Hypogammaglobulinemia Associated with Accelerated Catabolism of IgG Secondary to its Interaction with an IgG-Reactive Monoclonal IgM

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ABSTRACT Hypogammaglobulinemia due to a new pathophysiological mechanism was studied in a patient with Sjögren's syndrome, a monoclonal IgM and a mixed (IgM-IgG) cryoglobulinemia. The IgM (IgM_{DK}) component of the cryogel possessed light chains of λ-type with highly restricted electrophoretic mobility analagous to those of a Waldenström's macroglobulin. IgM_{DK} reacted specifically with native IgG, with IgG subclasses 1, 2, and 4, and with the Fc piece of IgG to form a cryogel. Serum concentrations of IgG 1, 2, and 4 were 10% of normal, whereas the IgG3 level was slightly increased and the IgM level was markedly increased. Viscosity and analytical ultracentrifugation studies with the purified mixed cryogel (IgM-LgG) indicated soluble complex formation over a temperature range (36–38°C) attainable in vivo. Immunoglobulin turnover studies revealed a markedly elevated rate of IgM synthesis with a normal survival of IgM, IgA, and IgE. IgG3, which failed to form complexes with IgM_{DK} at body temperature, had a normal synthetic rate and survival. In contrast, the other IgG subclasses showed reduced synthesis and shortened survival. These studies are the first indicating a short survival of some IgG subclasses with a normal survival of another. The hypogammaglobulinemia appears to be due in part to a new mechanism of accelerated protein catabolism: The rapid elimination of IgG due to its interaction with an IgG-reactive monoclonal IgM.

INTRODUCTION

Over a dozen immunological deficiency diseases with associated hypogammaglobulinemia have been described. A reduction in the serum concentration of one or more of the immunoglobulin classes may occur secondary to a variety of pathophysiological mechanisms that affect the rates of synthesis, the distribution, or the rates of catabolism or loss of these proteins (1). Hypogammaglobulinemia may result from decreased synthesis of all major classes of immunoglobulins (2–13). Alternatively, a defect in immunoglobulin synthesis may be restricted to one or two classes of immunoglobulins, as in patients with ataxia telangiectasia (14) or the other dysgammaglobulinemias (12, 15). A second major pathophysiological mechanism resulting in hypogammaglobulinemia is excessive loss of serum proteins into the urinary, respiratory, or gastrointestinal tracts (7, 16–18). A third major mechanism resulting in hypogammaglobulinemia is hypercatabolism of immunoglobulins. This hypercatabolism may involve different classes of serum proteins, as in the recently reported syndrome of familial hypercatabolic hypoproteinemia (19). Alternatively, the hypercatabolism may be restricted to a single class of proteins as in the isolated hypercatabolism of IgG in patients with myotonic dystrophy (20). The cause of the hypercatabolism of serum proteins in these disorders has not been defined.

We now report on another cause of hypogammaglobulinemia with reduced immunoglobulin survival. The hypogammaglobulinemia, affecting three subclasses of IgG, (G1, G2, and G4) is associated with an abnormal immunoglobulin-immunoglobulin interaction. The patient studied has Sjögren's syndrome with a mixed (IgM-IgG) cryoglobulinemia and a high serum level of a monoclonal IgG-reactive IgM, a slightly elevated concentration of IgG3, and a markedly reduced concentration of the other IgG subclasses. The patient's IgM forms complexes with IgG1, IgG2, and IgG4 subclasses

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over a range of temperatures attainable in vivo. The rate of synthesis of these IgG subclasses is markedly depressed. The survival of these subclasses is shortened in the patient presumably due to the formation in vivo of IgM-IgG complexes which are rapidly catabolized. IgG3 subclass molecules do not complex with the patient's IgM at body temperature and have a normal survival and synthetic rate.

METHODS

Patient. The patient, D. K., is a 73 yr old white woman with the diagnosis of Sjögren's syndrome who presented with an 8 yr history of recurrent fever, usually associated with cold exposure, and a 5 yr history of recurrent parotitis with saccular parotid sialectasis, dacryocystitis, otitis media, and anemia. Her clinical findings have been published (21). Lobectomy for a cavitary right upper lobe lesion revealed an infiltrate of plasma cells and small lymphocytes consistent with the diagnosis of pseudolymphoma (21). Additional features of her illness are a rheumatoid factor titer by bentonite flocculation test of $>1:8192$, depressed total serum hemolytic complement, elevated serum IgM, depressed IgG, and a striking mixed cryogelglobulinemia consisting of IgM and IgG. Five attempts to immunize her with typhoid antigen and two attempts with Foshay tularemia and *Escherichia coli Vi* antigens were unsuccessful. She was successfully sensitized to dinitrochlorobenzene.

Isolation and characterization of the components of the patient's cryogel. The mixed cryogel was isolated by chilling the patient's citrated plasma to 4°C in an ice bath. It was freed of other serum proteins by repeatedly dissolving it in pH 7.0, 0.01 M sodium phosphate buffer made 0.05 M in NaN_3 at 40°C followed by regelling at 4°C.

The two components of the cryogel were purified by first redissolving the gel in 1.0 M KSCN followed by ultracentrifugation at 40°C. The IgM component was isolated from the pelleted material by DEAE-cellulose chromatography at 40°C. The IgG component was obtained from the supernatant of the ultracentrifugation by DEAE-cellulose chromatography.

Analytical ultracentrifugation of the purified cryogel or its subunits was performed at various temperatures in the Spinco Model E ultracentrifuge (Beckman Spinco, Palo Alto, Calif.) at 56,100 rpm. The association of the purified IgM_{DK} from the patient's cryogel with an IgG1 myeloma protein or an IgG3 myeloma protein was studied at rotor temperatures from 25° to 40°C as described by Stone and Metzger (22).

The components of the cryogel were identified by immunoelectrophoresis and double diffusion methods using sheep antisera to human immunoglobulin heavy chains and to human K and λ-light chains.

Analytical polyacrylamide disc gel electrophoresis of the light chains obtained from IgG_{DK} and IgM_{DK} was performed using a 7% monomer polyacrylamide gel in 10 M urea according to the method of Reisfeld and Small (23).

Specificity of the interaction between IgM_{DK} and serum proteins

D. K. serum (1.0 ml) was mixed with ^{125}I-labeled serum proteins at 40°C, incubated for 10 min, then cooled in an ice bath before centrifugation at 3000 rpm at 4°C. The supernatant was removed and discarded and 5 ml of 0.15 M saline was added to the cryogel. The cryogel was then warmed to 40°C to bring it into solution. This process was repeated five times. The radioactivity remaining in the gel was then determined in a well-type gamma ray scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Additional studies were performed to determine which purified proteins would cause purified IgM_{DK} to gel. In these studies 0.1 mg of IgM_{DK} radiolabeled with ^{125}I was incubated with various proteins at a molar ratio of 7:1 (protein to IgM_{DK}). The reactants were incubated in 0.3 ml of pH 7.0, 0.01 sodium phosphate, 0.1 M sodium chloride buffer at room temperature for 1 hr, refrigerated at 4°C overnight, and then centrifuged at 2000 rpm at 4°C for 1 hr. The radioactivity retained in the precipitate was then determined.

Viscosity measurements. Viscosity measurements employed an Ostwald capillary viscosimeter with a water free-fall time of 39.0 sec at 50°C. Temperature control was maintained at ±0.2°C in a water bath monitored by a precision total immersion thermometer (Fisher Scientific Co., Pittsburg, Pa.).

Quantitation of immunoglobulins. Immunoglobulin concentrations were determined by the radial diffusion method of Mancini, Carbonara, and Heremans (24) at 40°C using Hyland Immunoplates (Hyland Div., Travenol Labs, Inc., Costa Mesa, Calif.). IgG concentrations were also determined in the presence of 0.001 M dithiothreitol with results agreeing closely with those determined at 40°C in the absence of a reducing agent. IgG3 levels were determined by a solid phase radioimmunoassay described by Mann, Granger, and Fahey (25). The sum of IgG1, 2, and 4 levels were determined from the difference between the total IgG level and the IgG3 level.

Preparation of labeled proteins for turnover study. The preparation of IgG, IgA, IgM, IgG3, and albumin for labeling was performed by techniques that have been described previously (8, 9, 14, 26). IgE was obtained from the serum of a patient with an IgE myeloma protein by DEAE-cellulose chromatography. Albumin was prepared from normal serum by Geon[1] and Pevikon[2] block electrophoresis. Each of the preparations was analyzed by radioimmunoelectrophoresis and Ouchterlony double diffusion, using antisera to whole human serum, albumin, IgG, IgA, IgM, and transferrin. All of the preparations were found to be free of contaminating proteins using these techniques.

Iodination of the above proteins was performed with either ^{131}I or ^{125}I by the iodine monochloride technique of McFarlane (27). All preparations were calculated to have an average of less than one atom of iodine per molecule of protein in the final product. The products contained less than 1% nonprecipitable radioactivity.

Study protocol. Each of the radiolabeled serum proteins was administered to the patient and to at least three control subjects who had diseases not affecting serum protein metabolism. From 10 to 50 μCi of the iodinated proteins were administered intravenously from a calibrated syringe and serum samples were obtained 10 min after administration and daily thereafter. Urine specimens were collected in 24-hr lots. Serum and urine samples were counted with appropriate standards to within ±3% counting error in an automatic gamma ray scintillation counter.

Calculation of the data. The time course of decline of radioactivity from the serum and whole body was plotted semilogarithmically. These curves were used to determine

[1] Geon Resin, B. F. Goodrich Co., Niagara Falls, N. Y.
[2] Pevikon, Superfosfat, Fabrika, Aktiebolog, Stockholm, Sweden.

the plasma volume, total circulating protein pool, total exchangeable protein pool, fraction of the circulating pool catabolized per day, the survival $t_{\frac{1}{2}}$ and the synthetic rate of the protein, according to the method of Nosslin (28).

RESULTS

Characterization of the gel. The purified cryogel isolated from the plasma of D. K. examined by analytical ultracentrifugation at 40°C in pH 8.6 tris-HCL buffer was shown to contain two major sedimenting components, one with a sedimentation constant of 7S and the other of 18S. The 7S component was identified immunologically as IgG (IgG_{DK}) and shown to be composed of electrophoretically heterogeneous molecules containing both κ- and λ-light polypeptide chains. The purified 18S component was identified as IgM (IgM_{DK}), and in contrast to the IgG component was demonstrated to be antigenically homogeneous with respect to light polypeptide chains containing only λ-antigenic determinants. The light polypeptide chains prepared from IgM_{DK} when examined by alkaline urea polyacrylamide disc gel electrophoresis were of highly restricted mobility analogous to those of a Waldenström's macroglobulin, However, the light chains from IgG_{DK} were electrophoretically heterogenous. Thus, the IgM component of the mixed cryogel had the characteristics of a monoclonal protein while the IgG was comparable to normal heterogeneous IgG.

Specificity of the interaction of IgM_{DK} with IgG and IgG subunits

Neither the isolated IgG_{DK} nor the isolated IgM_{DK} would form a cryogel in vitro in the pH 7 and ionic strength solvent conditions studied. The IgM_{DK} would, however, form a cryogel if normal serum or normal IgG was incubated with it. The IgG_{DK} would neither form a cryogel under these circumstances nor if incubated with other purified IgM proteins. It was thus felt that the cryogel of patient D. K. formed as a result of the interaction of the patient's abnormal monoclonal IgM protein with normal IgG.

The ability of IgM_{DK} cryogels to trap serum proteins in the gel was studied by adding radioiodine labeled purified serum proteins to D. K. serum as described in methods. No significant quantities of added radiolabeled albumin, ceruloplasmin, IgA, IgD, IgE, normal IgM, or λ- or κ Bence Jones proteins were associated with the cryogel after five washings. In contrast, 50–85% of normal IgG and of myeloma IgG of the G1, G2, and G4 subclasses remained associated with the gel after five washings (Table I). Significantly lower quantities (3%) of three different IgG3 myeloma proteins were associated with the gel following this procedure. Low but significant quantities (3–7%) of mouse IgG, rabbit IgG, and canine IgG were trapped by the D. K. cryogel.

TABLE I

*Per cent of Radioiodinated Serum Proteins Associated with D. K. Serum Cryogel**

	Per cent remaining in gel
IgG (normal)	50, 52, 85‡
IgG1	55
IgG2	64
IgG3	3
IgG4	53

* Radioactive proteins were added to D. K. serum which was cooled to cause gelling. The supernatant was removed, the gel was brought back into solution by heating, 5 ml of saline was added, and the mixture was then regelled. This procedure was repeated five times.

‡ Three different preparations of IgG.

Other studies were performed directed at determining which purified proteins were capable of causing purified IgM_{DK} to gel. In these studies, the percentage of 0.1 mg of ^{125}I-IgM_{DK} that formed a cryogel following the addition of a 7-fold molar excess of various serum proteins was determined (Table II). No significant gelling of purified ^{125}I-IgM_{DK} occurred upon addition of albumin, ceruloplasmin, IgA, IgM, IgD, IgE, λ- or κ Bence Jones proteins or nonhuman IgG molecules. However, from 18 to 71% of IgM_{DK} gelled following addition of normal IgG or myeloma IgG of G1, G2, or G4 subclasses. Little or no cryogel formed on addition of IgG3 myeloma protein. The addition of Fc piece of an IgG1 throughout an IgG to IgM molar ratio range of 1:1 to 10:1 resulted in rapid gelling at 4.0°C. Addi-

TABLE II

*Per cent of Purified $^{125}IgM_{DK}$ in Gel after Addition of Purified Serum Proteins**

	$^{125}IgM_{DK}$ in gel
	%
IgG (normal)	71.6
IgG1 myeloma Pe	26.2
IgG1 myeloma Pw	29.8
IgG2 myeloma Dw	65.1
IgG2 myeloma Sa	36.9
IgG3 myeloma Vi	0
IgG3 myeloma Mc	4.6
IgG3 myeloma Be	1.0
IgG4 myeloma Dw	18.0
IgG4 myeloma Me	34.7
IgG3 (heavy chain disease fragment)	0.9

* Purified proteins were added in a molar ratio of 7:1 to 0.1 mg of IgM_{DK}.

TABLE III

Albumin, IgA, IgE, and IgM Metabolism in Patient D. K.

Subject	Protein studied	Serum concentration	Plasma volume	Total circulating pool	Total exchangeable pool	Survival time	Fraction of circulating pool catabolized/day	Synthetic rate
		mg/ml	*ml/kg*	*mg/kg*	*mg/kg*	*$t_{\frac{1}{2}}$ days*		*mg/kg per day*
D. K.	Normal albumin	32	39	1200	3300	20.3	0.095	114
Controls (12)*	Normal albumin	41 ±5	42 ±3	1700 ±200	4100 ±500	17 ±2	0.096 ±0.01	163 ±25
D. K.	Normal IgA	0.96	42	40	80	4.8	0.28	11
Controls (21)	Normal IgA	2.53 ±1.4	39 ±5	95 ±50	228 ±130	6.4 ±1.3	0.25 ±0.04	24 ±15
D. K.	Normal IgM	11.0	38	420	490	4.7	0.17	71
D. K.	D. K. IgM	11.0	36	400	490	3.7	0.23	92
Controls (10)	Normal IgM	0.93 ±0.5	39 ±5	37 ±20	49 ±20	5.1 ±1	0.18 ±0.04	6.6 ±3
D. K.	Myeloma IgE	0.000032	38	0.0012	0.0025	2.9	0.60	0.00072
Controls (9)‡	Myeloma IgE	0.000076 (0.000006–0.000912)	39 ±5	0.0030 (0.00023–0.036)	0.0046 (0.00036–0.055)	2.3 ±1	0.81 ±0.24	0.0024 (0.00019–0.029)

* Number of control subjects indicated in parentheses. Control values given are means ±1 standard deviation.

‡ IgE values for serum concentration, pool sizes and synthetic rates are given as geometric means with 95% confidence interval given in parentheses.

tion of the Fab piece of this G1 protein over a similar range of molar ratios failed to produce gelling and was incapable of inhibiting gel formation with Fc piece. Thus the cryogel formed secondary to the interaction of IgM_{DK} with the Fc piece of IgG.

Characteristics of the interaction of IgM_{DK} with IgG. Several characteristics of the interaction of IgM_{DK} with the Fc fragment of IgG were consistent with an antigen-antibody reaction rather than a specific but nonimmunological interaction. The stoichiometry of the IgM_{DK} interaction with an IgG1 was investigated over a wide range of IgG–IgM molar ratios. 1.0×10^{-10} moles of IgM_{DK} labeled with ^{131}I was added to each of several 3.0 ml tubes and the volume in each tube brought to 0.2 ml with pH 8.0 borate-saline or pH 6.0 phosphate saline buffer. The tubes were incubated in an ice bath for 30 min and gels were pelleted by centrifugation at 4°C for 30 min at 5000 × *g*. Supernatants were carefully removed with Pasteur pipets and the wall of each tube was washed with ice cold buffer. Each tube containing the pelleted gel was then counted in a well type gamma scintillation counter and the ^{125}I values were corrected for counts contributed by ^{131}I.

The results (Fig. 1) indicate a maximum of 5 moles of IgG interacted with 1 mole of IgM in pH 6.0 phosphate buffered 0.05 M saline. However, a maximum of 10 moles of IgG interacted with 1 mole of IgM in pH 8.0 borate saline. At all ratios of IgG to IgM studied (i.e. molar ratios 1:1–30:1) there was incomplete precipitation of both IgG and IgM. Examination of the supernatant from a tube with an IgG to IgM molar ratio of 6:1 in the analytical ultracentrifuge revealed that all the unprecipitated IgM_{DK} was present as a 22S complex. A specific anti-IgG was added to a portion of this supernatant and quantitatively precipitated the IgM_{DK} indicating that no uncomplexed IgM was present in the supernatant.

In order to investigate the possible characteristics of cryogel formation in vivo, the influence of different temperatures on the in vitro interactions was studied.

TABLE IV

Survival of IgG in Patient D. K.*

Subject	Serum IgG	Plasma volume	Total circulating IgG pool	Total exchangeable IgG pool	Survival time	Fraction of circulating pool catabolized/day	Synthetic rate
	mg/ml	*ml/kg*	*mg/kg*	*mg/kg*	*$t_{\frac{1}{2}}$ days*		*mg/kg per day*
D. K. no therapy	1.3	40	52	104	12.0	0.115	6.0
D. K. (penicillamine 750 mg/day)	1.3	36	47	94	12.5	0.115	5.4
D. K. postpenicillamine	1.3	35	46	82	12.0	0.105	4.8
Controls (25)	12.1 ±2.6	42 ±6	490 ±120	1090 ±260	22.9 ±4.0	0.063 ±0.01	33 ±11

* Values for IgG are for IgG 1, 2, and 4 subclasses.

When fresh plasma from the patient was gradually cooled from 37°C, gross gelling was visible at 33°C. Because soluble complexes could be demonstrated at low temperatures in the gelling tests it was suspected that such complexes existed in the absence of visible gelling at higher temperatures. Therefore, purified IgM_{DK} was added to an IgG1 and examined in the analytical ultracentrifuge from 40° to 35°C. Soluble complex formation began at temperatures between 38–36°C.

In contrast to the observations with IgM_{DK} and IgG1, soluble complex formation detectable in the ultracentrifuge was not observed with IgM_{DK} and IgG3 unless the temperature was below 28°C and gross gelling was never observed at any temperature.

In an attempt to study complex formation in the presence of other serum proteins, the viscosity characteristics of fresh whole plasma D. K. were examined over a wide temperature range. The data plotted in Fig. 2 show a change in viscosity behavior at approximately 37°C as manifested by a change in slope to one of greater negativity.[3]

Protein turnover studies. The results of the radioiodinated albumin, IgA, IgM, and IgE turnover studies are shown in Table III. Patient D. K. had a somewhat reduced serum protein concentration, total circulating protein pool, and total body pool of albumin, IgA, and IgE. In each case, the synthetic rate of these proteins was reduced to between 46 and 70% of normal. The half-time of survival and the fractional catabolic rate of these proteins, however, were within the normal range. The serum concentration, total circulating and total body pools of IgM and the rate of synthesis of IgM were markedly increased as assessed in studies using normal IgM or IgM isolated from patient D. K. The half-time of survival and the fractional catabolic rate of normal IgM were normal in the patient. The IgM of patient D. K. showed a catabolic rate comparable to normal IgM after the first 24 hr when slightly accelerated catabolism was observed.

The different subclasses of IgG had quite different metabolic behavior in patient D. K. The survival of IgG isolated from normal serum [4] was studied in patient D. K. on three occasions (Table IV); at one time when she was on no therapy, a second time when she was receiving penicillamine 750 mg/day, and a third time immediately following penicillamine therapy. On each occasion, the serum concentration of the IgG subclasses other than G3, the total circulating IgG pool, and the total body IgG pool were reduced to 10% of normal (Table IV). The synthetic rate of IgG molecules of subclasses other than IgG3 was reduced to 16% of normal. In contrast to the studies with the other immunoglobulins and albumin in this patient the survival half-time of IgG was markedly reduced to 12–12.5 days compared to the normal of 22.9 days and the fractional catabolic rate was significantly increased to 11.2% of the circulating pool per day, approximately twice the rate seen in control individuals (6.3%). The low serum IgG concentration of the G1, G2, and G4 subclasses is thus due to a combination of decreased IgG synthesis and accelerated IgG catabolism.

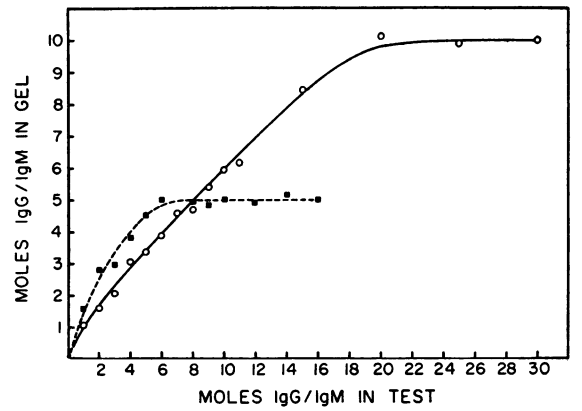

FIGURE 1 Moles of IgG per mole of IgM in the washed cryogel is plotted *versus* moles of IgG added per mole of IgM. The experiments performed in 0.01 M sodium phosphate buffered 0.05 M NaCl at pH 6.0 are indicated by the dashed line (- -■- -) and those performed in 0.21 M sodium borate buffered 0.16 M NaCl at pH 8.0 are indicated by the solid line (—○—). Each point in both solvents represents the average of five determinations. The pH 6.0 data indicate a valence of 5 for IgM_{DK} and the pH 8.0 data indicate a valence of 10.

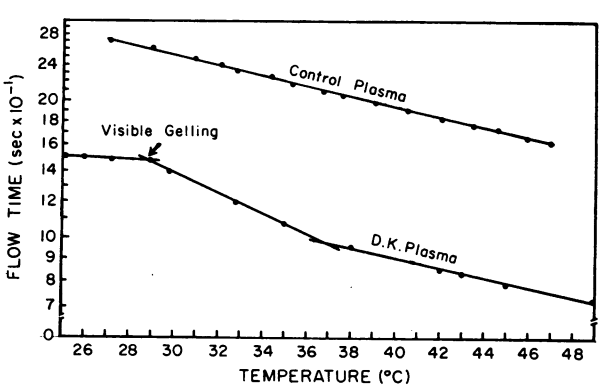

FIGURE 2 Viscosity expressed as flow time $\times 10^{-1}$ sec is plotted *versus* temperature. A data point at 53°C is not shown. The control plasma from a patient with Waldenström's macroglobulinemia does not contain a paraprotein with recognized antibody specificity.

[3] Validity of choosing two slopes between 29–53°C to fit the data was tested by examining the scatter of points about the two slopes and comparing them to the scatter about a single regression line drawn through all the points. The two slopes depicted on the graph between 29–48°C are significantly different ($t = 7.54$, 9 degrees of freedom, $P < 0.001$).

[4] Over 97% of this material was G1, G2, and G4.

TABLE V

IgG3 Metabolism in Patient D. K.

Subject	Serum IgG3	Plasma volume	Total circulating IgG3 pool	Total exchangeable IgG3 pool	Survival time	Fraction of circulating pool catabolized/day	Synthetic rate
	mg/ml	*ml/kg*	*mg/kg*	*mg/kg*	*$t_{\frac{1}{2}}$ days*		*mg/kg per day*
D. K.	1.3	38	49.4	82.4	17	0.068	3.4
Controls (7)	0.50 ±0.14	42 ±4	20 ±4.8	31.2 ±7.0	7.1 ±0.7	0.168 ±0.006	3.4 ±0.7

The metabolism of radioiodinated purified gamma globulin of the IgG3 subclass was studied in patient D. K. and controls. In patient D. K. the serum IgG3 concentration, total circulating and total body IgG3 pool sizes were increased (Table V). The IgG3 synthetic rate was normal in contrast to the markedly reduced synthetic rates observed with the other IgG subclasses. The survival of IgG3 in normal recipients is much shorter than that of the other subclasses with a $t_{\frac{1}{2}}$ of survival of 7.1 days and a fractional catabolic rate of 16.8% of the intravascular pool per day. In patient D. K. however, the IgG3 survival $t_{\frac{1}{2}}$ was prolonged to 17 days and the fractional catabolic rate was reduced to 6.8% of the intravascular pool per day. This is the first report of a short survival of some subclasses of IgG with normal or prolonged survival of another subclass (IgG3).

DISCUSSION

The serum of D. K. contained a large amount of a mixed IgM–IgG cryogel. Recently a similar cryogel has been extensively studied and shown to probably represent the immune reaction of a homogeneous IgM antibody with IgG (*22*, *29*). In those studies the following criteria were proposed for an antibody: (*a*) The protein must be well characterized as a known immunoglobulin; (*b*) the "antibody" must have a clearly defined specificity; (*c*) the entire preparation of antibody must be active; (*d*) antigen should be bound by only those fragments of the antibody possessing the antigen-binding site—F(ab') 2μ and Fab μ in the present case; (*e*) the reaction between antibody and antigen should exhibit a stoichiometry consistent with the number of potential antigen-binding sites. An IgM antibody therefore should exhibit a valence of 10.

Most of these criteria are satisfied in studies with the 18S IgM$_{DK}$. It is quantitatively precipitated by a specific antiserum to human μ chain and contains exclusively λ-light chains with restricted electrophoretic heterogeneity. These are properties characteristic of Waldenström's macroglobulins. Intact IgM$_{DK}$ had a clearly defined specificity reacting with determinants on the Fc portion of IgG resulting in the formation of soluble complexes at 37°C and an insoluble gel at temperatures below 33°C. Soluble complexes of IgM$_{DK}$ and IgG3 occurred only at temperatures below 28°C and no gel was produced at temperatures as low as 4°C. Because IgM$_{DK}$ was isolated from whole plasma through complex formation and cryogelling with IgG present in the plasma the entire preparation was active. Furthermore, the IgM$_{DK}$ which remained in the supernatant after gelling was shown to be present as a 22S complex which could be quantitatively precipitated by the addition of a specific anti–IgG antiserum.

The fourth criterion proposed by Metzger was not fulfilled. A number of studies were performed in order to determine the region of IgM$_{DK}$ that reacted with IgG. These methods included boundary analysis in the ultracentrifuge (*22*), inhibition of gel formation (*29*) and binding of radiolabeled IgM$_{DK}$ and its fragments to insoluble IgG. The F(ab') 2μ and Fab μ tryptic fragments of IgM$_{DK}$ were not capable of binding IgG or the Fc fragment of IgG. However, there was no evidence of binding occurring in other portions of the IgM molecule remote from the binding sites; i.e., the 7S reductive subunit of IgM$_{DK}$ with cleaved intersubunit and with inter-μ-chain disulfide bonds and the pentameric Fcμ fragment with intact disulfides were also incapable of binding IgG. It is considered most likely that the affinity of the intact IgM$_{DK}$ for the antigenic sites on IgG is of a low order of magnitude and that any operations which reduce the number of binding sites per molecule result in a loss of detectable binding.

Even though isolated Fab μ was incapable of detectable IgG binding the valence studies were compatible with 5 or 10 active antigen-binding sites per intact IgM molecule. A valence of 5 was obtained in pH 6.0, 0.01 M sodium phosphate which was the solvent in which gelling was most rapid and complete. This result is comparable to that reported by Metzger (*29*). At pH 8.0 equivalent gel formation required 18–24 hr at 4°C. Under these conditions 10 moles of IgG were bound per mole of IgM$_{DK}$. It is likely that at pH 6.0 rapid gelling prevented binding by all antibody sites while the less efficient gel formation at pH 8.0 allowed complete valence filling. This interpretation would support the hypothesis that

all 10 potential binding sites are active in the intact IgM_{DK} molecule.

In summary, the data characterizing IgM_{DK} indicated it was a highly homogeneous antibody with specificity for antigenic determinants on the Fc portion of native IgG. The examination of mixtures of IgM_{DK} and IgG1 myeloma protein in the analytical ultracentrifuge over a wide range of temperatures demonstrated soluble complex formation at 37°C in the absence of gel formation. The viscosity experiments utilizing fresh whole plasma indicated comparable behavior in the presence of the other serum proteins. It was of considerable interest that temperatures substantially lower than usually attainable in vivo were required for complexing of IgM_{DK} and IgG3. This in vitro difference in the binding by IgM_{DK} of IgG3 as compared to the rest of the IgG subclasses suggested the possibility of similar differential behavior in vivo.

D. K. had a number of alterations in IgG concentration, synthesis, and catabolism that appear to be related to the presence of the IgG-reactive IgM molecules and to the in vivo formation of complexes. The serum IgG concentration was markedly reduced. This reduction was selective in that the concentration of the IgG3 subclass was increased to 1.3 mg/ml (about twice the normal mean) while the total serum concentration of the remaining subclasses was 1.3 mg/ml (a value 10% of normal). Thus in this patient IgG3 accounted for 50% of the total IgG concentration rather than the 8% seen in normal individuals (30).

IgG turnover studies showed the reduced concentration of the major subclasses of IgG (except IgG3) to be due to two factors, decreased synthesis and shortened IgG survival. A number of factors may play a role in the reduced IgG synthesis rates observed. In patients with monoclonal IgG or IgM proteins the concentration and rate of synthesis of the nonmyeloma IgG is usually somewhat decreased. In most cases however the rate of synthesis of IgG1 molecules is approximately 65% of normal in patients with macroglobulinemia and 50% of normal in patients with multiple myeloma (26, 31). The IgG synthetic rate was however much lower in the present case than in most patients with a monoclonal gammopathy and other potential causes for decreased IgG synthesis were examined.

The allotype suppression studies of Dray (32) and Mage and Dray (33) suggest an additional mechanism for the profound decrease in IgG synthesis observed in patient D. K. In these studies, pregnant female rabbits homozygous for one allotype (eg b_4b_4) were immunized with the paternal immunoglobulin of another allotype (eg b_5b_5). The genotypically heterozygous (b_4b_5) offspring that developed in a maternal environment containing antipaternal type immunoglobulin produced much less allotype of the paternal (b_5) than of the maternal allotype (b_4). A similar effect was produced if the antisera to one of the allotypes was injected into the heterozygotes after birth. In analogous studies Cooper, Kincade, Lawton, and Bochman (34), have shown that IgM synthesis in chickens can be suppressed by the injection of anti-μ-antibodies into eggs. These studies suggest that antibody to an allotype or a class of immunoglobulin may interact with immunoglobulin-like receptors on the surface of immunologically competent cells thereby reducing the quantity of this type of immunoglobulin synthesized. This could occur by killing or preventing the differentiation and proliferation of immunoglobulin synthesizing cells or by specifically interfering with the intracellular regulatory mechanisms controlling immunoglobulin synthesis. By analogy with these experimental studies the production of autoreactive IgM molecules by patient D. K. that react at body temperature with IgG 1, 2, and 4 subclasses may act in a similar way to contribute to the markedly reduced rates of synthesis of these molecules.

Another factor resulting in the reduced IgG concentrations was a shortened survival of the normally predominent IgG subclasses with IgG survival half-times of 12.5 days compared to the normal of 22.9 days. In general the survival of all subclasses of IgG in man and certain animals varies inversely with the IgG concentration (1, 8, 10, 26, 35, 36). That is, as the concentration of IgG rises the survival decreases (fractional catabolic rate increases) until a limiting $t_{\frac{1}{2}}$ of approximately 10 days is reached at very high IgG levels. On the basis of her total serum IgG concentration of 2.6 mg/ml a survival $t_{\frac{1}{2}}$ of 35 days rather than the observed 12.5 days would have been expected. Thus the patient had a significantly shortened IgG survival contributing to the observed hypogammaglobulinemia. The short IgG survival does not appear to be secondary to any previously described mechanisms. The patient did not have proteinuria. Excessive gastrointestinal protein loss could also be excluded since the patient had a normal ^{51}Cr albumin test (37). In addition, the patient had none of the clinical features associated with the other syndromes (familial hypercatabolic hypoproteinemia (19), the Wiskott-Aldrich syndrome (38) or myotonic dystrophy (20)) associated with excessive endogenous protein catabolism. Thus, the short survival of IgG in patient D. K. is by a new and previously undescribed mechanism. This short survival of IgG may be the result of IgM-IgG complex formation in vivo with rapid catabolism of the complexes formed. The ultracentrifugation and viscosity studies that indicated complex formation between the autoreactive IgM_{DK} and IgG at a high temperature (36–38°C) support the hypothesis that such complexes are being formed in vivo.

The metabolism of the IgG3 subclass in patient D. K. contrasts markedly with that of the other major subclasses. The IgG3 serum level and total circulating pool were elevated. The synthetic rate was normal while the survival $t_{\frac{1}{2}}$ was longer than normal. As noted previously the survival of IgG3 in normal recipients is much shorter than that of the other subclasses with a $t_{\frac{1}{2}}$ of survival of 7.5 days (26, 39). In patient D. K. the survival $t_{\frac{1}{2}}$ was 17 days. IgG3 participates in the concentration-catabolism effect discussed above in which the expected IgG survival of any subclass is determined by the total IgG level (26). The 17 day survival $t_{\frac{1}{2}}$ of IgG3 which does not form complexes with IgM_{DK} at body temperature was that which would be predicted on the basis of the total IgG concentration of 2.6 mg/ml. These studies in patient D. K. are the first to demonstrate a pathological process that results in a significantly shortened survival of some subclasses of IgG while the survival of the remaining subclass is that expected on the basis of the IgG serum level. These findings are best explained by the fact that IgM_{DK} reacts with the IgG 1, 2, 4 subclasses at body temperature to form rapidly catabolized complexes while IgM_{DK} does not react with IgG3 to form such complexes at body temperature.

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