Quantitative Determination of Serum Immuno- globulin D in Gammopathy

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Serum immunoglobulin D (IgD) level was determined in 10 normal subjects, 5 cases of IgD-myeloma and 23 cases of various autoimmune diseases by the antibody-agar plate method using the antiserum against the heavy chain of IgD, which had been prepared in a rabbit immunized with an IgD-myeloma protein.

The serum IgD level was below 0.05 mg/ml in normal subjects and above 10 mg/ml in IgD-myeloma cases. In one case, IgD levels were serially followed; the level dropped to 1.0 mg/ml by administration of cyclophosphamide. More than a half of cases of various autoimmune diseases showed the serum IgD levels of above 0.05 mg/ml. The highest level in this autoimmune disease group was 0.14 mg/ml in a case of aortitis. All of the 4 cases of aortitis studied showed IgD levels of above 0.05 mg/ml. Whether serum IgD elevation in autoimmune diseases is an expression of general elevation of immunoglobulins or represents the elevation of antibodies specific for these diseases awaits further studies.

A new type of myeloma protein, IgD-myeloma protein, was discovered in 1965.¹ Since then, case reports of IgD-myeloma have been gradually increasing.²⁻⁶ The normal counterpart of IgD-myeloma protein, immunoglobulin D (IgD, γ D) was also discovered.⁷ Accordingly, IgD may be expected to be present in sera of various diseases other than IgD-myeloma, and it would be significant to know whether noticeable increase of IgD is seen or not in gammopathies other than IgD-myeloma.

Quantitation of IgD can be performed by single radial immunodiffusion technique, if specific antiserum against the heavy chain of IgD is available.^{8,9} This report describes the results of serum IgD quantitation in various diseases, in which electrophoretically broad-banded hypergammaglobulinemia (polyclonal gammopathy¹⁰) is frequently observed and autoimmune basis of their etiology is strongly suspected.

MATERIALS AND METHODS

Materials

Sera were obtained from the following cases and were stored at -20°C .

- 1) Normal subjects. Sera from ten healthy individuals were used as controls.
- 2) IgD-myeloma cases. Sera from five cases of IgD-myeloma were available. In one case, serum IgD levels were serially followed with the administration of cyclophosphamide.
- 3) Diseases in which autoimmune basis is strongly suspected as their etiology and in which polyclonal gammopathy is frequently observed. For this group, 6 cases of systemic lupus erythematosus, 3 cases of rheumatoid arthritis, 4 cases of Sjögren's syndrome, 4 cases of aortitis (pulseless disease), one case of thrombotic thrombocytopenic purpura, one case of dermatomyositis, and 4 cases of liver cirrhosis were selected. In all of these cases, sera were obtained soon after admission to the hospital so that the values of IgD obtained might represent ones in active stage of the diseases.

*Methods*1) *Preparation of anti-IgD serum*

IgD-myeloma protein was obtained from a type-L IgD-myeloma serum by a modification of block electrophoresis as described by Fahey and McLanghlin.¹¹ For this, agar was employed as the supporting medium, and pH 8.6 veronal buffer of 0.05 ionic strength was used. The portion of IgD-myeloma protein was visible as a transparent zone in agar after electrophoresis. This portion of IgD-myeloma was separated by cutting from other portions and solved in physiologic saline. The amount of IgD was roughly calculated from the total protein concentration of the serum and the percentage of IgD peak determined by cellulose acetate electrophoresis. About 5 mg of IgD-myeloma protein thus obtained were used for immunizing a rabbit. This procedure was repeated several times at 2-week intervals. The antiserum thus obtained contained not only precipitating antibodies against the heavy chain of IgD, but also antibodies against the light chain and IgG. Therefore, the prepared antiserum was adsorbed by Cohn F-II and type-L Bence Jones protein, and further by normal human serum. Thus the antiserum became specific to the heavy chain of IgD, and on immuno-electrophoresis it gave a precipitate line only to IgD-myeloma serum, but not to normal human serum (Fig. 1).

Fig. 1. Immuno-electrophoresis with anti-IgD serum.

a: IgD-myeloma serum b: anti-IgD serum c: normal serum

2) Quantitation of IgD

The antibody-agar plate method was employed.⁸ The antiserum was incorporated into the agar in a ratio of 1:15. The test sera and IgD standards were placed in the antigen wells, and the precipitate rings were measured after diffusion at room temperature for 16 hours. The initial standard serum was

Fig. 2. Antibody-agar plate for IgD quantitation.
Top line: standards. Middle and bottom lines: test samples.

kindly supplied by Dr.J.L. Fahey, National Cancer Institute, Bethesda, Maryland, U.S.A., and IgD-myeloma serum and its two-fold dilutions were employed, as demonstrated in Fig. 2. The logarithm of IgD concentrations was proportional to the diameter of the precipitate ring.

3) Quantitation of IgG, IgA and IgM

For this, Hyland Immunoplates with attached standards were employed as described previously.¹²

RESULTS

1) Normal subjects

Ten sera from normal subjects gave visible precipitate rings, but their diameters were too small to give reliable IgD values. In all of these sera, the IgD level was below 0.05 mg/ml.

2) IgD-myeloma cases

In all cases of IgD-myeloma, IgD is distinctly increased, while other immunoglobulins are rather decreased, as shown in Table 1. This is natural, since IgD myeloma is monoclonal gammopathy of IgD. In one case of IgD-myeloma, IgD levels were serially followed in the course of cyclophosphamide administration, as shown in Fig. 3. The percentage of plasma cells in bone marrow smears was examined for comparison with the serum IgD level at the corresponding time.

3) Polyclonal gammopathies

As shown in Table 2, the IgD level was more than 0.05 mg/ml in 12 out of 23

TABLE 1.

Diagnosis	Serum immunoglobulin (mg/ml)			
	G	A	M	D
IgD-myeloma 1*	10.6	1.4	0.7	14.5
"				9.1
"				9.1
"				1.2
"				1.0
"				1.5
"				1.8
"				2.4
IgD-myeloma 2	11.6	1.4	0.5	12.5
IgD-myeloma 3	12.2	1.3	0.7	10.0
IgD-myeloma 4				60.0
IgD-myeloma 5				11.5
Normal ^a	18.5±3.9	3.6±0.7	1.3±0.4	>0.05

* Serum IgD levels of Case 1 were followed serially.

TABLE 2.

Diagnosis	Serum immunoglobulin (mg/ml)			
	G	A	M	D
SLE 1				<0.05
SLE 2	21.0	2.8	1.1	<0.05
SLE 3				<0.05
SLE 4	30.5	2.9	1.0	0.05
SLE 5	20.0	2.9	0.9	<0.05
SLE 6	43.0	4.0	1.6	0.12
RA 1	17.0	2.3	1.1	<0.05
RA 2	30.5	3.1	1.2	<0.05
RA 3	17.5	3.8	1.2	0.07
Sjögren 1				<0.05
Sjögren 2	51.0	5.2	3.0	<0.05
Sjögren 3	40.5	5.5	1.5	0.06
Sjögren 4	34.0	3.6	1.5	<0.05
Aortitis 1	30.0	3.2	2.5	0.14
Aortitis 2	21.0	5.6	4.0	0.07
Aortitis 3	42.5	5.1	2.6	0.08
Aortitis 4				0.07
TTP	21.0	2.4	1.5	<0.05
Dermatomyositis	30.5	3.6	1.5	0.05
Liver cirrhosis 1	30.0	3.9	3.5	<0.05
Liver cirrhosis 2	22.6	7.3	8.5	0.07
Liver cirrhosis 3	22.0	3.9	9.5	0.05
Liver cirrhosis 4	28.0	4.1	1.4	0.07

cases. The IgD level was not necessarily related to the levels of other immunoglobulins in the same serum. The highest value of IgD of this group was 0.14 mg/ml. This value was significantly higher than the normal, but far below that in IgD-myeloma, in which the lowest value was 1.0 mg/ml. It is noticeable that all cases of aortitis showed the values above 0.05 mg/ml.

DISCUSSION

In the present study, the normal value of IgD was estimated to be below 0.05 mg/ml. Rowe and Fahey⁷ reported that the median serum IgD concentration among healthy subjects was 0.03 mg/ml, and that the normal values ranged from less than 0.003 mg/ml. This suggests that there are normal individuals with high serum IgD level.⁷ It seems, however, that a more accurate method such as radioimmunoassay¹³ is required to establish the normal values.

It was reported that there was minor antigenic difference between IgD-myeloma proteins and normal IgD.³ However, the error caused by this antigenic difference seems really insignificant for the quantitation of IgD,⁷ and it will be neglected when the IgD level is serially followed in individual cases, as shown in Fig. 3.

Fig. 3. Serial determination of serum IgD levels (IgD-myeloma 1).

In some disorders such as connective tissue diseases, liver diseases, infections, etc., electrophoretically broad-banded hypergammaglobulinemia is observed, and the above disorders are called polyclonal gammopathies.¹⁰ It is well recognized that simultaneous increases of IgG, IgA and IgM are seen in these disorders, while only one kind of immunoglobulin is increased and other immunoglobulins^{14,15} are decreased in monoclonal gammopathies. In polyclonal gammopathies, it may be assumed that IgD is also increased. But, this point has not been clarified, because IgD was identified recently and quantitation of IgD has not yet been

generalized, as compared with that of IgG, IgA and IgM.

Our study indicates that IgD is significantly elevated in some of polyclonal gammopathies. It seems reasonable to assume that this IgD elevation is only a phenomenon in company with the elevation of other immunoglobulins. It should be noted, however, that all of 4 cases of aortitis showed IgD elevation in our study. This may be analogous to IgM elevation in infectious mononucleosis observed by Wollheim and Williams.¹⁶

Compared with other immunoglobulins, the antibody activity of IgD has not yet been clarified. Kohler and Farr reported that IgD was more increased in reaginic patients than in non-reaginic ones.¹⁷ However, the role of IgD as reaginic antibody was questioned. On the contrary, IgE is now regarded on more sound grounds as a carrier of reaginic activity.^{18,19} The role of IgD in immunological phenomena will be clarified probably by the development of a more refined technique of IgD quantitation.

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