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Immunoglobulin G, A, and M Light Chain Ratios in some Humoral Immunological Disorders

Á. HARALDSSON, M. JAMINON, J.A.J.M. BAKKEREN, G. B. A. STOELINGA & C. M. R. WEEMAES
Department of Paediatrics, University Hospital Nijmegen, Nijmegen, The Netherlands

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The total kappa/lambda immunoglobulin light chain ratio and the kappa/lambda ratios within each of the serum immunoglobulin classes G, A, and M were measured in thirteen patients with humoral immunological disorders. Of those patients, eight had common variable immunodeficiency whereas five patients had other forms of humoral immunological deficiencies. Eleven patients had abnormal antibody response in vivo. All but three of the thirteen patients had clearly abnormal light chain ratios in one or more of the immunoglobulin classes.

We conclude that humoral immunologcal disorders, usually characterized by abnormal heavy chain production and a disturbed antibody response, may frequently have a concomitant abnormal synthesis of the light chains resulting in an abnormal kappa/lambda light chain ratio.

Á Haraldsson, Department of Paediatrics, University Hospital Leiden, Postbox 9600, 2300 RC Leiden, The Netherlands

The total immunoglobulin kappa/lambda (κ/λ) light chain ratio has been found to deviate from the normal in a variety of infectious diseases [1], autoimmune disorders [1–4], and other immunological abnormalities [1, 5]. A κ/λ light chain imbalance has also been described in some humoral immune deficiencies, usually superimposed on an altered heavy chain production affecting all or some of the immunoglobulin classes [5, 6]. These studies have hitherto primarily been done on total κ/λ ratio. As IgG usually represents the bulk of the total serum immunoglobulins, the total κ/λ ratio reflects primarily the IgG κ/λ ratio.

Light chain restricted antibody responses which primarily contain either kappa- or lambda-bearing immunoglobulins have been demonstrated following stimulation by certain antigens [7–9]. Some immunological disorders presenting with abnormal heavy chain concentrations also have a defective antibody response [10]. The light chains have not been investigated in this context.

Recently methods have been described for measuring not only total κ/λ ratio but also polyclonal IgG, IgA, and IgM κ/λ ratios separately [11, 12], encouraging further work on the issue. In addition, reference values for polyclonal IgG, IgA, and IgM κ/λ ratios according to age are known [13].

In order to investigate a possible relationship between abnormal heavy and light chain production, κ/λ ratios in IgG, IgA, and IgM as well as total κ/λ ratio were studied in patients with some humoral disorders. Abnormal light chain ratios in these patients might help explain the abnormal antibody response found in vivo.

PATIENTS AND METHODS

The total number of patients enrolled in the study was thirteen (Table I). Eight patients had common variable immunodeficiency (CVID), two patients had immunodeficiency with hyper IgM and three patients had a functional antibody deficiency. Of those patients with functional antibody deficiency, one had antibody deficiency with normal immunoglobulin concentrations, one had IgM deficiency and diminished antibody production, and one patient had antibody deficiency, IgG_2, IgG_4 and IgM deficiency as well as growth hormone deficiency (Table I).

The serum samples were collected when the patients

TABLE I. Clinical symptoms and immunological results in thirteen patients with various humoral immunological disturbances

Patient no.	Age (years)	Sex	Other complications	B cells (%)	IgG (g/l)	IgA (g/l)	IgM (g/l)	IgD (U/l)	IgE (U/l)	Iso aggl.[1]	DTP[2]	HPH[3]	T cells (%)	Stimulation by antigen	PHA	PWM
CVID																
1	4	M	IgG 1,2 and 4 def.,	0.3	0.8	<0.02	0.4	88	1	neg.	neg.	ND[4]	N[5]	pos.	N	N
2	4.5	F	hyper IgG3	<1	3.6	1.8	1.3	102	14	neg.	neg.	neg.	N	pos.	N	N
3	4	M	Neutropenia	11	—[6]	<0.02	6.7	<2	<2	pos.	neg.	ND	N	pos.	N	N
4	4.5	M	Haemol. anaemia	7	2.1	0.4	0.2	<2	<2	ND	ND	neg.	N	ND	decr.	N
5	9	F	Juvenile RA, intractable diarrhoea	8	1.6	<0.02	2.9	<2	<2	neg.	neg.	neg.	N	pos.	N	decr.
6	14	M	Chr. aggr. hepatitis, alopecia totalis, RA	3	—[6]	<0.02	2.0	<2	<2	pos.	neg.	ND	N	decr.	decr.	N
7	16	M	IgG2 and 4 def.	7	5.4	<0.02	0.4	6	0.5	low	neg.	neg.	N	pos.	N	N
8	17	F	IgG2 and 4 def.	23	5.6	<0.02	0.2	<2	<2	ND	low	neg.	N	pos.	N	N
Immunodeficiency with hyper IgM																
9	24	F		16	<2	<0.02	17.5	<2	<2	pos.	pos.	neg.	N	pos.	N	N
10	28	M		9	<2	<0.02	17.3	<2	<2	pos.	pos.	pos (M)[7]	N	pos.	N	N
Antibody deficiency syndrome																
11	12	M		1	12.6	4.7	0.2	144	85	low	low	neg.	N	pos.	N	decr.
12	1.5	F	Chronic diarrhoea	12	6.6	3.4	1.0	1	5	neg.	low	ND	N	pos.	N	N
13	3	F	Growth hormone def. IgG4 and IgM def.	2	3.9	1.1	0.2	25	<2	neg.	neg.	neg.	N	pos.	N	N

[1] Isoagglutinin titre; [2] DTP: antibodies to diphtheria, tetanus and poliomyelitis after vaccination; [3] HPH: IgG, IgA, and IgM antibodies to helix pommatia haemocyanin after vaccination; [4] ND: not done; [5] N: normal; [6] Immunoglobulin substitution; [7] Only IgM antibodies.

were still presenting some immunoglobulins in the serum but not receiving immunoglobulins or immunomodulating treatment except for patients 3 and 6 who were already receiving immunoglobulin substitution (Table I). Both were receiving monthly doses of intramuscular injections of immunoglobulins. Their IgG κ/λ ratio is not shown as this reflects only the substitution. Patients 7 and 8 had IgG levels of 5.4 and 5.6 g/l respectively when serum was sampled for this study. However, both patients had antibody deficiency and their IgG concentration was decreasing, reaching the lowest level in patient 7 of 2.2 g/l before treatment with immunoglobulins was started. The serum samples of all the patients had been obtained at different times and kept frozen at −20°C until measured.

All patients had recurrent infections. Patient 4 also had autoimmune haemolytic anaemia and patient 5 developed severe juvenile rheumatoid arthritis. Patients 5 and 12 had protracted diarrhoea. Patient 6 had recurrent herpes zoster infections, chronic aggressive hepatitis, alopecia totalis as well as rheumatoid arthritis. Patient 3 had agranulocytosis (Table I).

The concentrations of immunoglobulin G, A, and M were measured by ELISA [11] and the total κ/λ ratio was measured by nephelometry as described earlier [5]. The κ/λ ratios for each of the serum polyclonal immunoglobulin classes G, A, and M were measured by using a solid-phase ELISA-sandwich method [11]. Affinity-purified goat antiserum directed against human gamma, alpha or mu heavy chains were used to capture IgG, IgA or IgM, respectively. Peroxidase-labelled goat anti-κ or anti-λ antiserum was used as a second antibody. As standard serum, pooled serum from 500 healthy Dutch blood donors was used. As control serum another batch of pooled serum from 500 healthy Dutch blood donors was measured at three different dilutions which were expected to lie at different points on the standard curve. The absorption was measured at 492 nm and the concentration of the κ and λ light chains was determined from a standard curve.

The results of the IgG, IgA, and IgM κ/λ ratio determinations were compared to a reference group [13] consisting of 134 healthy individuals, 114 children aged one month to 16 years and 20 adult blood donors.

RESULTS

The κ/λ light chain ratios deviated from our reference values in ten of the thirteen patients for one or more of the immunoglobulin classes or for the total κ/λ ratio (Fig. 1). Patients who developed CVID in early childhood (patients 1–5) tended to have more disturbed ratios than those presenting later in life (patients 6–8). For patients 3 and 6, IgG concentration and IgG κ/λ ratio is irrelevant as it reflects the immunoglobulin substitution whereas the IgM κ/λ ratio primarily concerns the patients' own immunoglobulin synthesis. Patient 12 who had protracted diarrhoea had only a raised IgA κ/λ ratio with the other ratios within the normal range. Patient 13 with antibody deficiency syndrome and growth hormone deficiency expressed increased κ/λ ratios for all classes measured. Patient 1 had the lowest κ/λ ratios but did not differ clinically from the other patients with agammaglobulinaemia. Usually, in a given patient, the ratios were either all raised or all decreased.

All patients except patients 9 and 10 had a disturbed antibody response in vivo, measured as antibody titres against diphtheria, tetanus or poliomyelitis after immunization and/or as isoagglutinin titres (Table I). Patient 4 has not been immunized but his antibody response against various respiratory tract micro-organisms (adenovirus, coronavirus, influenza A and B, parainfluenzae 1, 2 and 3, respiratory syncytial virus and mycoplasma) is absent. Peripheral blood lymphocyte stimulation by antigens in vitro was tested in twelve of the patients and was normal in all but one. Antigen synthesis after helix pommatia haemocyanin vaccination was negative in eight out of the nine patients tested. The proliferative response of peripheral blood lymphocytes to PHA or PWM in vitro was normal, or only slightly disturbed, in all patients (Table I). The number of B cells varied but the number of T cells (E-rosette forming cells or $CD3^+$ cells) were normal in all patients (Table I). When tested, $CD4^+$ and $CD8^+$ cells were normal except for patients 10 and 12 expressing rather low $CD4^+$ and $CD8^+$ cells, respectively.

DISCUSSION

Our findings indicate that in patients with CVID or other humoral disturbances the production of the light chains as well as of the heavy chains is affected. In the four patients who developed CVID in childhood before puberty and did not receive immunoglobulin treatment, all κ/λ ratios were abnormal, either increased or decreased. Only in the two patients with IgG_2, IgG_4, IgA and IgM deficiency, developing late onset agamaglobulinaemia after puberty, were all κ/λ ratios normal. Therefore, it can be suggested that the regulation of the light chains is affected in different ways, depending on the cause of the agammaglobulinaemia.

In an earlier study, an age-related increase of the total κ/λ ratio, and IgG and IgM κ/λ ratio was found whereas a decrease of the IgA κ/λ ratio was

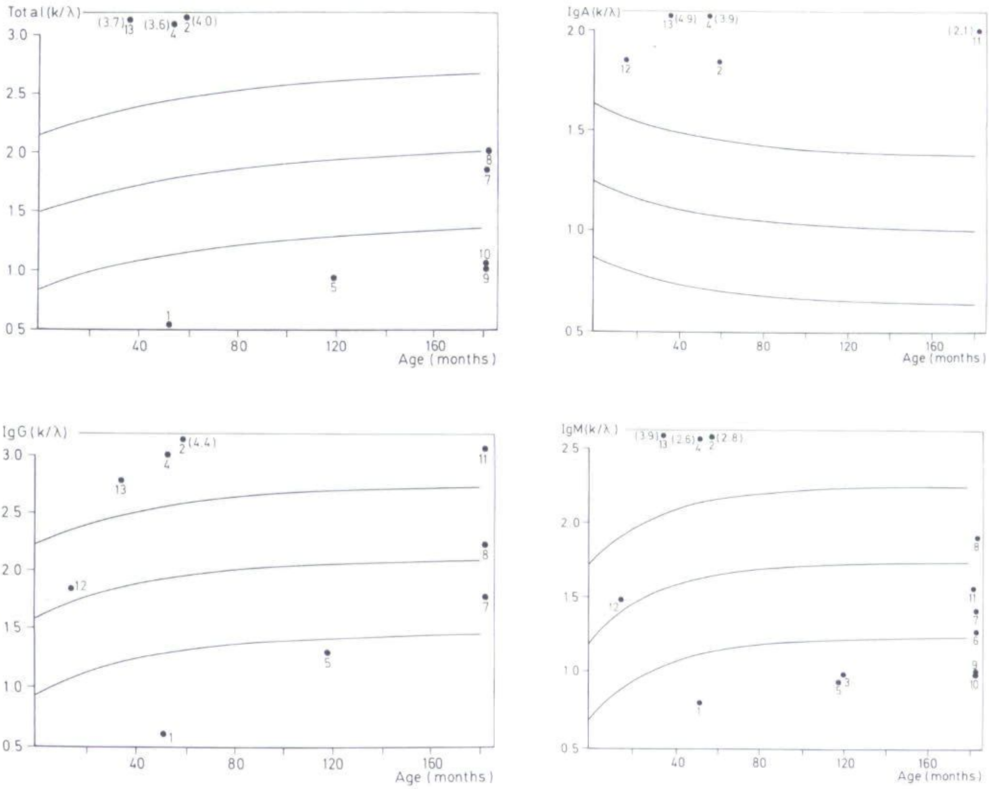

FIG. 1. κ/λ ratios of total immunoglobulins, IgG, IgA, and IgM in patients with humoral immunological disorders. Patients 1–8: CVID; patients 9 and 10: immunodeficiency with hyper IgM; patients 11–13: antibody deficiency syndrome (solid lines indicate 5%, 50% and 95% confidence limits [13]).

noted [13]. Apparently, in healthy children the regulation of the light chain synthesis is different for those various immunoglobulin classes. In pathological situations, however, abnormal κ/λ ratios were demonstrated for all serum immunoglobulins measured in several patients, indicating a more general defect in the light chain regulation.

In patients 11, 12 and 13 with antibody deficiency, the κ/λ ratios were either normal or raised; decreased κ/λ ratios were not found. One might expect that the light chains play an important role in the antibody response. However, the primary response was impaired after immunization in ten of the eleven patients tested, independent of whether the patients had normal or abnormal κ/λ ratios. The response to a booster immunization was only positive in two sibs with agammaglobulinaemia with hyper IgM. Both expressed strongly increased IgM levels and a decreased IgM κ/λ ratio. The isoagglutinin titre—also an IgM antibody—was normal in these patients. This was also demonstrated in patient 3 who had a slightly decreased IgM κ/λ ratio. This indicates that despite a disturbed κ/λ ratio the antibody response may be normal. However, although the IgM κ/λ ratio was decreased in these three patients, they all expressed high concentrations of IgM κ as well as IgM λ as their serum level of IgM was extraordinarily high. Whether normal antibody levels can be produced with normal or decreased serum immunoglobulin levels and abnormal light chain ratios remains questionable.

We conclude that light chain production is affected in some humoral immunological disorders presenting with a disturbed heavy chain concentration. Furthermore we conclude that both heavy and light chains can most probably be simultaneously but independently stimulated or inhibited in some immunological disorders. Disturbed light chains may contribute to impaired antibody response in vivo or may possibly be a result of this abnormal response.

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