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Immunology

Milk precipitins, circulating immune complexes, and IgA deficiency

(bovine milk/immune deficiency/antigen–antibody complexes)

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ABSTRACT **Twenty-two patients with selective IgA deficiency were studied for the presence of serum precipitins to bovine milk, bovine and fetal calf serum, and circulating immune complexes. Fifty-nine percent had circulating immune complexes, 50% had milk precipitins, 23% had precipitins to bovine serum, and 13% had precipitins to fetal calf serum. All patients with precipitating antibodies against milk or against bovine or fetal calf serum had circulating immune complexes and the precipitin titers correlated with the amount of circulating immune complexes. After one IgA-deficient patient had drunk 100 ml of milk, studies of sequential serum samples showed the presence of casein in the circulation at 60 min and the appearance of increasing amounts of immune complexes for 120 min. These findings are interpreted to indicate that in human beings the IgA system may provide a major barrier to absorption of immunogens from the gastrointestinal tract.**

It has been known for some years that the gastrointestinal tract of the newborn is permeable to macromolecules and that undigested food antigens such as cow's milk can enter the circulation and cause the production of antibodies (1–3). Occasionally, normal infants up to the age of 3 mo develop precipitating antibodies after the introduction of cow's milk into the diet, but these antibodies disappear with age and are ultimately found in only 1–2% of the general population (3, 4). However, in one immunodeficiency disease, selective IgA deficiency, milk precipitins are commonly found (4).

This study was undertaken to investigate the possibility that individuals who are deficient in IgA and have developed milk precipitins may have a continued permeation of food antigens into the blood stream, resulting in the periodic or persistent circulation of immune complexes.

METHODS AND PATIENTS

Fifteen patients with selective IgA deficiency were seen in the Immunodeficiency Clinic of the Memorial Sloan-Kettering Cancer Center. Sera from these patients were taken when they had had no current or recent infection. Another seven patients, who had been previously diagnosed as having malignancies, were found to have a deficiency of IgA as part of their initial or subsequent evaluation. Another group of five patients, all having circulating immune complexes but no immunoglobulin abnormalities, were included as control samples in precipitation studies. Three of these were known to have disseminated cancers; two of them did not have cancer but did have progressive pulmonary fibrosis of unknown origin. All patients studied here drank milk and/or consumed milk products, and none had a known allergy to milk. All sera were obtained from patients after an overnight fast.

IgG, IgM, and IgA were determined by radial immunodiffusion (5) with prepared plates for normal-range or low-level immunoglobulin analysis (Behring Laboratories, Somerville, NJ). IgE was quantitated by a radioimmunoassay from Pharmacia (Piscataway, NJ).

Milk precipitins were detected by micro double-diffusion in 1% agar gel (6). Fresh raw milk was centrifuged (Beckman microfuge) for 10 min to separate the particulate and lipid layers away from the central zone of clearer liquid. This latter part was removed and frozen in aliquots of 0.3 ml at −20° until thawed for use. After incubation of the agar slides at 37° for 24 hr, they were dialyzed against 0.85% saline with several changes for 24 hr and then in distilled water for another 24 hr. The slides were dried, stained with Ponceau S, and destained with 7% acetic acid. For a rough quantitation of milk precipitins, serum samples found to be positive when tested against the milk antigen were diluted 1:2, 1:4, and 1:8 with normal saline and retested. Each serum was also tested in agar diffusion against whole bovine serum and fetal calf serum by the methods described above.

Serum samples from IgA-deficient patients and from patients with cancers or other immunologic disorders were tested for the presence of immune complexes by the Raji cell radioimmunoassay (7). These tests were all performed without knowledge of the results of immunoglobulin analysis or results of precipitin tests. One IgA-deficient patient, S. R., was further tested by taking serum samples after giving him 100 ml of pasteurized milk. The samples, taken at 0, 30, 60, 80, and 120 min after he had drunk the milk, were tested for the presence of antigen–antibody complexes and milk precipitins and were also diffused in agar against rabbit antisera to anti-β-lactoglobulin, anti-α-lactalbumin, anti-α_{s1}-casein, anti-β-casein, and anti-κ-casein. These antisera were the gift of Harold M. Farrell, Jr. of the United States Department of Agriculture. Identical tests were performed with two normal male volunteers of age similar to that of S. R.

RESULTS

Table 1 gives the immunoglobulin, immune complex, and milk precipitin data for 22 patients with IgA deficiency whose ages and primary diagnoses are included. Thirteen of the 22 patients on this list had circulating immune complexes (59%), and 11 of these 22 (50%) had milk precipitins. *No* patient had milk precipitins and a negative test for immune complexes, but two patients with immune complexes had no milk precipitins (patients L. P. and J. S.). In these two patients the amount of circulating immune complexes was quite low.

Table 2 gives the correlation between the presence of milk precipitins and precipitins to bovine and fetal calf serum. Five patients had precipitins to whole bovine serum (23%) and three had precipitins to fetal calf serum (13%). Negative milk and bovine serum precipitin tests were found in sera of five patients known to have circulating immune complexes but who did not have immunoglobulin abnormalities.

Fig. 1 shows the timed serum samples of patient S. R., tested by double diffusion in agar against antisera specific to milk

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Table 1. Sex, age, primary diagnosis, immunoglobulin levels, and presence of circulating immune complexes and milk precipitins for the IgA-deficient patients studied

Patient	Sex	Age, yr	Primary diagnosis	IgG*	IgA*	IgM*	IgE*	Immune complexes†	Milk ppt. (dilution)
K. Ba.	F	62	Lymphoma	860	0	76	30	50	+
K. Bl.	F	5	IgA deficiency	540	13	32	ND	<16	0
M. C.	F	35	IgA deficiency	1575	0	355	6	<16	0
L. C.	M	59	IgA deficiency	1760	0	100	25	<16	0
J. D.	M	35	IgA deficiency	775	32	55	78	<16	0
J. G.	F	6	IgA deficiency	1940	0	90	47	280	+(1:2)
M. J.	F	62	Lymphoproliferative disorder	760	0	24	135	<16	0
P. K.	F	27	IgA deficiency	2900	0	432	5	3200	+(1:8)
E. L.	M	65	Immunoblastic lymphadenopathy	1450	10	246	ND	480	+(1:2)
A. N.	F	19	IgA deficiency	1500	0	172	2500	1520	+(1:4)
B. N.	M	65	Lymphoma	1100	0	470	140	<16	0
R. O.	M	44	IgA deficiency	1318	0	131	120	<16	0
L. P.	F	64	Lymphoma	780	34	148	135	50	0
A. R.	F	43	IgA deficiency	1640	0	64	48	1648	+(1:4)
M. R.	M	3	Severe combined immunodeficiency; S/P bone marrow transplantation	1480	0	158	110	3080	+(1:8)
J. R.	F	5	IgA deficiency	1380	0	112	40	<16	0
S. R.	M	19	IgA deficiency	1134	0	90	90	110	+
J. S.	M	50	IgA deficiency	910	31	165	38	32	0
F. T.	M	28	Vocal cord papillomas	1800	0	105	75	832	+(1:2)
H. W.	F	12	IgA deficiency	1760	0	214	165	1300	+(1:2)
J. Wn.	M	50	Melanoma	1600	0	490	60	<16	0
J. Ws.	M	11	IgA deficiency	1500	0	120	ND	832	+(1:2)

* Values are mg/100 ml. Normal immunoglobulin ranges for adults: IgG, 800–1800 mg/100 ml; IgA, 90–450 mg/100 ml; IgM, 60–280 mg/100 ml, and IgE, 10–506 IU. ND, not done.

† Measured in μg equivalent to heat-aggregated human IgG per ml.

proteins. He had no detectable milk antigens in the circulation before drinking milk or 30 min afterwards. However, at 60 min, precipitin lines appeared on diffusion against both κ-casein and α_{s1}-casein. These lines were not visible at 90 or 120 min. On another agar slide (not shown) serum from S. R. at 60 min, but at no other interval, had a precipitin line against β-casein.

Table 2. IgA-deficient patients of Table 1, showing the correlation between serum precipitins to milk, bovine serum, and fetal calf serum

Patient	Milk	Bovine serum	Fetal calf serum
K. Ba.	+	0	0
K. Bl.	0	0	0
M. C.	0	0	0
L. C.	0	0	0
J. D.	0	0	0
J. G.	+	0	0
M. J.	0	0	0
P. K.	+	+	0
E. L.	+	+	+
A. N.	+	+	0
B. N.	0	0	0
R. O.	0	0	0
L. P.	0	0	0
A. R.	+	+	0
M. R.	+	+	+
J. R.	0	0	0
S. R.	+	0	0
J. S.	0	0	0
F. T.	+	0	0
H. W.	+	0	0
J. Wn.	0	0	0
J. Ws.	+	0	0

The serum samples of patient S. R. were also assayed for the presence of circulating immune complexes. These results are given in Table 3. Immune complexes were detectable in the serum sample 0 and 30 min after he had drunk milk, but the complexes were not detected at 60 min. At 90 min, complexes reappeared at a high level, and at 120 min 1280 μg equivalent of heat-aggregated human IgG per ml was detected. At the one interval that milk proteins were present in the serum, immune complexes were not found. One normal control had trace amounts of immune complex (64 μg/ml) detectable at 60 min, which then disappeared. Neither control had free milk antigen detectable at any time interval.

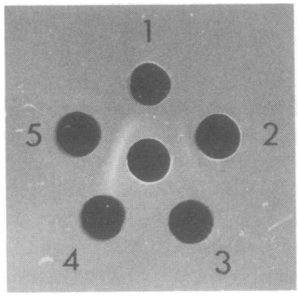

FIG. 1. Serum samples of S. R., taken at 0, 30, 60, 90, and 120 min after he had drunk milk, were diffused against rabbit antisera specific to milk proteins. In the 60-min sample seen here, κ-casein and α_{s1}-casein are detected. On another plate (not shown) β-casein was also identified in the 60-min sample. 1, Normal rabbit serum; 2, anti-α_{s1}-casein; 3, anti-α-lactalbumin; 4, anti-β-lactoglobulin; 5, anti-κ-casein.

Table 3. IgA-deficient patient S. R., who was given 100 ml of milk to drink

Time, min	Immune complexes*	
	S. R.	Control
0	184	<16
30	192	<16
60	<16	64
90	800	<16
120	1280	<16

Serum samples were taken at 0, 30, 60, 90, and 120 min, and tested for the presence of immune complexes. The normal control was tested similarly.

* Measured in μg equivalent to heat-aggregated human IgG per ml.

DISCUSSION

IgA deficiency is a common immunologic abnormality which may affect as many as 1 out of every 700 individuals (8). The majority do not have symptoms referable to deficient immunity, but for the minority who do have symptoms, sinopulmonary infections, allergic reactions, and frequent gastrointestinal and arthritic complaints are common (9). Individuals with IgA deficiency have an increased incidence of connective tissue diseases (10) and malignancies (11), and there is a greater tendency toward the production of autoantibodies (12).

The association of IgA deficiency and the presence of precipitating antibodies to constituents of cow's milk is well known (4). Presumably, a lack of IgA in the intestinal secretions permits the entrance of certain undigested food antigens into the circulation. IgA produced by intestinal plasma cells normally has antibody activity for milk and other food antigens (13, 14), which may be one reason why only traces of such substances normally enter the circulation.

In this study it appears that not only are milk and other bovine protein precipitins a common feature of IgA deficiency, but that an even higher proportion (59% of our patients) may have circulating immune complexes. In fact, all patients with milk precipitins and IgA deficiency had such complexes.

From the strong correlation between circulating complexes and milk precipitins it seemed possible that the complexed antigen in the circulation of our patients could be a milk protein. We first compared the amounts of circulating complexes with the density and number of precipitin lines and the degree to which a patient's serum could be diluted before the Ouchterlony reaction disappeared. The four patients with the strongest precipitation reactions also had the highest values for circulating immune complexes (patients P. K., A. N., A. R., and M. R.). This did not prove that milk was the circulating antigen, since a mucosal abnormality that could allow one food antigen to enter the circulation could presumably allow others to penetrate equally.

For a more direct approach, we analyzed sequential serum samples from one patient with IgA deficiency and a low level of circulating immune complexes who was given a small amount of milk to drink. Sixty minutes after milk ingestion, α_{s1}-, β-, and κ-casein milk proteins were detectable in his serum. Circulating immune complexes, present at low amounts at 0 and 30 min, disappeared at 60 min and reappeared at 90 min in larger quantity. At 120 min the amount of immune complexes was even more elevated. The casein was present only at the time interval in which immune complexes disappeared, which may suggest immune complex dissociation in antigen excess. One normal control, interestingly, had a small amount of immune complexes in the circulation at 60 min but no evidence of milk antigens by agar diffusion at any time. It should be emphasized that the IgA-deficient patients were studied at a time when they had had no recent or current infection, which could presumably also lead to the appearance of circulating immune complexes.

These data appear to indicate that in IgA deficiency a commonplace food antigen such as cow's milk protein may enter the blood stream, become complexed to pre-existing antibody, and be circulated as an immune complex for an uncertain (possibly persistent) period of time. It, however, remains uncertain which of the milk proteins may be involved in immune complex formation. The volume of milk was a small one, which might easily be taken several times in one day. It is difficult at present to assess the relevance of this observation in terms of the diseases to which IgA-deficient patients are prone, although by testing for milk precipitins and immune complex formation in greater numbers of IgA-deficient patients, it may be possible to correlate these phenomena with the development of autoimmune disease, connective tissue pathology, and possibly with malignancies.

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