

HUMAN ANTIBODIES AGAINST BOVINE IMMUNOGLOBULIN M IN IgA DEFICIENT SERA

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SUMMARY

Analysis of the sera of seventeen patients with a selective deficiency of IgA revealed that 24% gave a precipitin reaction on gel diffusion with normal bovine serum and milk. None of 100 normal adults and only one of approximately 500 patients with various diseases without an IgA deficiency had precipitins when studied by this same technique. Analysis of the nature of this reaction revealed that an IgG type antibody present in the human sera was reacting with the Fc fragment of the IgM present in bovine milk and serum.

An immunologically identical reaction occurred with goat and sheep, and reactions of partial identity with horse and pig sera. No reactions were found with sera from other species including man. In two of the IgA deficient sera additional but much weaker precipitin lines were noted with cow milk. These data are consistent with the hypothesis that oral immunization with the highly antigenic macroglobulin present in cow milk was facilitated by the mucosal antibody defect accompanying the IgA deficiency.

INTRODUCTION

During the course of investigations of human sera for the presence of a secretion specific component, lactoferrin, a precipitin reaction was noted between a goat antilactoferrin antiserum and one of the 500 human sera tested. Studies on the nature of this precipitin reaction revealed that it was not due to the antilactoferrin antibodies in the goat serum, but was a reaction between the human serum and a component present in the sera and milk of normal goats and cows. The human serum reacting with these animal sera was obtained from a patient with regional ileitis and on further analysis of immunoglobulin content this patient was found to have a selective deficiency of IgA. This paper extends an earlier report (Tomasi *et al.*, 1971; Yurchak *et al.*, 1970) of this case to other patients with IgA deficiency and describes further studies on the nature of this precipitin system.

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MATERIALS AND METHODS

Sera. Sera were obtained from normals and patients with various diseases, which are listed in Table 1. All sera were stored at -20°C until tested. The IgA deficient sera were from patients with various clinical disorders, the nature of which are briefly outlined in Table 2.

Secretions. In three of the five IgA deficient cases $20\times$ concentrated parotid saliva and duodenal secretions did not contain IgA on Ouchterlony analysis.

TABLE 1. Precipitins for bovine immunoglobulin M

Disease	No. sera	No. positive	Per cent positive
Normal adults	100	0	0
Ulcerative colitis	19	0	0
Idiopathic steatorrhea	2	0	0
Miscellaneous diseases	440	0	0
Regional ileitis	14	1	7
Selective deficiency of IgA	17	5	24
Agammaglobulinaemia	9	0	0

TABLE 2. Patients with precipitins for bovine immunoglobulin M

Patient	Diagnosis	Serum immunoglobulin (mg/ml)		
		IgG	IgA	IgM
E.H.	Normal	17.0	0	2.0
J.W.	Disseminated lupus erythematosus	9.6	0	5.4
C.B.	Recurrent infections, allergies	22.3	0	1.1
J.K.	Regional ileitis	20.6	0	1.4
C.D.	Mucocutaneous moniliasis	10.1	0.20	0.80
M.H.	Regional ileitis	14.1	4.0	1.1
Normal value (mean)		12.0	1.6	1.0

Determination of immunoglobulin levels

Antisera specific for the major immunoglobulin classes were made in rabbits from purified preparations of immunoglobulins and rendered specific by appropriate absorption, as previously described (Tomasi *et al.*, 1965). Quantitative determinations of the immunoglobulin levels in the various human sera were performed using rabbit antisera in the radial diffusion technique (Tomasi & Zigelbaum, 1963).

Isolation of the cow and goat immunoglobulins

The IgM was isolated from bovine serum and colostrum by essentially identical techniques which have been previously described in detail (Sullivan *et al.*, 1969). Similar procedures

were used for the isolation of the goat and sheep IgM. Briefly the technique involved: high-speed centrifugation in the Spinco Model L ultracentrifuge, using the No. 40 rotor at 40,000 rev/min for 18 hr. The pellet and a small amount of overlying fluid containing the majority of the macroglobulins were removed and fractionated on Sephadex G-200 in 0.14 M sodium chloride. The void volume fractions containing primarily the α_2 macroglobulin and IgM along with a small amount of IgA polymers, were then pooled and fractionated by pevikon block electrophoresis. The IgM containing fractions were determined using specific antisera. The Fc fragment of cow IgM was produced by high temperature proteolysis (trypsin 1:20 at 56°C for 30 min) as previously described for human IgM (Plaut & Tomasi, 1970).

The isolation of cow IgG and the production of antisera specific for the various cow immunoglobulins have been previously reported (Sullivan *et al.*, 1969). Antisera against the cow proteins showed extensive cross reactions with the immunoglobulins of the goat and sheep and were therefore used to identify the immunoglobulins in these species.

Fractionation of human sera were performed on Sephadex G-200 and the location of the reacting human antibody determined by the technique of reverse immunoelectrophoresis. Cow glycoprotein α_1 was obtained from colostral whey as described by Groves & Gordon (1967). Alpha lactalbumin and β lactoglobulin was purchased from Nutritional Biochemical.

RESULTS

In the original survey of human sera only one of approximately 500 sera from patients with various diseases showed a reaction with the goat antihuman lactoferrin antiserum. This patient had a selective deficiency of IgA and a clinical syndrome consistent with the diagnosis of regional ileitis. Subsequent analyses of sixteen IgA deficient sera revealed four additional patients who had a precipitin reaction with the antilactoferrin serum and also with normal goat and cow serum (see Tables 1 and 2). Subsequently, one additional patient with severe regional ileitis gave a reaction immunologically identical by Ouchterlony analysis with that of the patients with IgA deficiency. This patient had an elevated serum IgA level. A typical immunoelectrophoretic pattern of the reaction between an IgA deficient serum and cow serum is shown in Fig. 1(a). The specificity of the antibodies for the Fc fragment of cow macroglobulin is demonstrated by Fig. 1(b).

The reaction between the patients' sera was also obtained using three normal goat sera and an immunologically identical reaction with four normal cow sera and cow and goat milk. Figs 2A and 2B illustrate a Sephadex G-200 chromatograph of cow serum and cow colostral whey and Fig. 2C shows the location of the precipitin reaction with one of the IgA deficient sera, as determined by Ouchterlony analyses on the pooled chromatographic fractions obtained from the cow serum column. Note that with this human serum a precipitin reaction occurred only with the void volume peak containing the high molecular weight cow serum protein. Identical results were obtained with the pooled colostral whey column fractions. Immunoelectrophoresis of the cow serum and colostral Sephadex G-200 void volume peak is shown in Fig. 2D. Typical IgM type precipitin arcs are evident. In two of the six IgA deficient sera precipitin reactions were also noted on Ouchterlony with the 7S and 2S regions of the chromatograph. These were shown to be due to reactions with cow IgG and α lactalbumin, using immunological identity with the purified proteins on Ouchterlony analysis. The identity of the cow serum and colostral reactions is shown in

Fig. 3(a). This figure also illustrates precipitin systems with two representative IgA deficient sera, one showing a single IgM reaction, the other an additional milk specific system which proved to be α lactalbumin. Fractionation studies performed on goat and sheep serum and milk using Sephadex G-200 yielded essentially identical results to those illustrated with the cow.

In order to demonstrate that the cow protein reacting with the human sera was indeed IgM and not the small amount of polymeric IgA contaminating these preparations, an antisera made against a human monoclonal macroglobulin protein (μ -chain specific) which was found to cross react to the extent of approximately 50% with cow macroglobulin by quantitative complement fixation (Mehta & Tomasi, 1969), was used to absorb a cow IgM preparation. Similar absorption experiments were performed using antihuman IgG and IgA antisera which showed good cross reactions with the corresponding cow proteins. It was found that only the human antimacro antiserum removed the antigen indicating that it

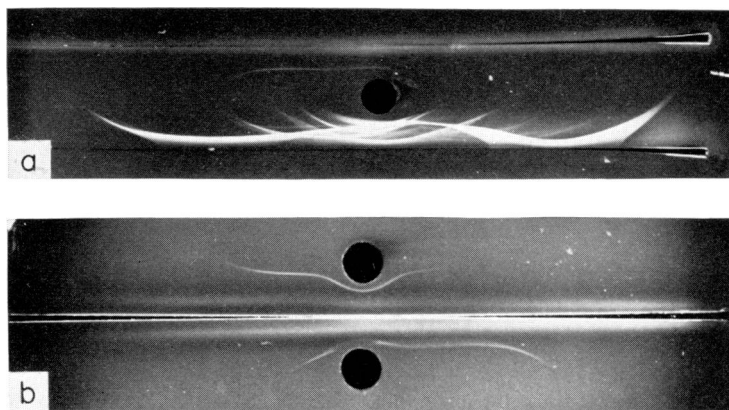


FIG. 1. Immunoelectrophoresis. (a) Centre well, normal bovine serum; upper trough, IgA deficient serum J.W.; lower trough, rabbit antiserum; (b) upper well, bovine IgM; lower well, trypsin Fc fragment of bovine IgM; trough, IgA deficient serum J.W.

shared antigenic determinants with the human IgM. The nonidentity with the cow IgA system is also demonstrated in Fig. 3(b) which shows bilateral spurs of the human antibody and antiserum when reacting with a cow macroglobulin preparation.

To determine the nature of the reactive material in the human IgA deficient sera, two of these sera were fractionated by Sephadex G-200 chromatography, and the fractions tested with whole cow and goat sera. The reactive material was found in the 7S region of the chromatograph, and on reverse immunoelectrophoresis was shown to be an IgG type antibody. Reduction (0.1 M β -mercaptoethanol) did not destroy the antibody activity in the human sera but similar reduction of the cow IgM preparation resulted in increased diffusion of the antigen and straightening of the typical curved macroglobulin arc suggesting depolymerization to 7S subunits without a loss of antigenic determinants.

Sheep, goat, and cow sera gave reactions of immunological identity with all of the positive IgA deficient sera. The horse and pig were antigenically deficient as shown by reactions of

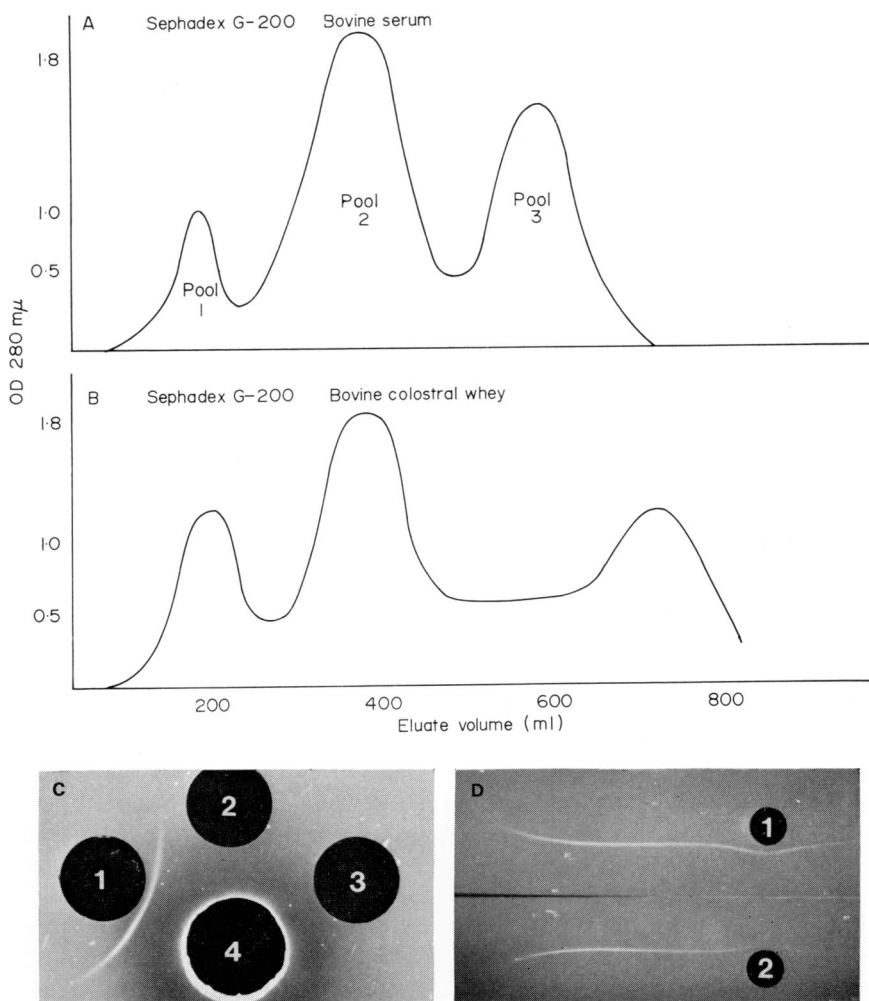


FIG. 2. Sephadex G-200 chromatograph of bovine serum (A) and bovine colostral whey (B). (c) Wells 1, 2, 3 contain pools 1, 2 and 3 respectively of the bovine serum G-200 chromatogram. Well 4 contains IgA deficient serum J.W.; (d) upper well, pool 1 from bovine serum G-200 chromatogram and lower well, void volume peak from bovine colostral whey G-200 chromatogram. Trough IgA deficient serum J.W. Note the reaction of the IgA deficient serum J.W. only with pool 1 (void volume) of bovine serum in (c) and the typical macroglobulin arcs of both the void volume from serum and colostral whey on immunoelectrophoresis in (d).

partial identity with the cow. Other species including human, monkey, rabbit, mouse, rat and chicken were completely negative. No reaction was observed between the IgA deficient sera and cow glycoprotein a, a secretion specific component thought to be analogous to human secretory component. In one of the human sera, antibodies against cow IgA were found and this serum is presently under more extensive study.

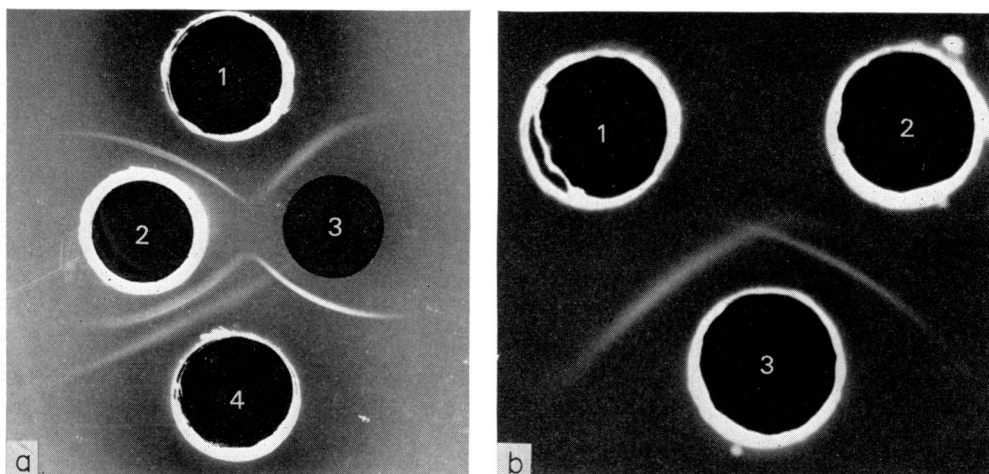


FIG. 3. Ouchterlony gel diffusion. (a) Well 1, IgA deficient serum J.W.; well 2, bovine colostrum whey; well 3, bovine serum; well 4, IgA deficient sera J.K.; (b) well 1, antiovine secretory IgA; well 2, IgA deficient serum C.B.; well 3, bovine colostrum whey Sephadex G-200 void volume. For interpretation see results section in text.

DISCUSSION

The data presented in this paper show a high incidence of precipitating antibodies against cow IgM, occurring in patients with a selective deficiency of IgA. Thus far the only patient with normal IgA and cow precipitins had extensive bowel disease, due to regional ileitis. However, as shown in Table 1, other patients with regional ileitis and various bowel diseases did not have precipitins at least as tested by gel diffusion. Undoubtedly, as reported by others (Buckley & Dees, 1969), more sensitive techniques such as haemagglutination would detect precipitins in a higher percentage of these patients.

The reason for the high incidence of cow IgM precipitins in patients with IgA deficiency is not known. It is assumed that these antibodies result from antigenic stimulation from ingested dairy products. It should be noted in this regard that the IgM reaction has been demonstrated with commercially pasteurized milk as well as unpasteurized colostrum and mature milk. One hypothesis consistent with the data is that IgA deficient individuals also have a secretory IgA deficiency (Stobo & Tomasi, 1967) and that normally the secretory IgA antibodies produced in the gut in some way limit the mucosal absorption of ingested antigens particularly macromolecular antigens such as IgM. Why the cow macroglobulin system is especially prominent in these sera is unclear. Perhaps the macroglobulin is a particularly strong antigen. However, antibodies against other milk antigens are also present in some of the IgA deficient sera as well as normal sera and in the sera from patients with various diseases (Peterson & Good, 1963; Rothberg & Farr, 1965). It is possible that the lower level of antibodies to other milk proteins compared to the IgM in the IgA deficient sera are actually related to an increased absorption of the smaller molecules, thus resulting in removal of antibodies by antigen and/or in partial immunological tolerance. However, further work is needed, using more sensitive and quantitative techniques before any definitive statements can be made regarding circulating antigen and the question of whether these

patients do indeed absorb more mucosally deposited antigens, i.e. have a 'leaky' GI and/or respiratory tract mucosa. Another although perhaps less likely possibility that should be mentioned is that the anti IgM antibodies are produced in response to an unrelated but cross reacting antigen such as a microorganism.

The site of synthesis of the anticow IgM antibody which appeared to be primarily of the IgG type was not determined. However, since many patients with IgA deficiency have both IgG and IgM in their secretions and cells producing these classes of immunoglobulins in their lamina propria (Heremans & Crabbé, 1967), it is possible that the antibodies produced are derived, at least in part, from local production in the GI tract. Increasing evidence is accumulating in animals for the passage of antibodies synthesized in the GI tract into the circulation (Vaerman & Heremans, 1970), although its occurrence in man has not been established.

In most of the published cases with milk precipitins the antigens responsible have been bovine serum albumin, γ -globulin, lactoglobulin, or lactalbumin (Holland *et al.*, 1962). However, Lopez & Hyslop (1968) reported a reaction between IgA deficient sera and normal sera derived from bovidae species which may have been due to IgM although the antigen(s) was not specifically identified. Also, a high incidence of cow milk haemagglutinins in IgA deficient individuals has been previously found by Buckley & Dees (1969), although again the nature of this antigen(s) was not established. This study confirms and extends these reports. A hypothesis similar to the one put forth here regarding the secretory system and its role in the absorption of nonviable antigens was postulated by both of these groups.

Another interesting but speculative point pertinent to these findings is the question of the relation of IgA deficiency to various diseases, many of which are characterized by autoimmune type phenomena (Tomasi, 1968). It is possible that absorption of various ingested antigens (including milk proteins) resulting from a deficiency of the secretory system might lead to immune complex disease or possibly to the formation of antibodies which cross react with various human tissues.

Finally, a point of practical importance concerning the detection of human IgA or its quantitation by the radial diffusion technique when the antisera used are produced in goats. If the IgA deficient serum contains anti IgM antibodies then the reaction of this antibody with the goat IgM will give the impression (false positive) that the human serum contains a significant amount of IgA.

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