

Mechanism of allergy to components of commercial bovine thyrotropin

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This report describes a systemic immediate-type allergic reaction to commercial thyrotropin in a patient with hypothyroidism and chronic candidiasis. Immunologic studies of the patient's serum revealed precipitating antibodies against bovine albumin and gamma globulin, and the thyrotropin preparation was found to be contaminated with these proteins. Skin testing with purified bovine albumin and commercial thyrotropin produced wheal-and-flare reactions indicating the presence of reaginic antibodies but did not evoke Arthus or delayed-type responses. The patient's serum contained elevated concentrations of thyrotropin consistent with the diagnosis of primary hypothyroidism, but it did not inhibit the biologic activities of human or bovine thyrotropins. Although antibodies against bovine proteins are often found in human serum, they are only rarely associated with disease. In our patient a systemic allergic reaction was apparently the result of the interaction between anti-albumin antibodies and the albumin contaminating the TSH. The studies of the effects of the patient's serum on the biologic activities of bovine or human thyrotropins did not provide evidence for an anti-TSH that could be implicated in the pathogenesis of hypothyroidism.

Sera from apparently normal people often contain antibodies against bovine serum and milk proteins.¹ With certain exceptions,²⁻⁷ these antibodies have not been associated with clinical disorders and have been considered natural, benign consequences of ingestion of beef products.

This report concerns a young woman with hypothyroidism and chronic mucocutaneous candidiasis who experienced a systemic immediate-type allergic reaction following injection of commercial TSH. The investigations were directed at two questions: (1) which component of the thyrotropin preparation was responsible for the allergic reaction and (2) did her serum contain any factors that could inhibit the activity of TSH and thereby be implicated in the pathogenesis of hypothyroidism?

CASE HISTORY

The patient was a 24-year-old woman with chronic mucocutaneous candidiasis since the age of 3 weeks who was referred to the Clinical Center to participate in a study of this disorder.⁸ During the 2 years prior to admission she had noted progressive lassitude, had

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TABLE I. Immunologic functions of the patient

Humoral responses	Patient	Normal values	Cellular responses Delayed skin tests	Cm. induration at 24 hr.
Serum IgG mg./ml.	5.1	6.0 - 16.0	Candida	Neg.
IgA mg./ml.	0.82	0.6 - 2.0	Varidase	Neg.
IgM mg./ml.	0.83	0.5 - 1.5	Mumps	Neg.
IgE ng./ml.	182	5 - 2,045	PPD	Neg.
Parotid fluid			DNCB (2 wk. postsensitization)	Neg.
IgA mg./mg. protein	30.3	4.3 - 38.0		
Immediate-type cutaneous reactions			In vitro lymphocyte responses	
Candida	4+		Lymphocyte transformation stimulation ratio*	
BSA	3+			Skin test pos.
BGG	neg.		Patient	Controls
Isohemagglutinins			Candida	1.0 9.0 - 30.7
Anti-A	1:32	1:8 - 1:32	Varidase	1.1 23.5 - 62.0
Anti-B	1:32	1:4 - 1:32	PHA	50.0 94 - 744
Candida agglutinins	1:512	1:4 - 1:128	MIF production	
			Per cent inhibition	Skin test pos.
			Patient	Controls
			Candida	0 > 20%
			Varidase	0 > 20%

*Stimulation ratio = mean DPM of antigen-stimulated cultures divided by mean DPM of unstimulated cultures.

Special abbreviations used

TSH	=	thyroid-stimulating hormone
BSA	=	bovine serum albumin
BGG	=	bovine gamma globulin
LATS	=	long-acting thyroid stimulator
PPD	=	purified protein derivative
DNCB	=	dinitrochlorobenzene
DPM	=	disintegrations per minute
PHA	=	phytohemagglutinin
MIF	=	migration inhibition factor

gained 14 pounds, and had developed a generalized "bloated" or "puffy" sensation. The skin had become drier, but she had noted no changes in her hair. There were no symptoms of malabsorption and no past history of allergy. The patient experienced no adverse reactions after ingestion of beef or milk products. Physical examination disclosed a normally proportioned woman standing 145 cm. tall (her parents were also of short stature). Her face was puffy with nonpitting periorbital edema. The skin was dry, but the hair was normal. The lateral eyebrows were absent. The relaxation phase of the deep tendon reflexes was prolonged. Candidiasis was present in the nails, skin, and oral and vaginal mucous membranes.

The routine laboratory studies were unremarkable, and tests of intestinal absorption were normal. The results of the immunologic evaluation are summarized in Table I. The methods for obtaining these data have recently been described in detail.^{8,9} The studies disclosed essentially normal humoral immune responses and a marked, generalized defect in functions of the cellular immune system. No antibodies against thyroid tissue or thyroglobulin were detected in her serum.

The protein bound iodine was 2.2 μ g per 100 ml. (normal value 3.5 to 8.0), the T_4 (by column) 0.7 μ g per 100 ml. (normal value 3.0 to 7.0), and the T_3 binding was 23 per cent (normal value 25 to 35 per cent). Twenty-four hours after administration of ^{131} I, less than

1 per cent of the radioactivity was over the thyroid gland. Function of the other endocrine glands was normal.

Approximately 20 minutes following intramuscular administration of 10 units of bovine thyrotropin,* the patient complained of severe pain at the injection site and a generalized sensation of warmth. She became agitated and apprehensive. There were no respiratory symptoms. The skin was diffusely flushed, and there was marked diaphoresis but no urticaria. The injection site showed induration and 8 cm. of erythema. The vital signs were normal. All symptoms subsided in 30 to 40 minutes and did not recur. The thyroid uptake of ^{131}I following thyrotropin remained less than 1 per cent.

MATERIALS AND METHODS

Highly purified BSA and BGG were obtained from commercial sources. The purity of the proteins was confirmed by immunoelectrophoresis in agar gels and diffusion against rabbit antisera to whole bovine serum. In addition to the commercial TSH, lyophilized, partially purified bovine and human thyrotropins were provided by Dr. Robert Bates of the National Institute of Arthritis and Metabolic Diseases. These materials were dissolved in phosphate-buffered saline (pH 7.4) and sterilized by millipore filtration. Rabbit antisera to BSA and BGG were gifts from Dr. Ira Green of the National Institute of Allergy and Infectious Diseases.

Immunologic techniques

Serum samples that were collected prior to and for 3 weeks subsequent to the injection of thyrotropin were examined for precipitins against BSA, BGG, and bovine and human TSH by double diffusion in agar gels.¹⁰ Control studies employed sera from 4 other patients with chronic mucocutaneous candidiasis and normal thyroid function, and 5 normal subjects.

The concentrations of BSA and BGG in the commercial bovine TSH were measured by the method of Mancini, Carbonara, and Heremans.¹¹

Skin tests were done with sterile solutions of bovine TSH (2 units or 350 μg BSA per milliliter), human TSH (100 μg per milliliter), BSA (250 μg per milliliter), and BGG (250 μg per milliliter). A 0.02 ml. quantity of the test solution was injected intradermally, and the cutaneous responses were observed at 15 minutes and at 6 and 24 hours.

Serum TSH assays

The serum TSH concentration was determined by both bioassay and radioimmunoassay methods. The bioassay was performed by the method of McKenzie,¹² which measures the per cent change in blood radioactivity 2 hours following intravenous injection of test substances into weanling mice. Four standard doses of bovine thyrotropin (Armour) were employed with the bioassay, and the concentration of TSH in the patient's serum was expressed as milliunits per milliliter as derived from the dose-response curve. At least 5 animals were injected with each test dose. Serum TSH was also measured by the double-antibody radioimmunoassay¹³ using anti-TSH serum that had been absorbed with an excess of human chorionic gonadotropin and was provided by the National Pituitary Agency Endocrinology Study Section. Human Thyrotropin Research Standard A from the Division of Biologics Standards was used as a standard.

Effect of serum on biologic activity of thyrotropin

The patient's serum collected prior to the allergic reaction or normal serum was incubated with either bovine TSH or partially purified human TSH at 37° C. for one hour, or at 4° C. for 16 hours.¹⁴ The incubation mixtures were then centrifuged and the supernatants bioassayed for TSH activity as described above and for activity of the LATS by measuring changes in blood radioactivity 8 hours after injection of the unknown or standards.¹⁴

*Thyropar, Armour Pharmaceutical Co., Chicago, Ill.

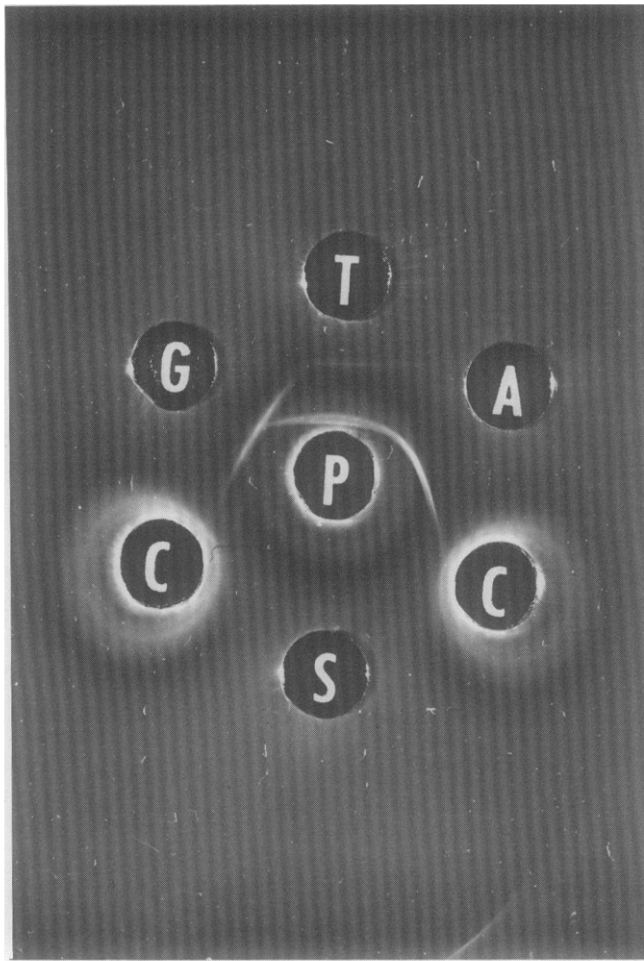


FIG. 1. Double-diffusion analysis in agar gel. **P**, Patient's serum collected prior to administration of TSH. **A**, Bovine albumin. **G**, Bovine gamma globulin. **T**, Commercial thyrotropin. **C**, Control serum. **S**, Saline. Note the lines of identity extending between the patient's serum and albumin and thyrotropin, and between the patient's serum and gamma globulin and thyrotropin.

RESULTS

By the double diffusion assay, precipitins were demonstrated between the patient's sera collected both before and after the allergic reaction and commercial thyrotropin, BSA and BGG (Fig. 1). The precipitins against the commercial TSH showed reactions of identity with both BSA and BGG. Control sera from other candidiasis patients and normal subjects did not produce precipitin lines against the bovine proteins or commercial TSH. By the quantitative immunodiffusion technique,¹¹ the commercial TSH contained 1,750 μg BSA and 22 μg BGG per milliliter.

Fifteen minutes after intradermal injection, bovine thyrotropin produced a

TABLE II. Effect of incubation with patient's or control serum on the biologic activity of standard thyrotropins

Hormone added	Biologic activity* following incubation with	
	Control serum	Patient serum
None	None detectable	0.4 $\pm$ 0.02
Bovine TSH	0.3 $\pm$ 0.05	0.65 $\pm$ 0.09
Human TSH	1.2 $\pm$ 0.3	1.4 $\pm$ 0.4

*Mean and S.E. expressed as milliunits per milliliter.

1.2 cm. diameter wheal with pseudopodia surrounded by a 3.5 cm. diameter flare. The 15 minute response to a BSA skin test showed a 1 cm. diameter wheal without pseudopodia and a 1.5 cm. flare. No immediate reactions occurred with BGG, human thyrotropin, or the diluent, and none of the antigens produced cutaneous responses at 6 or 24 hours. Furthermore, none of these test antigens evoked immediate or delayed-type responses following injection into 3 euthyroid, control subjects.

By the bioassay, the patient's serum contained 0.4 ± 0.02 mU. of TSH per milliliter, whereas the TSH concentration by radioimmunoassay was $0.29 \pm .02$ mU. per milliliter. Both of these values were significantly elevated when compared to euthyroid normal serum (0 to 0.01 mU. per milliliter by radioimmunoassay and not detectable by bioassay). As shown by the data in Table II, there was no evidence that the patient's serum had any neutralizing or enhancing effect on the biologic activities of either bovine or human thyrotropins. Furthermore, TSH preparations that had been incubated in the patient's serum did not show the LATS activity found in TSH-anti-TSH complexes.¹⁴

DISCUSSION

Although it is well known that sera from many normal children and patients with a variety of disorders contain antibodies to proteins of bovine milk and serum, it is rarely possible to assign pathogenic roles to these antibodies. In 1962, Heiner, Sears, and Kniker² reported a study of anti-milk antibodies in over 2,000 pediatric patients with a wide variety of respiratory disorders. Within this population they identified 8 patients with a clinical syndrome of chronic respiratory disease, pulmonary infiltrates, hemoptysis, iron-deficiency anemia, and retarded growth. In healthy children precipitins were uncommon. Subsequently, Hinkle, Hong, and West³ and Holland and associates⁴ reported 27 cases of a similar disorder, and in all but one of their cases the antibody activities were directed against bovine serum albumin and gamma globulin but not against α -lactalbumin or β -lactoglobulin. Although these observations were provocative, in no instance has a role for antibodies been established in the pathogenesis of the disorder.

It is known that release of the pharmacologic mediators of immediate-type allergy may occur through 2 immunologic pathways.¹⁵ One involves reaginic antibodies of the immunoglobulin class IgE. The presence of these antibodies may be shown by the wheal-and-flare responses following skin testing. The second mechanism involves formation of immune complexes, complement fixa-

tion, and formation of histamine-releasing substances (anaphylatoxins). In these cases, precipitating antibodies are frequently found in the serum.

Although pain at the injection site is a common consequence of the thyrotropin test,¹⁶ our patient also had symptoms suggesting an anaphylactoid reaction. The results of the immunologic studies were compatible with this diagnosis. Skin testing with bovine thyrotropin and purified bovine albumin produced wheal-and-flare responses and suggested that the offending antigen was the contaminating bovine albumin rather than bovine TSH. In this respect, our findings differed from those of Sherman and Werner,¹⁷ who observed a patient with an immediate-type allergic reaction to thyrotropin in which the inciting antigen was apparently bovine TSH itself rather than a contaminating protein. However, the sera from our patient also contained precipitating antibodies against bovine albumin and gamma globulin, a finding that raised the alternative possibility that the second mechanism of immunologic histamine release mediated through immune-complex formation could have occurred in our patient.

Endocrine failure occurs commonly in patients with chronic mucocutaneous candidiasis, and many of these patients have serum "autoantibodies" that react with endocrine tissues.^{8, 18, 19} While the pathologic processes producing the endocrinopathies have not been defined, it is clear that they are not the result of invasion of the organs by *Candida albicans*. It has been shown that repeated administration of bovine TSH to normal subjects stimulates production of antibodies against TSH, and these antibodies neutralize the effect of exogenous TSH on thyroid uptake of iodine.²⁰ The second part of this study was a search for a similar antibody against TSH in a patient with hypothyroidism of unknown etiology. No evidence for anti-TSH in the patient's serum was found, and the etiology of the thyroid failure remains unknown.

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