

Chapter 12

A Substance in the Plasma of Normal Humans which Interferes in a Radioimmunoassay for α -Lactalbumin

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INTRODUCTION

One problem frequently facing the radioimmunoassayist is to determine whether a substance which competes in the assay is identical to the antigen being measured. Some information can be gained by comparing slopes of inhibition curves but with low levels of inhibitor this is not always possible. Another approach is to compare different methods of radioimmunoassay (RIA). An important step in an RIA is the separation of free from bound label and comparison of assays using different methods of separation can sometimes give additional information about the product detected.

We have established two radioimmunoassays for human α -lactalbumin, a protein specific to milk. Both assays use lactalbumin labelled with ^{125}I and a rabbit antiserum. In the one assay, free is separated from bound label by precipitating the rabbit gamma globulins with a donkey antiserum (double-antibody RIA); in the alternative assay, the gamma globulins are precipitated using a 10% solution of polyethylene glycol (PEG assay).

If a sample of plasma or serum contains, not lactalbumin, but an antibody to lactalbumin, these will compete with the rabbit antibodies for labelled lactalbumin. If the rabbit antibodies are precipitated specifically with a second antibody, a decrease in precipitated label will be observed - a result which could be misinterpreted as demonstrating the presence of lactalbumin. In contrast, if the gamma globulins are precipitated non-specifically with PEG the antibodies in the plasma will cause an increase in the amount of label precipitated. Also, in the absence of added rabbit antiserum, the antibodies in the plasma sample alone will cause an increase in label bound.

Using our two assays we have observed the phenomenon described above and have shown that about half the population have antibodies to lactalbumin circulating in their blood. These antibodies are directed against bovine lactalbumin and cross-react with human protein. We have also shown that they have the properties of IgG.

This paper gives a summary of our results. Full details will be published elsewhere.

METHODS

Lactalbumin was purified from human milk by the method of Phillips and Jenness¹. Bovine lactalbumin was purchased from the Sigma Chemical Co. Both lactalbumins were labelled with ^{125}I using lactoperoxidase coupled to Sepharose and hydrogen peroxide². The rabbit anti-human lactalbumin serum was raised for us by Sera-lab Ltd. It was used in the RIA at a dilution of 1 in 25 000.

Table 1. Fraction^a of labelled lactalbumin precipitated in the presence of plasma in two alternative radioimmunoassays.

Plasma	Sex	PEG Assay			Interpretation	
		Rabbit Antiserum		Double Antibody	Lactalbumin	Binding Protein
		Present (i)	Absent (ii)	(iii)		
None	—	1.0	0.25	1.0	—	—
A	F	1.12	0.93	0.67	Absent	Present
B	F	1.00	0.27	1.00	Absent	Absent
C	F	0.75	0.22	0.64	1.3 $\mu\text{g l}^{-1}$	Absent
D	M	0.98	0.22	0.95	Absent	Absent
E	M	1.19	1.02	0.72	Absent	Present
F	F ^b	0.29	0.26	0.21	240 $\mu\text{g l}^{-1}$	Absent
G	F ^b	0.67	0.68	0.41	Present	Present

^aExpressed as fraction of maximum label precipitated

^b30 weeks pregnant

Antibody, labelled lactalbumin and either dilutions of known standards or unknown plasmas were incubated at 4 °C. In the double-antibody RIA, the rabbit gamma globulins were precipitated by the addition of a donkey anti-serum (Wellcome), incubation at 4 °C for 24 h, centrifugation and aspiration of the supernatant. In the PEG - RIA, a solution of ice-cold PEG was added to give a final concentration of 10% w/v and the resulting precipitate collected by centrifugation. The limit of detection of lactalbumin was 100 pg per tube of 500 ng l⁻¹ plasma.

Plasmas from pregnant or from lactating women containing high levels of lactalbumin (> 100 $\mu\text{g l}^{-1}$) gave inhibition curves which paralleled those obtained using purified lactalbumin.

RESULTS

Samples of plasma from 15 males and 15 females were collected and the following RIA data recorded: the amount of label precipitated by PEG (i) in the absence of rabbit antiserum, (ii) in the presence of the rabbit antiserum, and (iii) the amount precipitated in the double-antibody RIA. Table 1 shows some representative results. The plasmas from males could be divided into two groups: those which had no effect on the amount of label precipitated in either assay and those which inhibited binding in the double-antibody RIA but gave increased binding in the PEG assay (D and E in Table 1). Our interpretation of this result is that the first group have neither lactalbumin or anti-lactalbumin in their blood; the latter group have anti-lactalbumin. The females could be divided into three groups: those who had neither antigen nor antibody, those who had antibody and those who had low levels (about 1 $\mu\text{g l}^{-1}$) of lactalbumin (plasmas inhibited binding in both RIAs). We also looked at plasmas from 22 women who were over 30 weeks pregnant. While 16 of them contained high levels of lactalbumin, seven plasmas increased the amount of label precipitated by PEG in the absence of rabbit antiserum but inhibited binding in the presence of antiserum in both assays (for example G in Table 1). We surmise that these plasmas contain antibodies of low avidity together with low levels of lactalbumin. The results are summarized in Table 2.

Table 2. People with detectable lactalbumin and those with antibody to lactalbumin in their blood.

Sex	Status	Number With				Total
		Lactalbumin	Antibody	Neither	Both	
Male	Normal	None	8	7	None	15
Female	Normal	3 ^a	7	5	None	15
Female	Pregnant	22 ^b	6	None	6	22

^a0.7, 0.8 and 1.0 $\mu\text{g l}^{-1}$ lactalbumin

^bSixteen plasmas > 50 $\mu\text{g l}^{-1}$ lactalbumin; the other six had both lactalbumin and antibody

Table 3. Competition for human antibodies between human and bovine lactalbumin.

Labelled lactalbumin	I_{50} ^a in $\mu\text{g l}^{-1}$ for lactalbumin	
	Human	Bovine
Human	10	5
Bovine	1000	12

^aThe amount of unlabelled lactalbumin needed to give 50% inhibition of binding of label to the antibodies in a human plasma.

Table 4. Properties of human antibody to bovine lactalbumin.

Precipitated by 45% saturated ammonium sulfate
 Did not bind to DEAE in 0.02 M phosphate buffer, pH 7.0
 Co-eluted with IgG from Sephadex G-200

Because it is known that some people have antibodies to bovine milk proteins including lactalbumin (for example, see Ref. 3), we investigated the reaction of a human plasma with bovine lactalbumin. Since we wanted a large quantity of plasma, we obtained several batches of out-dated blood from the Blood Transfusion Service, prepared plasmas and checked them for anti-bodies to human lactalbumin. One of the plasmas which clearly demonstrated an antibody was chosen for further study. Using the neat plasma as the source of antibody in a PEG RIA, about 50% of either bovine or human lactalbumin was bound. Competitive inhibition studies showed that the antibodies in the plasma had a higher avidity for bovine than for human lactalbumin (Table 3). Although bovine protein inhibited the binding of labelled human lactalbumin, the human protein did not inhibit the binding of labelled bovine lactalbumin to the human antibody.

We have also partially purified the antibody in human plasma. The antibody co-purified with IgG. These properties are summarized in Table 4.

SUMMARY

It has been reported that many apparently healthy men have detectable levels of lactalbumin in their blood^{4,5}. These observations were based on a double-antibody RIA and in view of the present results the observed inhibition was probably due, not to lactalbumin, but to antibody.

In this paper we have summarized our results which show that some people have antibodies to bovine lactalbumin in their blood. Surprisingly these antibodies cross-react with human lactalbumin and consequently in a radioimmunoassay can compete with added antibody for labelled lactalbumin.

These results illustrate a problem that can occur with a double-antibody RIA. Comparing slopes of inhibition curves was of little value since plasmas which gave suitably high levels of inhibition genuinely contained high concentrations of lactalbumin. The problem was only resolved by using an RIA which used a different method of separating bound from free label. When unexpected results are obtained using a radioimmunoassay (in this case, the apparent presence of a milk-specific protein in plasma of males), the use of an alternative assay should be considered. This method of analysis could also be considered when the presence of an antibody ₆ to the substance being studied has been suggested as a result of other experiments.

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DISCUSSION

E.D. BRANSOME: Perhaps it would be of interest if the converse problem — that of an interfering antigen — was mentioned. Niswender's laboratory in the United States has tried to develop a radioimmunoassay for rhesus monkey luteotropic hormone (LH) in blood; their efforts have been thwarted by the presence of an antigen in the monkeys' blood which was present in hypophysectomized animals and which was bound nonetheless by their antisera. Their initial findings were not unlike yours. The sword of specificity seems unfortunately to be two-edged.