician had reinstituted 20 U insulin the week before we saw her at our clinic. She was transferred to our medical center because she developed urticaria, angioedema, and chest tightness with each insulin injection. On endpoint intradermal titration, she was equally reactive to porcine and human insulin. As HI was not commercially available at the time, she was treated with porcine insulin. Her insulin dose was reduced to 5 U and was increased by 2 U morning and evening until control of diabetes was achieved.

Cutaneous testing. Patients were tested by the prick method with various insulins (porcine, beef, and HI) at a concentration of 100 U/ml. Skin test results were graded 0 to 4.¹⁰ Patients were also tested by the intradermal method with serial tenfold dilutions of the above insulins beginning with a concentration of 0.001 U/ml. A positive endpoint was defined as the most dilute solution producing a 5 mm wheal and 10 mm flare.

ELISA. The insulin ELISA procedure was identical to that outlined above for chymopapain, except that the microELISA plates were coated with various pure, regular bovine or porcine insulins (100 µg/ml; Eli Lilly, Indianapolis, Ind.) or HI rather than chymopapain.

RESULTS

Chymopapain

Cutaneous testing. None of the nonatopic controls and none of the 61 patients scheduled for chemonucleolysis had positive skin tests to chymopapain. All subjects had negative reactions to the diluent control and positive reactions to the histamine control. All patients tolerated chemonucleolysis without anaphylaxis.

ELISA. Fig. 1, *a*, demonstrates that patient B has IgE against chymopapain. The titer endpoint was 4096 in patient B, who had anaphylaxis. This patient also demonstrates IgG against chymopapain (Fig. 1, *b*). His titer endpoint was 20,480.

Insulin

Cutaneous testing. Results of cutaneous testing of patients X and Y are shown in Table I. Each patient was most reactive to bovine insulin. Patient Y was equally reactive to porcine insulin and HI, while patient X was more skin reactive to HI than to porcine insulin.

ELISA. As is evident from Fig. 2, *a*, both patients X and Y have IgE directed against each of the insulins. The titer endpoint for patient X is 10 for bovine and

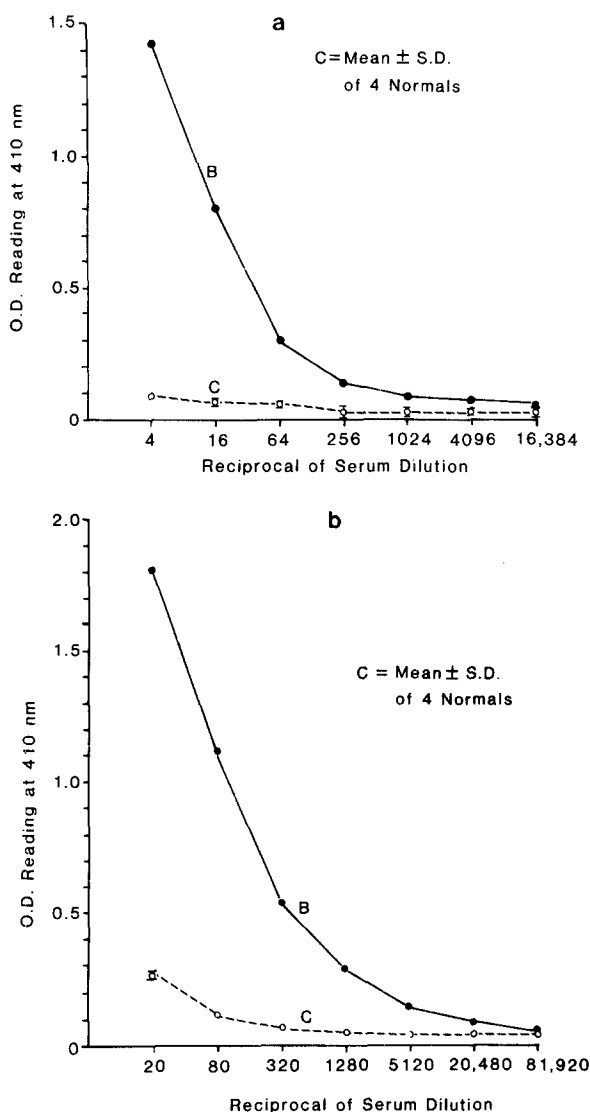


FIG. 1. Results of antibodies against chymopapain as determined by ELISA in patient B (●) and in four nonatopic controls (○). In both panels the *abscissa* is the serum titer and the *ordinate* is the optical density. *a*, IgE against chymopapain; *b*, IgG against chymopapain.

porcine insulin, while it is 50 for human insulin. The endpoint for patient Y is 100 for each insulin. Both patients also demonstrate IgG against each of the insulins. Each patient's titer endpoint to bovine insulin was 500, while endpoint titers to the other two insulins were 1000.

DISCUSSION

Because large numbers of patients are expected to seek chemonucleolysis, it is essential to prevent anaphylaxis and attendant morbidity and death in these patients. Risk in patients should be predictable by cutaneous testing, in vitro assays, or both, just as

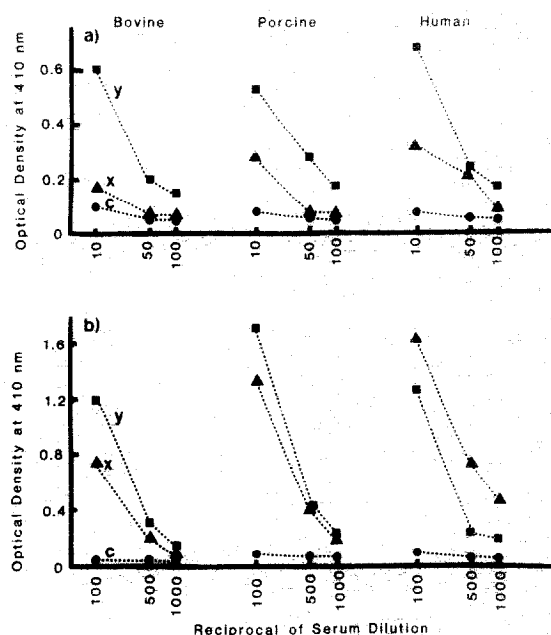


FIG. 2 Results of antibodies against insulin (bovine, porcine, HI) as determined by ELISA in patient X (▲), patient Y (■), and in two nonatopic controls (C, ●). In both panels the *abscissa* is the serum titer and the *ordinate* is the optical density. **a**, IgE against insulin; **b**, IgG against insulin.

patients who are at risk for anaphylaxis from other proteins like foreign serum can be identified by immediate skin test reactivity.¹¹ Pretreatment with cimetidine and diphenhydramine, as suggested in the package insert, did not prevent severe anaphylaxis in patient B. Thus this pretreatment regimen alone is not sufficient prophylaxis.

Cutaneous tests have several advantages over *in vitro* assays. First, physicians or nurses can learn with very limited training to perform and interpret skin tests. Second, the skin tests can be read within 15 or 20 minutes, so there is little delay. Finally, the skin test reagents are readily available and cost less than *in vitro* assays. Chymopapain is unsuitable for intradermal testing because it is a proteolytic enzyme. Consequently, our protocol requires prick testing. We are currently using only two concentrations, 1 and 10 mg/ml, as we no longer believe it is necessary to use the more dilute solutions. If no IgE against chymopapain is demonstrable by skin test, it is unlikely that the patient could have anaphylaxis. On the other hand, if the patient has positive skin tests to chymopapain, the patient would be at risk for anaphylaxis.

A prospective study will be required to confirm the value of skin testing and *in vitro* assays for preventing chymopapain anaphylaxis. We are aware of studies

of this type that have been initiated at the following universities: Cincinnati, Medical College of Wisconsin, Pennsylvania, Scripps Clinic, and Tulane. During these studies the dilemma of what to do with a patient with a positive skin test who is also surgically at a high risk will likely arise. It is unclear as to whether the presence of IgE antibody to chymopapain is an absolute contraindication to its use or whether it would be preferable to use a pretreatment regimen such as the one that has reduced the incidence of anaphylactic reactions to radiocontrast media.¹² Current knowledge and future required assessments in the area of chymopapain anaphylaxis are outlined in Table II.

Systemic insulin allergy is a problem that has been well described for many years.^{9, 13-15} This problem is one of low incidence but it may result in such serious consequences as uncontrolled diabetes or anaphylaxis if it is not properly managed. Principles of management have been outlined in detail elsewhere.⁹ In brief, if a patient has systemic insulin allergy, it must be determined whether insulin administration is essential. Once that has been determined, the patient should be hospitalized and the approach to insulin administration depends on whether or not the patient has received insulin within 24 hours. If the patient has received insulin within 24 hours, the insulin dose can simply be reduced to one third or, for extreme caution, to one fifth of the dose that caused anaphylaxis. The dose can then be increased by 2 to 5 U per day until control of diabetes is achieved. If the patient has not received insulin for several days, desensitization from miniscule doses is required. The least allergenic insulin for a given patient can be chosen on the basis of intradermal cutaneous endpoint titration to various insulins. It should be noted that because of the availability of skin testing and delay of results from *in vitro* analyses, the latter play no role in the clinical management of patients. *In vitro* analyses are valuable only as research tools.

The introduction of HI has been associated with speculation that this would be the drug of choice for diabetics who have systemic insulin allergy.⁴ Several studies have demonstrated that HI is less immunogenic than animal insulins.^{16, 17} Moreover, there are reports of its successful use in patients with systemic allergy to animal insulins.¹⁸ However, there are patients like ours who have more cutaneous reactivity to HI than to porcine insulin and who consequently may have serious allergic reactions to HI. Thus HI cannot be substituted at therapeutic doses in patients with systemic allergy to animal insulins.

We previously described patients with cutaneous

TABLE II. Summary of current status

Insulin allergy		Chymopapain allergy
Mechanism	IgE	IgE
Risk	Anaphylaxis	Anaphylaxis
Frequency	Rare	1%; serious concern; not prevented by cimetidine/diphenhydramine pretreatment suggested in package insert
Value of skin test	Titration shows comparative sensitivity to animal and HI and which should be used for desensitization	Not enough data yet; very likely predictive; requires limited training and experience to do and interpret (nurse can do)
Value of in vitro test	Clinical: none; delay in therapy; experimental value only	Probably will be predictive, but not enough data yet; delay of diagnosis; more expensive than nurse doing skin testing in office
Future assessment required	What is incidence of people who react to animal insulin who are sensitive to HI to similar degree? What is incidence of systemic insulin allergy in patients initially treated with HI?	Need prospective study of skin test and serology; this would best be a collaborative study at several centers. What should be done in patients with positive skin test who are at high risk for surgery? Absolute contraindication or prophylaxis?

reactivity to synthetic human insulin and to human insulin isolated from human pancreas.¹⁹ This paradox of people being allergic to human insulin is explained by the fact that the specificity of an antigenic site on a protein may result from the primary amino acid sequence or from a secondary or tertiary conformational structure.¹ Although the primary amino acid sequence of HI is the same as that of endogenous human insulin, the processing of HI appears to induce conformation changes that render the protein immunogenic.

As the use of HI becomes more widespread, it will be important to determine whether patients who are started on HI rather than on animal insulins will become allergic to HI. As more experience is acquired with HI in patients with systemic allergy to animal insulin, it will be possible to determine the incidence of allergy to HI in those patients. Table II presents an outline of the current knowledge about patients with systemic allergy to animal insulins.

In summary, the basic science of protein immunology has formed the foundation for the approach to clinical problems of anaphylactic reactions to two protein drugs—chymopapain and insulin. We are developing methods to identify the population at risk for chymopapain anaphylaxis and thus prevent anaphylactic deaths from chymopapain. We have also developed an approach to management of patients with systemic allergic reactions to animal insulins and are ascertaining the role of HI in the management of those patients.

REFERENCES

1. Goodman JW: Immunogenicity and antigenic specificity. In Fudenberg HA, editor: Basic and clinical immunology, ed 3. Los Altos, 1980, Lange Medical Publications, p 44
2. Willis, J, editor: Chymopapain approval. FDA Drug Bull 12:17, 1982
3. Lancaster H: Struggle for approval of back drug shows frustrations of FDA review. Wall Street J, April 1983, p 27
4. Hennessy J: More studies show human insulin's benefit. JAMA 249:328, 1983 (medical news)
5. Drenth J, Jansonius JN, Keokoek R, Swen HM, Wolthers BG: Structure of papain. Nature 218:929, 1968
6. Novey HS, Marchioli LE, Sokol WN, Wells ID: Papain-induced asthma—physiological and immunological features. J ALLERGY CLIN IMMUNOL 63:98, 1979
7. Voller A, Bidwell DE, Bartlett A: Enzyme immunoassays in diagnostic medicine: therapy and practice. Bull WHO 53:55, 1976
8. Sepulveda R, Longbottom JL, Pepys J: Enzyme linked immunosorbent assay (ELISA) for IgG and IgE antibodies to protein and polysaccharide antigens of *Aspergillus fumigatus*. Clin Allergy 9:359, 1979
9. Grammer LC, Chen PY, Patterson R: Evaluation and management of insulin allergy. J ALLERGY CLIN IMMUNOL 71:250, 1983
10. Booth BH: Diagnosis of immediate hypersensitivity. In Patterson R, editor: Allergic diseases. Philadelphia, 1980, JB Lippincott, p 88
11. DeSwarte RD: Drug allergy. In Patterson R, editor: Allergic diseases. Philadelphia, 1980, JB Lippincott, p 568
12. Greenberger PA, Patterson R, Simon R, Lieberman P, Wallace W: Pretreatment of high risk patients requiring radiocontrast media studies. J ALLERGY CLIN IMMUNOL 67:185, 1981
13. Mattson JR, Patterson R, Roberts M: Insulin therapy in patients with systemic insulin allergy. Arch Intern Med 135:818, 1975
14. Reaves ML, Galloway JA, deShazo RD: Complications of

- insulin therapy and their treatment. *In* Skyler JS, editor: Insulin update. Amsterdam, 1982, Excerpta Medica, pp 295-306
15. Galloway JA, Bressler R: Insulin treatment in diabetes. *Med Clin North Am* 62:663, 1978
 16. Fineberg SE, Galloway JA, Fineberg NS, Rathburn MJ: Immunologic improvement resulting from the transfer of animal insulin treated subjects to human insulin (recombinant DNA). *Diabetes Care* 5:107, 1982
 17. Fireman P, Fineberg SE, Galloway JA: Development of IgE antibodies to human (recombinant DNA), porcine, and bovine insulins in diabetic subjects. *Diabetes Care* 5:119, 1982
 18. Falholt K: Binding capacity of insulin-specific IgE antibodies. *In* Proceedings of the Eleventh Congress of the International Diabetes Federation. Amsterdam/Oxford/Princeton, 1982, Excerpta Medica, vol 11, p 58 (abstr)
 19. Patterson R, Lucena G, Metz R, Roberts M: Reaginic antibody against insulin: demonstration of antigenic distinction between native and extracted insulin. *J Immunol* 103:1061, 1969