A Low Molecular Weight Immunoglobulin Antigenically Related to 19 S IgM *

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Summary. Sucrose density gradient analysis of the fresh sera of patients with hereditary ataxia telangiectasia, disseminated lupus, and Waldenström's macroglobulinemia revealed the presence of an immunoglobulin possessing IgM determinants but having a sedimentation coefficient of approximately 7 S. Bio-Gel chromatography of patients' sera confirmed the presence of two distinct populations of IgM. The low molecular weight IgM possessed incomplete isohemagglutinin activity that was resistant to treatment with reducing agents. Gel diffusion analysis revealed that the 7 S IgM showed immunological identity with both 19 S IgM and the subunits of the 19 S IgM produced by reduction. Approximately 10 to 15% of the patient's total IgM was low molecular weight. Evidence is presented that the 7 S IgM was not produced from the patient's serum 19 S IgM on *in vitro* incubation. A simple rapid technique is described, using double diffusion in polyacrylamide gels, which permits the determination of low molecular weight IgM in sera and other fluids. Using this technique, the sera of 52 patients with disseminated lupus were surveyed, and 17% of the patients were found to contain low molecular weight IgM. The low molecular weight IgM occurred with particular frequency in male patients with disseminated lupus and in those patients with low or absent serum IgA.

Studies of the salivary immunoglobulins of patients with ataxia telangiectasia and disseminated lupus suggest an inverse relationship between the levels of IgA and IgM. In patients lacking salivary IgA, IgM was the major immunoglobulin present. No correlation was observed between salivary immunoglobulin levels and the severity of sinopulmonary infections in these patients.

Introduction

Immunoglobulin systems containing both high and low molecular weight components that are antigenically closely related or identical have been described in several species. Lower vertebrates such as the smooth dogfish (1) and lemon shark (2) appear to possess a single immunoglobulin system with both high and low molecular weight components showing closest similarity to the human IgM system. Studies of horse serum have also suggested the existence of a low molecular weight γ-globulin antigenically related to the horse 19 S IgM (3). The occurrence of a similar system in humans was suggested by the findings of Killander (4) and especially those of Rothfield, Frangione, and Franklin (5). The latter workers showed that certain patients with disseminated lupus possess antinuclear factors of the IgM type having a sedimentation coefficient of approximately

TABLE I

Immunoglobulin levels and isohemagglutinin titers in serum and saliva of patients
with ataxia telangiectasia

| Patient | In-fections | Serum γ-globulins | | | | Salivary γ-globulins | | | Isohemagglutinins | | | |
| | | | | | | | | | Serum | | Saliva | |
		IgG	IgA	IgM	7 S IgM	IgG	IgA	IgM	A	B	A	B
			mg/ml				mg/100 ml					
Normal (mean)	0	10.0	1.6	1.0	0	0	3.0	0	8	– 64	4	– 8
M.F.	4+	8.3	0	5.8	+	0	0	4.3	32	16	4	4
L.C.	2+	8.1	0	3.2	+	0	0	4.4	16	16	4	4
J.V.	1+	9.2	0	2.6	0	0	0	9.7	32	16	4	4
J.M.	1+	11.0	0	2.9	+	0	0	3.9	8	8	2	2
S.B.	1+	6.2	0	5.4	+	nd*	nd	nd	64	†	nd	
G.S.	1+	8.4	0.28	2.7	0	0	Trace	1.3	‡	16	‡	0
R.S.	2+	6.8	1.3	3.0	0	0	1.5	0.86	‡	16	nd	

* nd = not done.
† Patients were blood type B.
‡ Patients were blood type A.

7 S. Also, low molecular weight components possessing IgM determinants have been described in 2 patients with dysproteinemias whose total IgM levels were elevated (6, 7).

The present report demonstrates naturally occurring antibody activity (isohemagglutinins) in low molecular weight IgM fractions and suggests that its occurrence may be more common than previously recognized. The low molecular weight IgM is present in significant amounts in the serum of patients with several disorders including hereditary ataxia telangiectasia, disseminated lupus, and Waldenström's macroglobulinemia. Most importantly, although the low molecular weight IgM appears to be closely related immunologically to the 7 S subunit produced from the macroglobulin by reduction, evidence will be presented suggesting that it is not produced from 19 S IgM, at least by in vitro degradation. The low molecular weight IgM seems to occur particularly in patients with low or absent serum IgA who also have elevated levels of total IgM, although this is not invariably the case. Studies are also presented suggesting a reciprocal relationship between IgA and IgM in the secretions of these patients. In those with low levels of IgA in their secretions, IgA is replaced solely or principally by IgM.

Methods

Parotid saliva was collected with a Curby parotid cup. Samples of 20 to 100 ml were concentrated 50-fold by negative pressure dialysis. Serum samples were routinely collected approximately 1 hour before use.

Antisera were prepared by immunization of rabbits with purified antigens in Freund's adjuvant or were purchased commercially [1] and made specific for each of the immunoglobulins tested by further absorption with purified immunoglobulin preparations. Quantitative determinations of the immunoglobulins were performed using the immunoplate method previously described (8). With this method, as little as 0.02 mg per ml of IgG and 0.03 mg per ml of IgA can be detected. The amount of low molecular weight IgM present in serum was difficult to quantitate as the standards for the plate technique are based on the diffusion rate for 19 S IgM. For this reason, the concentrations of low molecular weight IgM are expressed as ring diameters obtained on the quantitative plates.

Analytical and density gradient ultracentrifugation, starch block electrophoresis, and gel chromatography were performed according to methods previously described (9).

Isohemagglutinin titers are expressed as the reciprocal of the highest dilution giving a 2 + agglutination. The type of immunoglobulin in whole serum and saliva responsible for agglutination was determined by inhibiting the agglutination with antisera specific for IgG, IgA, or IgM as previously described (10). Incomplete agglutination was tested as follows. Red blood cells of the appropriate blood type were incubated with density gradient or column fractions at room temperature for 30 minutes. The suspension was centrifuged, the supernatant fluid was collected, and the cells were washed three times with saline. The washed, sensitized cells were resuspended in 0.1 ml of saline, and antisera specific for a given immunoglobulin class were added. After gentle mixing for ½ hour at room temperature the agglutination was read microscopically and graded 1 to 4 +. Reduction was performed with 0.1 M β-mercaptoethanol at 20° C for ½ hours followed by alkylation with 0.2 M iodoacetamide.
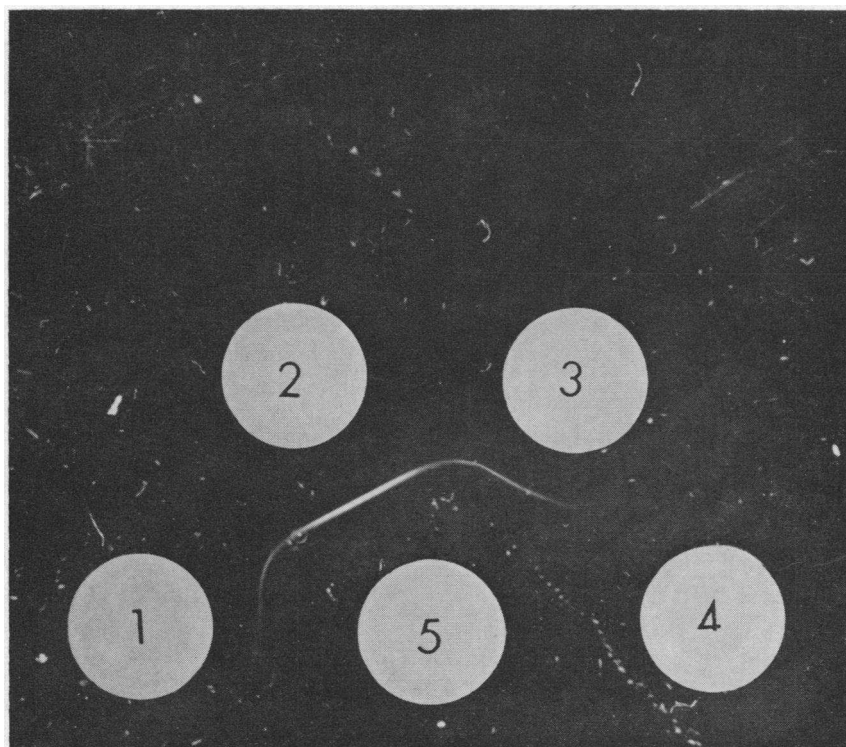
FIG. 1. POLYACRYLAMIDE GEL DIFFUSION. Antiserum directed against normal 19 S IgM was placed in well 5. Wells 1, 2, and 3: whole serum of patients having low molecular weight IgM. Well 4: normal human serum.

Enzymatic digestion of 19 S IgM was carried out using chymotrypsin C at pH 8 for 18 hours at 37° C. Details of the enzymatic studies of the 19 S IgM immunoglobulin will be the subject of a subsequent report (11).

Sera were surveyed for low molecular weight IgM by a technique involving double diffusion in polyacrylamide gel. A solution of 4% acrylamide [2] was poured into small petri dishes and allowed to polymerize for 20 minutes in an evacuated dessicator. After polymerization, the gel was crystal clear and firm enough to cut holes in with a no. 2 cork borer. Antisera specific for IgM were placed in the central well and the serum or fractions being tested in the peripheral wells. The plates were incubated for 48 hours at 37° C. The sieving action of the polyacrylamide prevents diffusion of 19 S but not the 7 S IgM. Since the antisera are specific for the μ chains of IgM, only low molecular weight molecules possessing IgM determinants are detected, even in complex mixtures such as serum.

Results

Table I shows the levels of the serum and salivary immunoglobulins in the patients with ataxia

[2] Canalco, Bethesda, Md.

telangiectasia. Sinopulmonary infections in these patients varied from mild otitis media or frequent colds to repeated episodes of pneumonia with chronic lung disease and were roughly graded according to frequency and severity from 1 to 4 +. Although serum IgG levels were essentially normal, the IgA levels were markedly decreased or absent in all patients except R.S. Significant elevations of serum IgM were evident in all of the patients. As shown in Table I, low molecular weight IgM was not detected in 3 of the patients with ataxia telangiectasia. These included patients J.V. and G.S., who had the lowest total serum IgM levels, and patient R.S., whose serum IgA level was within normal limits. It is interesting that G.S. and R.S. are brothers, one of whom had low IgA and the other essentially normal levels.

The sera of 52 patients with disseminated lupus were surveyed for the presence of low molecular weight IgM, using the polyacrylamide plate technique. An example of the use of this technique in

Incidence of 7 S IgM in patients with disseminated lupus

	No. patients	No. with 7 S IgM	% with 7 S IgM
Male	7	3	43
Female	45	6	14
Total	52	9	17
Low IgA*	3	3	100

* Serum IgA level of < 0.5 mg per ml.

detecting low molecular weight IgM is illustrated in Figure 1. The antisera were monospecific and detected only IgM. Evidence that the component being detected was low molecular weight IgM is the fact that, whereas pure preparations of 19 S IgM showed no precipitin line, reduced and alkylated 19 S IgM showed immunological identity with the component detected in the sera studied.

The results of the studies on the lupus patients are summarized in Table II. The incidence of low molecular weight IgM in the sera of these patients was approximately 17%. Of the 7 male patients, 3 or 43% had 7 S IgM, whereas only 14% of the female patients possessed this type of immunoglobulin. It is interesting and perhaps significant that all of the lupus patients having low serum IgA levels (less than 0.5 mg per ml, approximately 6% of the series) possessed the low molecular weight IgM.

Two of 12 patients with Waldenström's macroglobulinemia also demonstrated significant amounts of low molecular weight IgM. This is somewhat lower than the observations of Solomon (12) suggesting that approximately 40% of patients with macroglobulinemia have low molecular weight IgM.

Figure 2 shows a density gradient determination performed on the fresh serum of a patient, L.C., with hereditary telangiectasia. Although IgM is present in the 19 S region of the gradient, molecules possessing IgM determinants were also found in the 7 S region of the gradient. Similar density gradient patterns demonstrating the 7 S IgM were obtained using the fresh sera of all of the patients whose sera showed the presence of 7 S IgM by the polyacrylamide plate technique. Saline, or direct isohemagglutinins, were present in the 19 S region of the gradient and paralleled the 19 S IgM concentration (Figure 2). No direct

agglutination was found in the 7 S region of the gradient. However, red blood cells, of the appropriate type, sensitized with fractions from the 7 S region of the gradient were agglutinated using antisera specific for IgM but not with antisera directed against IgG or IgA. Both the direct and indirect agglutination could be inhibited with purified A or B substance, depending on the patient's blood type, and no agglutination of type O cells was observed. Addition of purified 19 S IgM inhibited both the direct and indirect agglutination, whereas addition of IgG or IgA had no effect on either type of agglutination. Reduction and
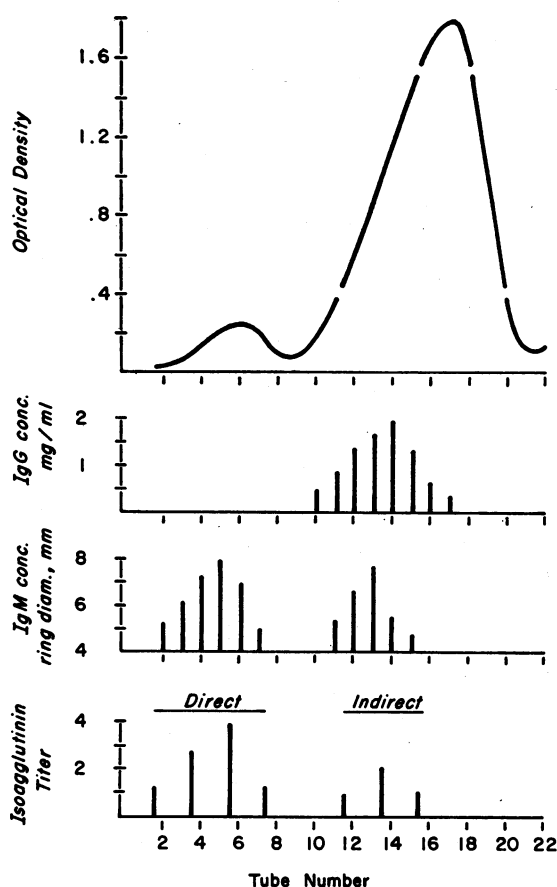

FIG. 2. SUCROSE DENSITY GRADIENT ANALYSIS OF THE SERUM OF PATIENT L.C. The distribution of IgG, IgM, and the direct and indirect isohemagglutinin titers is shown by the vertical bars. The concentrations of IgM are expressed as ring diameters in millimeters measured on the quantitative plates. Indirect agglutination was positive only when determined with specific anti-IgM antiserum. Similar results showing indirect agglutination were obtained with 4 other sera.

alkylation destroyed the saline isohemagglutinin activity, but had no effect on the incomplete iso-hemagglutination.

The serum of patient L.C. was fractionated by starch block electrophoresis. Fractions containing IgM were pooled, concentrated, and applied to a Bio-Gel P300 column. The elution pattern (Figure 3) demonstrates two distinct populations of IgM. The fall through or first peak contains high molecular weight IgM having an uncorrected sedimentation coefficient of 18.3 S. The second fraction of IgM was eluted early in the second peak with the IgG. The initial fractions of the second peak having a high ratio of IgM to IgG (approximately 1:1) showed a single symmetrical boundary in the ultracentrifuge, having an uncorrected sedimentation coefficient of 6.4 S. Density gradient analysis of this fraction (Figure 4) showed that the distribution of the low molecular weight IgM is similar to that of 7 S IgG.

Using the fraction from peak two of the Bio-Gel column containing essentially only IgG and 7 S IgM (as determined immunologically), we were able to determine the approximate concentration

FIG. 4. DENSITY GRADIENT ANALYSIS OF BIO-GEL PEAK 2. The early fractions of Bio-Gel peak 2 contain 7 S IgM and IgG in a ratio approximating 1:1. The distribution of IgG and IgM is shown by the vertical bars.

of 7 S IgM in the sera tested. The IgM concentration of the fraction was taken as the difference between the total protein concentration and the concentration of IgG. Various dilutions of the sample were then placed on quantitative plates, and a standard curve relating ring diameter to 7 S IgM concentration was constructed. From the relative concentrations of high versus low molecular weight IgM obtained on the density gradient and the Bio-Gel patterns, it was found that approximately 10% of the patient's total IgM was low molecular weight. The amounts of 7 S IgM in the other sera varied between 8 and 15%.

The patients' sera were incubated at 37° C for 96 hours to determine whether dissociation of 19 S IgM occurred in vitro. Incubation had no effect on the quantitative distribution of high versus low molecular weight IgM on density gradient analysis. Freezing and thawing or storage at 4°C for several weeks had no effect on the quantity of 7 S IgM, although this treatment did significantly de-
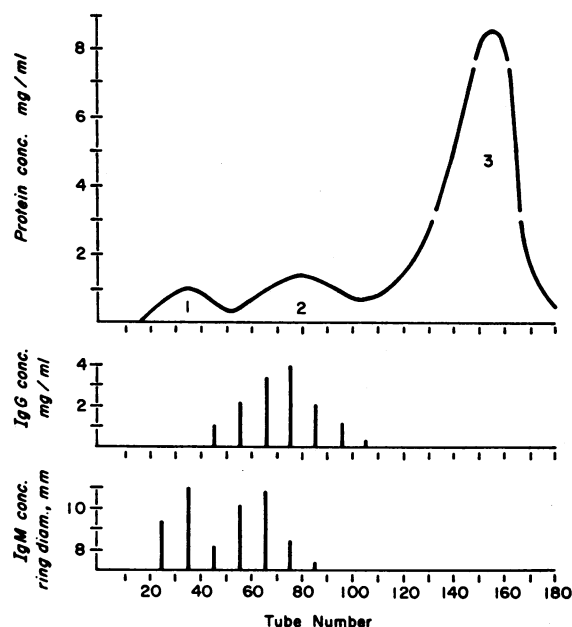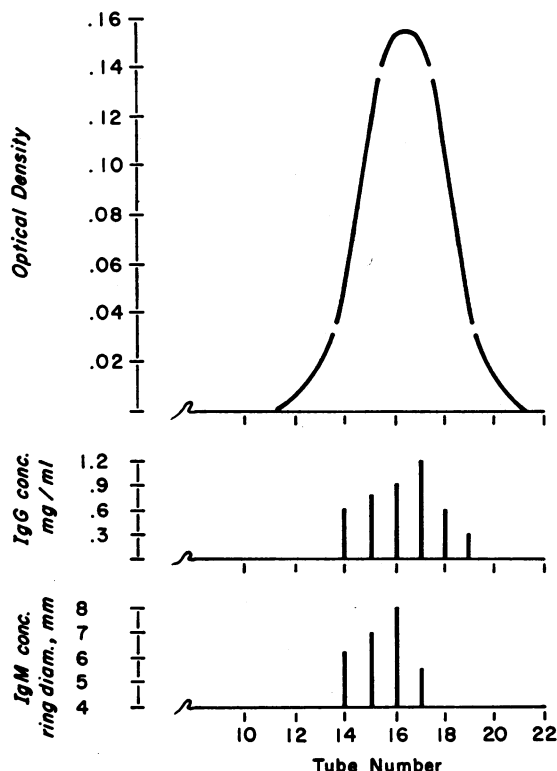

FIG. 3. BIO-GEL P-300 CHROMATOGRAPH. The serum of patient L.C. was initially fractionated by starch block electrophoresis. Fractions containing IgM were applied to the Bio-Gel column and eluted with 0.85% saline. The distribution of IgG and IgM is shown by the vertical bars.
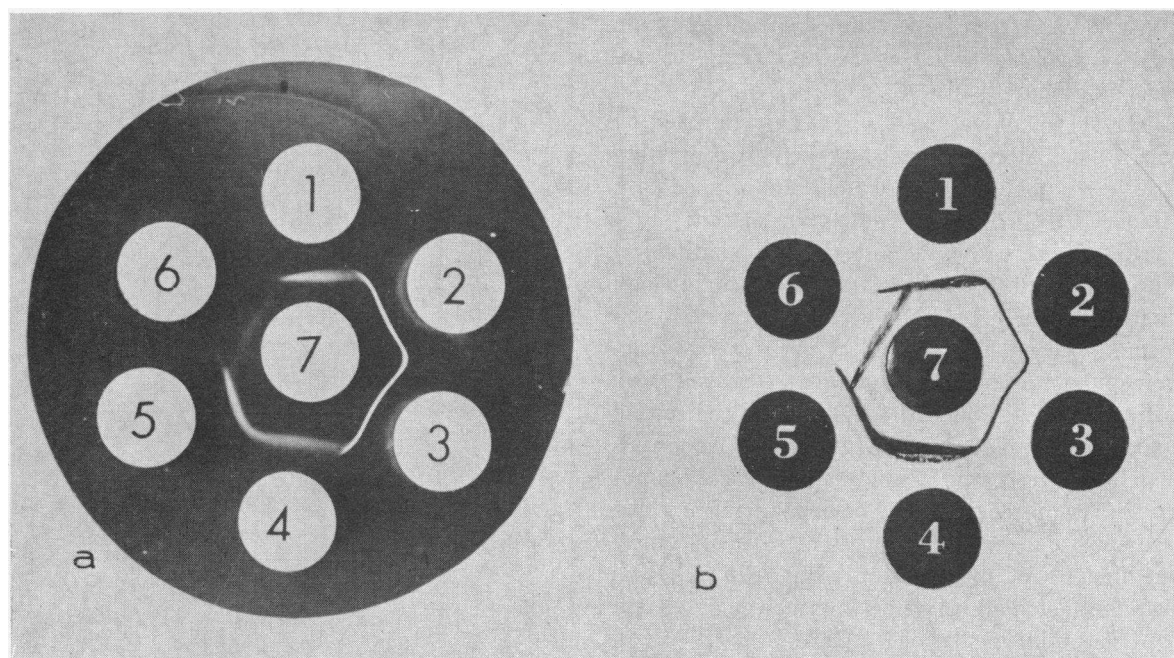
FIG. 5. *a*) OUCHTERLONY ANALYSIS OF THE LOW MOLECULAR WEIGHT IgM (WITH SCHEMATIC REPRESENTATION, *b*).
Antiserum directed against normal 19 S IgM was placed in the center well, no. 7. Wells 1 and 4: Bio-Gel peak 2
containing low molecular weight IgM. Well 2: 19 S IgM isolated from the same serum. Well 3: normal human
serum. Well 5: the subunits produced by reduction and alkylation of 19 S IgM. Well 6: the 6.6 S subunits pro-
duced from 19 S IgM by chymotrypsin C digestion.

crease the indirect isohemagglutinin titer. The
amount of 7 S IgM was constant in sera collected
at 3-week intervals although one patient's low
molecular weight IgM was significantly reduced
after an undiagnosed respiratory infection.

The presence of low molecular weight IgM is
not directly related to the absolute concentrations
of IgM. This is illustrated by the fact that 10 of
12 patients with Waldenström's macroglobuline-
mia, despite very high concentrations of IgM, had
no detectable 7 S IgM in their serum. No low mo-
lecular weight IgM was found in the serum of
several patients with Laennec's cirrhosis and infec-
tious mononucleosis who had total IgM levels com-
parable to the patients possessing low molecular
weight IgM. The low molecular weight IgM
could not be detected in any of 15 normal sera us-
ing the polyacrylamide gel technique. Analysis of
the sera of 3 normal subjects with a singular de-
ficiency of serum IgA also failed to demonstrate
the presence of low molecular weight IgM. Low
molecular weight IgM was not found in the
parents of patients L.C. and M.F.

As shown in Figure 5, gel diffusion analysis us-
ing antisera against normal 19 S IgM yielded a
pattern of identity between the patient's 7 S IgM,
19 S IgM isolated from the same sera, reduced and
alkylated 19 S IgM, and normal human serum.
Note especially that the 7 S IgM is not antigeni-
cally deficient to the 19 S IgM. However, the
6.6 S subunits produced by chymotrypsin C di-
gestion of 19 S IgM are deficient to both the 19 S
and the 7 S IgM. Identical results were obtained
using two other anti-IgM antisera.

IgA could not be detected in the saliva of those
patients whose serum lacked IgA (Table I). The
reason for the difference between these results
and those reporting normal salivary IgA lev-
els with absent serum IgA in patients with
ataxia telangiectasia (13) is not known. Our re-
sults are in agreement with those of South, Cooper,
and Wollheim (14).

In patients lacking salivary IgA, IgM was the
sole or major immunoglobulin present in the sa-
liva, and the concentration of IgM equaled or ex-
ceeded the total γ-globulin concentration of normal

saliva. As the concentration of IgA in the saliva increased, the concentration of IgM tended to decrease. This reciprocal relation between IgA and IgM has been noted in the saliva of several patients with other disorders not included in this report. IgG was not detected in the saliva of any of the patients, despite normal serum IgG concentrations.

No low molecular weight IgM was found in the saliva. This may indicate difficulties in quantitation rather than a lack of 7 S IgM. Small amounts of IgM are present in saliva relative to the amounts in serum, and if only 10% of the salivary IgM were low molecular weight, as in serum, it would not have been detected with the techniques employed.

It is interesting to note that the isohemagglutinin titers in the serum and saliva of the patients with ataxia telangiectasia were essentially normal and bear no apparent relation to the frequency of infection (Table I). Isohemagglutination in both the serum and saliva of the patients with ataxia telangiectasia could be inhibited with antisera against IgM, but not with antisera against IgG or IgA.

Discussion

Our findings that 17% of patients with disseminated lupus have 7 S IgM are comparable to those of Rothfield and her colleagues (5), who reported an incidence of 15% for low molecular weight IgM antinuclear factor in patients with systemic lupus erythematosus. Also of interest is the high incidence of males with 7 S IgM, findings again similar to those of Rothfield. The 7 S IgM seems to occur with particularly high frequency in lupus patients having low serum IgA, although more patients must be studied before a definite relationship is established.

The 7 S IgM found in the serum of these patients seems to occur naturally and is not formed from the high molecular weight IgM, at least as tested under *in vitro* conditions. Although the incubation experiments suggest that reductive cleavage does not occur *in vitro* in serum, it could occur *in vivo* perhaps intracellularly, by proteolysis, or by some type of disulfide interchange reaction. Since storage of preparations containing reasonably high concentrations of 7 S IgM does not result in reaggregation, it would be expected

that if free sulfhydryl groups are formed intracellularly, they are either blocked or buried, and thus are unreactive.

The low molecular weight IgM is similar to the 7 S subunits produced by reduction of the 19 S protein in two respects: 1) Unlike the 6.6 S subunits produced by enzymatic degradation, it shows immunological identity with 19 S IgM. It is not known, however, whether the naturally occurring 7 S IgM contains antigenic determinants that are either not present or are buried in the native 19 S IgM and its reductive subunit. Work is now in progress attempting to prepare antisera specific for the 7 S IgM. 2) The 7 S IgM may possess univalent antibody activity as suggested by the finding of only indirect isohemagglutination, although further studies are necessary to establish this point with certainty. It is of interest that univalent-like antibody activity has been reported with the 7 S reductive subunits by several workers (15–17).

The 7 S IgM is similar in its sedimentation properties to IgG. The small differences noted between the 7 S IgM and IgG in their distribution on the Bio-Gel column may indicate differences in either molecular size or shape. These findings are consistent with the observations of Miller and Metzger (18) demonstrating significant differences in the elution patterns of the H chains of IgM and IgG on Sephadex G-200. This was found to be a result of the higher molecular weights of the H polypeptide chain of IgM. However, whether the differences noted in our studies are indeed significant awaits verification by molecular weight determinations on purified preparations of 7 S IgM.

The evolution of the immune response is thought to parallel rather closely the development of the lymphoid system, particularly the thymus (19). As previously mentioned, both the smooth dogfish and lemon shark, whose thymic development is intermediate between the lowest vertebrate and man (20), have a single immunoglobulin system most closely related, by starch gel electrophoresis, to the human IgM system. In this species, both 7 S and 17 S immunoglobulins exist that are antigenically identical, a situation quite similar to the IgM system described in our patients. It is interesting, therefore, that thymic hypoplasia, lymphopenia, and dysplasia of the lymphoreticular system have been reported in ataxia telangiectasia

(21–23) and, although the evidence is less convincing, in disseminated lupus (24, 25). Whether patients having the low molecular weight IgM possess, for unknown reasons, a more primitive immune system is an interesting but unproven hypothesis.

In this small group of patients with ataxia telangiectasia and disseminated lupus, no correlation was observed between the severity of sinopulmonary infections and the levels of salivary γ-globulins. Although IgA was deficient or absent from the saliva of the majority of the patients whose serum lacked IgA, the concentration of salivary IgM was as great as or greater than the total γ-globulin concentration of normal saliva. The IgM present in saliva does have antibody activity as demonstrated by the occurrence of isohemagglutinins that are specifically inhibited by an anti-IgM antiserum. It seems unlikely that selective serum or salivary deficiencies of IgA in themselves can account for the increased susceptibility of these patients to infections. Normal individuals lacking serum or salivary IgA do not necessarily demonstrate an increased incidence of sinopulmonary infections (26, 27). Moreover, patient R.S., who had an essentially normal salivary IgA, did show an increased susceptibility to infections. It may be that the susceptibility to infections displayed by the patients with ataxia telangiectasia is more attributable to defects in delayed hypersensitivity than to immunoglobulin abnormalities. However, whether the IgM noted in the secretions of these patients has effective antibody activity (other than isohemagglutination) is not presently known. Further studies of the immunoglobulin levels, and particularly the secretory antibody response to various types of antigenic challenge in a larger group of patients, are necessary before definite conclusions can be reached.

Acknowledgments

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