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SELECTIVE IgA DEFICIENCY AND AUTOIMMUNITY

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SUMMARY

Laboratory and clinical data are presented in fifteen patients under the age of sixteen years with selective IgA deficiency, all of whom had normal serum IgG, IgM, IgD and IgE. Results indicate a high incidence of autoimmune disease, autoimmune phenomenon or unusual antibody formation. Three patients had thyroiditis, one cerebral vasculitis, one pulmonary haemosiderosis, one cystic fibrosis and nine had recurrent upper respiratory tract infections. Ten out of fifteen were positive for one or more 'autoantibodies.' Selective IgA deficiency cannot always be considered a benign entity and individuals with this defect warrant complete investigation.

INTRODUCTION

In surveys of 'normal' populations (Bachman, 1968; Hobbs, 1968) selective IgA deficiency occurs in an incidence of one in 700. Studies of individuals with this deficiency have indicated that this defect may be benign (Rockey *et al.*, 1964; Goldberg, Barnett & Fudenberg, 1968; Johansson, Högman & Killander, 1968). Some of the cases reported by West, Hong & Holland (1962), however, had associated diseases. The variations in symptomatology are due at least in part to additional immunological defects such as the cellular immune deficiency (Peterson, Cooper & Good, 1966) and IgE deficiency in patients with ataxia-telangiectasia (Ammann *et al.*, 1969). The present study describes the clinical and laboratory features of fifteen patients below the age of sixteen years with selective IgA deficiency as defined by levels of serum IgA less than 0·04 mg/ml, normal levels of IgG, IgM, IgD, and IgE and intact cellular immunity.

METHODS AND MATERIALS

All patients who had been diagnosed previously as having selective IgA deficiency were assessed for the presence of IgE. Those who had less than 0·04 mg/ml of IgA and who had

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normal IgE were placed in the category of selective IgA deficiency and retained in the study group.

Immunoglobulins were quantitated by a modification of the Mancini technique (Mancini, Carbonara & Heremans, 1965) using specific antisera prepared in our laboratories and originally standardized by purified immunoglobulin preparations. For routine analysis a pool of normal serum was used as the standard. IgD was assessed qualitatively using specific antiserum (Kallestadt Laboratories, Minneapolis, Minnesota) by the Crowle diffusion technique (Crowle, 1958).

The IgA was purified from a pool of fifty normal serum donors using the method of Van Munster & Stoelinga (1965). The protein content was determined by Kjeldahl nitrogen estimation and this preparation was used to standardize a serum pool obtained from twenty normal adults. Aliquots of the pool, frozen in small aliquots and stored at $-20°C$, were thawed and used only once. The immunoglobulin levels of the pool were further corroborated by Dr E. R. Stiehm, Los Angeles, California. Repeated use and corroboration of the values of this serum standard over the period of the study showed no change in the immunoglobulin level, and the fact that the diffusion rings remained constant in size over this period indicated that significant aggregation or other alteration of the immunoglobulins had not occurred during storage. The lower limits of quantitation in our laboratory at present are 0·02 mg/ml for IgA and IgM and 0·16 mg/ml for IgG, using the Mancini single radial diffusion technique.

IgE was evaluated utilizing the reversed passive cutaneous anaphylaxis test of Ishizaka (Ishizaka & Ishizaka, 1968). The test was found to be sensitive to levels of 50 ng/ml of IgE in the serum. Confirmation of normal serum IgE levels (>100 ng/ml) was obtained by radioimmunoassay techniques*.

Antibodies directed against IgA were detected using sheep red cells coated with purified IgA prepared from normal serum (Pressman, Campbell & Pauling, 1942; Van Munster & Stoelinga, 1965). These results were corroborated by Drs G. Vyas and H. H. Fudenberg. In their method, human 0+ red blood cells are coated by a panel of purified IgA myeloma proteins (Vyas *et al.*, 1969).

Antithyroid antibodies were measured by complement fixation and haemagglutination utilizing tanned red blood cells coated with thyroglobulin (Burroughs Wellcome Co., Tuckahoe, N.Y.). Rheumatoid factor was evaluated using IgG coated latex particles (Hyland Laboratories, Los Angeles, California).

Fluorescent antibodies directed against stomach parietal cells, smooth muscle, adrenal and liver were investigated, utilizing the indirect immunofluorescence technique with fluorescein labelled goat antihuman IgG. The test serums were diluted 1:10 for this test. All tissues were obtained from specimens at the time of surgery.

Milk precipitins were detected using double diffusion in agar by the Crowle technique with raw milk as the antigen.

Anti-deoxynucleoprotein antibodies were determined by a fluorescent procedure (Friou, 1962).

Quantitation of $\kappa:\lambda$ chain ratios was performed utilizing specific antisera. Values were compared to standards obtained on a pool of normal human sera quantitated by the N.C.I. Immunoglobulin Reference Center, Falls Church, Virginia.

Sera from age-matched normal individuals were used as controls.

* Performed by K. Ishizaka, Baltimore, Maryland and S.G.O. Johansson, Uppsala, Sweden.

RESULTS

Clinical

A total of fifteen patients with selective IgA deficiency were studied. Ages ranged from 3 to 16 years. Of these fifteen none could be considered 'normal'. Three had thyroiditis, one cerebral vasculitis, one pulmonary haemosiderosis, one cystic fibrosis and nine had recurrent respiratory tract infections.

Laboratory

Data are summarized in Table 1. Eight patients were positive for antibodies directed against IgA. Three sera were positive for rheumatoid factor. Antithyroid antibodies of the

TABLE 1. Antibodies in patients with selective IgA deficiency

	Antithyroid Ab.							
	C.F.*		Tanned cells		Rheumatoid factor		Anti-IgA	
15 patients	3		5		3		8	
40 controls	0		0		0		0	
	Fluorescent Ab.				Anti-DNP† Ab.	Milk Ab.	K/L‡ Ratios	
	S§	M	A	L			<1	>3
15 patients	2	2	0	0	0	9	2	0
40 controls	0	0	0	0	0	1	0	0

Ab. antibodies.
* CF: complement fixing.
† DNP: Deoxynucleoprotein.
‡ K/L ratio: $\kappa:\lambda$ ratio (normal = $1{\cdot}86 \pm 0{\cdot}28$).
§ S = stomach parietal cell, M = smooth muscle, A = adrenal, L = liver.

complement fixing variety were found in three patients and five patients were positive for haemagglutinating antithyroglobulin antibodies in titres of greater than 1:1024. Three of the five patients positive for antithyroid antibodies were clinically euthyroid and did not have a history of thyroiditis. Antibodies directed against parietal cells* and smooth muscle fibres of the stomach were found in two patients by the indirect immunofluorescent technique. None of the patients had anti-deoxynucleoprotein antibodies. Milk antibodies were found in nine patients utilizing the double diffusion technique. $\kappa:\lambda$ ratios were abnormal in two patients, being 0·6 and 0·2 respectively (normal = $1{\cdot}862 \pm 0{\cdot}28$), (Fahey & McKelvey 1965). Other immunoglobulin values (i.e. IgG, IgM, IgD and IgE) were normal in these patients. Cellular immunity was normal as indicated by one or more of the following: normal cutaneous delayed hypersensitivity, normal leucocyte response to phytohaemagglutin or normal small lymphocyte counts.

Table 2 presents data obtained on family 'S' and contrasts the results in the normal mother, father and sibling (K.S.) with three siblings (D.S., J.S., R.S.) with selective IgA deficiency. Anti-IgA antibodies, antithyroid antibodies, rheumatoid factor and milk

* Also positive for kidney tubule cytoplasm and therefore indicative of anti-mitochondrial antibodies.

precipitating antibodies were present in all three siblings with IgA deficiency. No history or evidence of thyroid disease was present in the three children at the time antithyroid antibodies were demonstrated. The father, mother and normal sibling with normal serum levels of IgA did not demonstrate any of these abnormalities.

Using the data of Cassidy, *et al.* (1968) compared with the known incidence of diseases of an autoimmune nature, the probability of the simultaneous occurrence of selective IgA deficiency and rheumatoid arthritis and systemic lupus is $P<0{\cdot}00001$.*

TABLE 2. Family 'S'

	IgA*	IgE	Anti-IgA Ab.	Rheumatoid factor	Antithyroid Ab.	Milk ppt. Ab.
Mother	120	N.D.	–	–	–	–
Father	400	N.D.	–	–	–	–
K.S.	130	normal	–	–	–	–
D.S.	<4	normal	+	+	+	+
J.S.	<4	normal	+	+	+	+
R.S.	<4	normal	+	+	+	+

* mg/100 ml.
N.D. = not done.

DISCUSSION

Our data emphasize the high incidence of autoimmune disease and autoimmune phenomena found in patients with selective IgA deficiency. Of particular significance is the increased incidence of autoimmune phenomena in our patients, all of whom were less than 16 years of age. This increased incidence for adults was noted by Hobbs. However, the study included a number of patients with values of IgA significantly higher than ours and the presence or absence of IgE was not tested. Fraser (1969) noted a significant incidence of autoimmune disease in selective IgA deficiency and agreed with Hobbs that some form of 'immune imbalance' exists in these patients. Although total serum immunoglobulin values other than IgA were normal, two patients had a reversal of the $\kappa:\lambda$ ratio, suggesting an imbalance of antibody production or catabolism. Further characterization of the immunoglobulins in these patients may reveal other abnormalities such as have been described in classic hypogammaglobulinaemia where electrophoretic, antigenic and functional defects have been found (Hong & Good, 1967).

A high incidence of antibodies directed against IgA was found in our studies as Vyas *et al.* (1969) have also shown. These authors have suggested that patients with anti-IgA antibodies fall into two categories, those with antibodies of limited specificity and those with broad reactivity. Antibodies of broad specificity would be expected to develop in those with complete absence of IgA, whereas, with normal serum levels of IgA, isotypic or idiotypic (limited specificity) antibodies would develop. Since the antibodies were detected by using an IgA coating derived from multiple donors, the differentiation between limited and broad

* Chi square method.

specificity could not be made by our methods. Whether or not the antibodies demonstrated in our patients were 'autoimmune' could only be determined by definition of isotypic specificity and typing of the patients' IgA. Unfortunately, such studies could not be performed.

The source of immunization is not immediately apparent. The great majority of our patients with anti-IgA antibodies did not have a history of exposure to blood products. Maternal foetal transfusion is a possibility, or the agent might have been colostral IgA ingested during infancy. Continuous immunization may also have occurred as a result of cross-reacting antigens ingested in bovine milk. In support of this latter possibility are the studies of Buckley & Dees (1969) who found that patients with selective IgA deficiency have a high incidence of milk precipitating antibodies. A total of nine of the fifteen patients in our series were also found to have antibodies to milk proteins. This observation may provide a clue to the susceptibility of IgA deficient patients to autoimmunity. A basic function of the gastrointestinal tract mucosa is selective absorption. This membrane is exposed to numerous antigens, some of which by virtue of their animal origin may cross react with human proteins and initiate autoimmune disease. A competent local antibody system should be able to exclude such antigens. For example, Rothberg (1969) has shown that the formation of antibodies to orally administered bovine albumin in newborn infants ceases with the maturation of the IgA system.

Another source of potential 'autoimmunogens' might exist in metabolic products which are normally excreted. Lerner & Dixon (1968) have shown that autologous urine injected into rabbits induces a severe glomerulonephritis due to antibodies formed against the glomerular basement membrane. In the absence of competent local immunity, diversion of antigenic material from its normal excretory pathway could produce similar results.

To determine whether or not an additional defect of IgE deficiency was also present, our patients were tested for cutaneous reactions with anti-IgE. Of sixteen patients with normal levels of IgG, IgM and IgD, only one was found to have an associated IgE deficiency and was excluded from study (Ammann, Roth & Hong, 1970). Combined IgA and IgE deficiency had been noted previously as a feature only in ataxia-telangiectasia (Ammann *et al.*, 1969). Thus it appears that what has previously been termed selective IgA deficiency does in fact represent an isolated immunoglobulin deficiency, as far as presently available methodology can determine.

It is apparent that not all autoimmune disease can be related to selective IgA deficiency and that other defects may be present. Nevertheless, our studies indicate that patients with selective IgA deficiency should not be considered normal *a priori* and that investigations for autoimmune and other diseases are warranted.

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