Metabolic Properties of IgG Subclasses in Man

ANDREAS MORELL, WILLIAM D. TERRY, and THOMAS A. WALDMANN

From the Immunology Branch, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT Metabolic properties of the four subclasses of human IgG were investigated by performing 47 turnover studies in individuals with normal IgG serum concentrations, as well as in patients with an increased level of one of the subclasses. Studies in 12 subjects with normal IgG serum concentration showed that the average biologic half-life of G_1, G_2, and G_4 was 21 days, while that of G_3 was only 7.1 days. Fractional catabolic rates of G_1, G_2, and G_4 were 6.9 to 8% of the intravascular pool per day. G_3, however, had a higher fractional catabolic rate, amounting to 16.8% of the intravascular pool per day. Distribution of the subclasses was such that the intravascular compartment contained 51–54% of the total body pools of G_1, G_2, and G_4, but 64% of the total body pool of G_3.

The short survival and high fractional catabolic rate of G_3 is an inherent property of these molecules, and is not due to denaturation during isolation and radiolabeling. This was demonstrated by studies of a patient with a serum G_3-myeloma protein. The survival of her own protein, separately labeled either in vivo with guanidoarginine-^{14}C or in vitro with ^{125}I, was determined in the patient. Survivals of the in vivo and in vitro labeled proteins were identical.

G_1 and G_3 serum concentrations and synthetic rates were determined. The mean serum concentration of G_1 was 6.8 mg/ml and that of G_3 was 0.7 mg/ml, while their synthetic rates were 25.4 and 3.4 mg/kg per day respectively. The low serum concentration of IgG_3 thus results from a combination of high catabolic and low synthetic rates.

Studies in 10 patients with multiple myeloma showed that an elevated serum concentration of any IgG subclass was associated with shortened biologic half-life and increased fractional catabolic rate of all subclasses. The implications of this concentration-catabolism relationship are discussed. The serum concentration of nonmyeloma IgG was usually low in myeloma patients and the synthesis of nonmyeloma IgG was somewhat decreased, suggesting that low serum concentrations of nonmyeloma IgG result from decreased synthesis, as well as from an increased fractional catabolic rate.

INTRODUCTION

Many metabolic features of human IgG have been demonstrated by turnover studies using IgG isolated from normal human serum. IgG, however, is heterogeneous and consists of four distinct species of molecules designated IgG_1, G_2, G_3, and G_4. All four of these subclasses are present in normal human serum, and in a normal indivdual the relative concentrations are: G_1, 65%; G_2, 23%; G_3, 8%; and G_4, 4% (1). Previous studies have, therefore, really determined the metabolic properties of a heterogeneous group of proteins.

Molecules of the four subclasses differ from one another in the antigenic and chemical structure of the carboxy terminal three-quarters of their gamma chains. One portion of this region, the Fc-fragment, determines a number of the biologic, nonantibody activities of IgG, including the rate of catabolism (2, 3). The subclasses differ in their Fc-fragments and therefore might also be expected to differ in metabolic properties. Investigation of characteristics of indivdual subclasses cannot be performed using normal serum IgG, because the molecules in normal serum have very similar charge and size and cannot be completely separated from one another. It is necessary, instead, to use G-myeloma proteins (G-MP) each of which serves as a representative of a subclass and can be obtained free of other subclasses.

Spiegelberg and coworkers (4, 5) have investigated one aspect of the metabolism of human IgG subclasses by comparing the biologic half-life of a number of isolated G-MP's in man and other species. They found that the average biologic half-life of G_1, G_2, and G_4 were similar, but that the half-life of G_3 was shorter than the others.

The purpose of the present study was to gather more information about the metabolism of the IgG subclasses

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Andreas Morell is a Fellow of the Swiss Center for Clinical Tumor Research, Bern, Switzerland.

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TABLE I
Clinical and Turnover

Initials	Sex	Age	Weight	Serum-IgG	Diagnosis	Survival $t_{\frac{1}{2}}$				Fraction of intravascular pool catabolized per day			
						G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4
		yr	*kg*	*mg/ml*		*days*							
M. G.	F	62	52.3	9.0	Malignant lymphoma		23	8.0			0.068	0.175	
A. W.	F	72	72.7	15.0	Mycosis fungoides		18	6.4			0.067	0.167	
W. A. B.	M	65	73.4	10.4	Metastatic carcinoma of the colon		21	6.3			0.066	0.159	
H. E. H.	M	61	64.7	10.0	Squamous cell carcinoma of the oral cavity		19	7.7			0.073	0.162	
J. H.	F	25	56.8	8.6	Metastatic choriocarcinoma			7.7				0.172	
P. E. W.	M	40	71.4	8.1	Metastatic melanoma			6.4				0.174	
J. J.	M	20	64.5	12.0	Choriocarcinoma	18			24	0.082			0.058
C. B.	F	65	56.0	8.3	Carcinoma of the breast	30			23	0.045			0.055
B. L. C.	F	42	61.5	6.4	Carcinoma of the breast	16			19	0.092			0.073
E. W.	F	36	73.0	8.7	Pheochromocytoma	26				0.06			
M. E. H.	F	59	52.5	13.5	Rheumatoid arthritis	22			20	0.10			0.09
E. L.	F	50	59.0	15.0	Sjögren's syndrome	15			17	0.10			0.07
Mean ±SD						21.2 ±5.4	20.2 ±1.9	7.1 ±0.7	20.6 ±2.6	0.08 ±0.02	0.069 ±0.003	0.168 ±0.006	0.069 ±0.014

and about the mechanisms that regulate their serum levels in man. 47 turnover studies with radiolabeled G-myeloma proteins of the different subclasses were performed in 24 individuals. The distribution between intra- and extravascular compartments, the rates of catabolism of all four subclasses, and the rates of synthesis of G_1 and G_3 were determined.

An attempt was made to answer the following three questions: (*a*) What are the metabolic characteristics of the four IgG subclasses in individuals with normal levels of IgG? (*b*) Is the apparently short survival of G_3 due to damage sustained by this labile molecule during isolation and radioiodination? (*c*) What is the effect of a high serum concentration of any one of the IgG subclasses on the synthesis and survival of all four subclasses?

METHODS

Isolation and radioiodination of proteins. Five myeloma proteins (types $G_1\lambda$, $G_2\kappa$, $G_3\kappa$, $G_3\lambda$, and $G_4\kappa$) were isolated from serum by zone electrophoresis and if necessary Sephadex G-200 gel filtration.[1] Purity of isolated proteins was assessed by immunoelectrophoresis and Ouchterlony analysis. Preparations contained no serum proteins other than IgG. Proteins were labeled with ^{131}I or ^{125}I by the iodine monochloride method of McFarlane (6). All preparations had an average of less than one atom of iodine per molecule and contained less than 1% nonprecipitable radioactivity. Iodinated preparations were made 3.5% in human albumin to minimize radiation damage. Preparations were sterile filtered and tested for sterility and pyrogenic activity before use.

Measurement of immunoglobulin concentrations. Serum concentrations of IgG, IgG_1, and IgG_3 were determined by a method of inhibition of immune binding (7). Monkey or rabbit antisera were absorbed so as to be specific for the heavy chains of IgG, G_1, or G_3. Specific antisera were made 14% in Na_2SO_4 and the precipitated globulin fractions were coupled to bromacetyl cellulose. These immunoadsorbents quantitatively bind their homologous radiolabeled antigens. The binding is inhibited by unlabeled antigen of the same specificity, and the degree of inhibition is quantitatively proportional to the concentration of the unlabeled antigen. IgA and IgM concentrations were measured by radial immunodiffusion (8).

Patient selection. 12 patients with a variety of neoplastic diseases were selected because they had relatively normal serum IgG concentrations (Table I). Ages ranged from 20 to 72 yr; four were male, and eight female. 10 additional patients were selected because their serum contained a myeloma protein, thus permitting metabolic studies in eight individuals with a high G_1 serum concentration, one individual with a high G_2 serum concentration, and one with a high G_3 serum concentration (Table II). Patients' ages ranged from 33 to 77 yr; three were female, seven male. No patients in either group had signs of significant gastrointestinal or renal protein loss.

Turnover study protocol. All subjects were hospitalized at the National Institutes of Health and studies were not performed during periods of acute illness. The serum concentrations of IgG, G_1, and G_3 remained constant in both the control and myeloma patients during the study periods, and all patients were therefore assumed to be in a steady state concerning IgG metabolism. 5 drops of Lugol's solution were given three times a day during the entire study to prevent uptake of radioactive iodine by the thyroid.

Subjects received pairs of subclass preparations intravenously, one preparation labeled with ^{125}I, the other with ^{131}I. The dose of radioactivity ranged from 10 to 25 μCi. A 10 min blood sample was drawn for plasma volume determination. Additional blood samples were collected at 4 hr, 8 hr, and then daily for 14 days following the administration of labeled protein. Daily 24 hr urine collections were obtained during the study. Serum and urine samples were counted

[1] Pharmacia Fine Chemicals Inc., Uppsala, Sweden.

Data of Control Patients

IgG subclass concentration		Plasma volume	Total circulating pool		Total body pool		Intravascular				Synthetic rate	
G_1	G_2		G_1	G_2	G_1	G_2	G_1	G_2	G_3	G_4	G_1	G_2
mg/ml		*ml/kg*	*mg/kg*		*mg/kg*		*%*				*mg/kg/day*	
	0.32	45		14.4		25.3		46	57			2.5
	0.71	34		24.2		33.6		62	72			4.0
	0.35	46		16.1		23.3		53	69			2.6
	0.76	37		28.1		44.7		52	63			4.6
	0.38	47		17.9		32.5			55			3.1
	0.46	42		19.3		27.9			69			3.4
8.5		42	357		744		48			52	29.3	
4.1		44	180		340		53			56	8.1	
4.5		41	184		355		52			52	16.9	
6.0		36	216		460		47				13.0	
9.0		46	414		882		47			48	41.4	
9.1		48	437		753		58			64	43.7	
6.9	0.50	42	298	20.0	589	31.2	51	53	64	54	25.4	3.4
±2.1	±0.17	±4	±108	±4.8	±212	±7.0	±4	±6	±6	±5	±13.7	±0.7

with appropriate standards in an automatic gamma ray well-type scintillation counter with a thallium-activated sodium iodide crystal. A pulse height analyzer allowed differentiation of the two isotopes in the samples.

In vivo labeled G_3-myeloma protein turnover study. A single intravenous dose of 100 μCi of guanidoarginine-^{14}C was given to patient G. B. who had a serum G_3-myeloma protein (Table II). Plasma samples were obtained daily. The myeloma protein was isolated from each plasma sample, and 5 mg of the protein were completely precipitated with 10% trichloracetic acid. Precipitates were dissolved in 0.5 ml of NCS reagent (Nuclear-Chicago),[2] 10 ml of a mixture of Liquifluor (New England Nuclear)[3] and toluene was added, and radioactivity was determined in a liquid scintillation counter (Packard Tri-Carb Model 4322).[4] The rate of decrease of specific activity of the G_3-myeloma protein reflects the biologic survival of this protein.

Calculation of the metabolic data. Whole body radioactivity was calculated by cumulative subtraction of the radioactivity appearing in the urine. Graphs of change in plasma and whole body radioactivity were constructed on semilogarithmic paper. The biologic half-life ($t_{\frac{1}{2}}$) of each labeled protein was determined graphically. The total circulating and total body protein pools, the fraction of the body protein pool remaining intravascular, the fraction of the intravascular pool catabolized each day (fractional catabolic rate or FCR), and the synthetic rate (turnover rate) were determined by a modification of the method of Matthews (9).

RESULTS

Subjects with normal IgG levels. Turnover data from studies in subjects with normal serum IgG concentrations are summarized in Table I. Five subjects received ^{131}I-$G_4\kappa$ and ^{125}I-$G_1\lambda$ simultaneously, one was injected only with ^{125}I-$G_1\lambda$, four received ^{125}I-$G_2\kappa$ and ^{131}I-$G_3\kappa$ simultaneously, and two received only ^{125}I-$G_3\lambda$.

The biologic half-life of the G_1, G_3, and G_4 proteins showed considerable variation when studied in different individuals. Average values, however, were similar for all three subclasses (Table I). The observed half-times of survival of 20.2 to 21.2 days are similar to those previously reported for whole IgG (23 ±4 days) (10). The survival of both G_3 proteins, however, was short, with a mean of 7.1 ±0.7 days (Table I).

The fraction of the intravascular pool catabolized per day (FCR) of the four subclasses is shown in Table I. Rates for G_1, G_3, and G_4 were between 0.05 and 0.10 of the intravascular pool per day, values which are similar to the FCR of whole IgG (0.067 ±0.015). The fractional catabolic rates of the G_3 proteins were considerably higher and ranged from 0.159 to 0.175 of the intravascular pool per day.

It was necessary to determine whether the short survival and high fractional catabolic rate of G_3 was due to damage of this labile molecule during isolation and radiolabeling, or whether this rate was indeed a metabolic property of native G_3. To accomplish this, an in vivo labeling procedure was utilized so that it was not necessary to isolate and label the G_3-myeloma protein prior to its metabolism in vivo. Patient G.B., whose serum contained a G_3-myeloma protein (Table II), was given a single intravenous dose of 100 μCi of guanidoarginine-^{14}C. On each of the 10 following days (except day 9) plasma samples were obtained, myeloma protein was isolated, and specific activity determined (see Methods). ^{14}C was rapidly incorporated into the myeloma protein, such that the specific activity was high 24 hr

[2] Nuclear-Chicago Corporation, Des Plaines, Ill.
[3] New England Nuclear Corp., Boston, Mass.
[4] Packard Instrument Co., Downers Grove, Ill.

TABLE II
Clinical and Turnover

Initials	Sex	Age	Weight	Serum-IgG	Myeloma protein (subclass, type)	Survival $t_{\frac{1}{2}}$				Fraction of intravascular pool catabolized/day			
						G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4
		yr	*kg*	*mg/ml*		*days*							
P. E.	M	50	67.3	14	$IgG_1\lambda$		11.0	4.0			0.15	0.26	
G. B.	F	47	51.0	33	$IgG_3\kappa$		8.0	3.5			0.15	0.38	
N. A. S.	M	51	73.8	49	$IgG_2\kappa$	7.0	10.6	3.0	10.5	0.14	0.11	0.27	0.11
D. P.	M	33	61.0	35	$IgG_1\kappa$	10.0	11.0	3.5	12.0	0.12	0.12	0.29	0.11
D. S.	F	77	38.5	17	$IgG_1\kappa$	13.5			14.0	0.11			0.11
L. C. D.	M	44	77.6	62	$IgG_1\lambda$	5.4			6.5	0.19			0.15
C. W.	M	46	95.5	27	$IgG_1\kappa$	10.5			13.0	0.13			0.10
M. J. K.	F	46	59.7	32	$IgG_1\kappa$	16.5			16.0	0.08			0.08
J. D. W.	M	76	54.8	45	$IgG_1\kappa$	18.0			18.5	0.07			0.07
D. D.	M	38	80.9	25	$IgG_1\kappa$	10.5			11.0	0.15			0.14
Mean ±1 SD						11.4	10.2	3.5	12.7	0.12	0.13	0.30	0.11

after administration and then progressively decreased with time. A semilogarithmic plot of the decrease of ^{14}C specific activity of the G_3-myeloma protein yields a straight line, with slope indicating a half-life of 3.5 days. This is similar to the half-life obtained with the patients exogenously labeled ^{131}I-G_3 (Fig. 1). These data show that the metabolic behavior of in vivo and in vitro labeled G_3 proteins are the same and indicate that the short survival and high FCR of G_3 are properties of native G_3 molecules.

In order to calculate the pool sizes and synthetic rates of G_1 and G_3, it was necessary to determine the serum concentration of these subclasses in all patients. For comparative purposes, the IgG, G_1, and G_3 serum concentrations were also determined in the sera of 20 normal blood donors. The average normal values ±1 SD were: IgG, 10.0 ±2.3 mg/ml; G_1, 6.8 ±2.6 mg/ml; and G_3, 0.7 ±0.3 mg/ml. Levels in the patients selected for normal IgG serum concentration generally fell within 1 SD of the control group (Table I). Using these serum concentrations, total circulating, and total body pools for IgG, G_1, and G_3 were calculated (Table I). The distribution of G_3 into the intravascular compartment was relatively high, and amounted to an average of 64% of the total body pool. By comparison, only 51, 53, and 54% of the total body pools of G_1, G_2, and G_4 were located in the intravascular compartments.

Synthetic rates for G_1 and G_3 in the patient group with normal IgG concentrations were calculated and compared with the synthetic rate of IgG, which has previously been reported as 34 ±11 mg/kg per day (10). Although there was a considerable range in the individual values for G_1 synthesis, the average rate was 25 ±14 mg/kg per day, which is 70% that of IgG. By contrast, the average G_3 synthetic rate was 3.4 ±0.7 mg/kg per day, or about 10% of the synthetic rate of IgG.

Myeloma patients. Table II summarizes the turnover data of the myeloma patients. As shown in previous studies (10, 11), myeloma patients have a higher plasma volume than normal individuals. In the present study, the plasma volume of the myeloma patients averaged 53 ±10 ml/kg, compared to 42 ±4 ml/kg in the control patients. The intravascular compartment contained 59, 56, and 59% of the total body pools of G_1, G_2, and G_4, and 77% of the total body pool of G_3.

The survival of each of the subclasses was significantly shorter in myeloma patients than in controls, and the FCR was approximately twice that of controls. A possible explanation for the shortened survival relates to IgG concentration, in that it has previously been noted that patients with elevated IgG serum concentrations have shortened survival of IgG (10, 12–15); that is, there appears to be an inverse relationship between serum IgG concentration and IgG survival. In the present study, survival times generally were shortest in recipients with the highest IgG levels. Studies in patients with elevated serum levels of G_1, G_2, or G_3 showed that survival of all four subclasses were decreased and catabolic rates increased (Figs. 2, 3). This relationship between serum concentration and catabolism of IgG is, therefore, applicable to elevation of G_1, G_2, or G_3 and is presumably valid also for elevation of G_4.

There were, however, some exceptions to this relationship. P. E., who had a low serum concentration of

Data of Myeloma Patients

Ig Concentration						Total circulating pool			Total body pool			Intravascular				Synthetic rate		
IgG					Plasma													
$G_{1\kappa}$	$G_{1\lambda}$	G_3	IgA	IgM	volume	$G_{1\kappa}$	$G_{1\lambda}$	G_3	$G_{1\kappa}$	$G_{1\lambda}$	G_3	G_1	G_2	G_3	G_4	$G_{1\kappa}$	$G_{1\lambda}$	G_3
		mg/ml			*ml/kg*		*mg/kg*			*mg/kg*			%				*mg/kg/day*	
5.9	5.0	0.92	1.10	1.20	46	271	230	42			54		48	78				11
1.7	0.9	30.0	0.43	0.32	59	100	53	1770			3052		62	58				673
0.7	0.3	0.15	0.20	0.18	52	36	16	8	50	22	9	72	55	86	66	5	2	2
33.0	0.6	0.22	0.26	0.32	62	2046	37	14	3197	58	18	64	59	77	61	246	4	4
15.0	0.7	0.20	0.16	0.32	68	1020	48	14	2000	94		51			51	112	5	
0.7	60.0	0.035	0.13	0.24	52	36	3120	2	65	4588		68			70	7	593	
26.0	0.2	0.053	0.11	0.05	52	135	10	3	2458	19		55			52	175	1	
30.0	1.0	0.26	0.36	1.90	51	1530	51	13	2942	98		56			57	122	4	
43.0	1.0	0.22	0.22	0.42	56	2408	56	12	4152	97		58			60	168	4	
22.0	1.2	1.10	0.50	0.68	29	638	35	32	1225	67		52			53	96	5	
			0.35	0.56	53 ±10							59	56	77	59			

$G_{1\lambda}$ myeloma protein (MP), had remarkably short survival times, whereas J. D. W. and, to a lesser extent, M. J. K. showed almost normal biologic half-lives of G_1 and G_4 in spite of clearly elevated serum IgG levels.

The synthetic rates of myeloma proteins in myeloma patients are high, as expected. In two patients, L. C. D. and G. B., synthetic rates of 593 and 673 mg/kg per day were observed, indicating a daily synthesis of 46 and 34 g of MP respectively in these patients.

The serum concentration of "nonmyeloma" IgG (e.g.

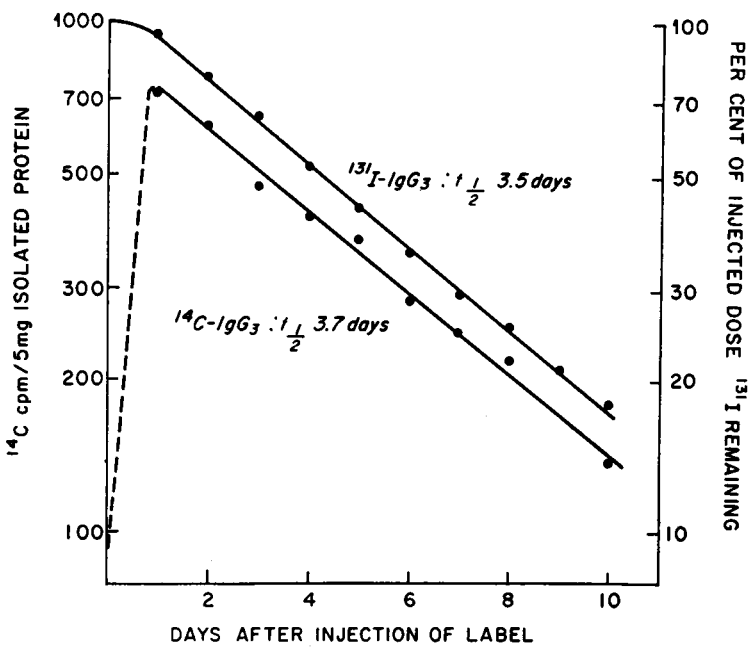

FIGURE 1 Survival of IgG_3 in a patient with elevated serum concentration of IgG_3. A turnover study of iodinated IgG_3 was performed in the usual manner. Upper curve shows residual whole body activity. In a separate study, the patient was given guanidoarginine-^{14}C and the myeloma protein was isolated from her serum on each of the next 10 days. The decrease of serum myeloma protein specific activity is shown in the lower curve.

IgG of the subclasses other than the myeloma protein, or of the same subclass but of the other light chain type) is usually markedly decreased (Table II). For example, the average $G_1\lambda$ serum concentration in eight patients with myeloma proteins of non-$G_1\lambda$ type was 0.7 mg/ml, as compared to an average of 2.8 mg/ml in nonmyeloma patients. The rates of synthesis of nonmyeloma IgG are about 1 SD below the average synthetic rates of control patients. The low levels of "nonmyeloma" IgG are, therefore, due not only to the increased fractional catabolic rate (Tables I and II) but also to reduced synthesis (Fig. 4).

DISCUSSION

The biologic half-lives and fractional catabolic rates of the G_1, G_2, and G_4 subclasses were similar to one another and to the values previously reported for IgG. It is not surprising that the survival of G_1 and G_3 should be similar to that of IgG, since G_1 and G_3 constitute approximately 90% of the IgG used in previous studies. In the only other reported turnover study of the IgG subclasses, considerably shorter survival times were reported for G_1, G_2, and G_4 (11.6, 12.4, and 11.3 days respectively) (4). This discrepancy may be due to differences in radiolabeling techniques, since these investigators used the chloramine-T method, and proteins labeled by that procedure may have shortened survival times.

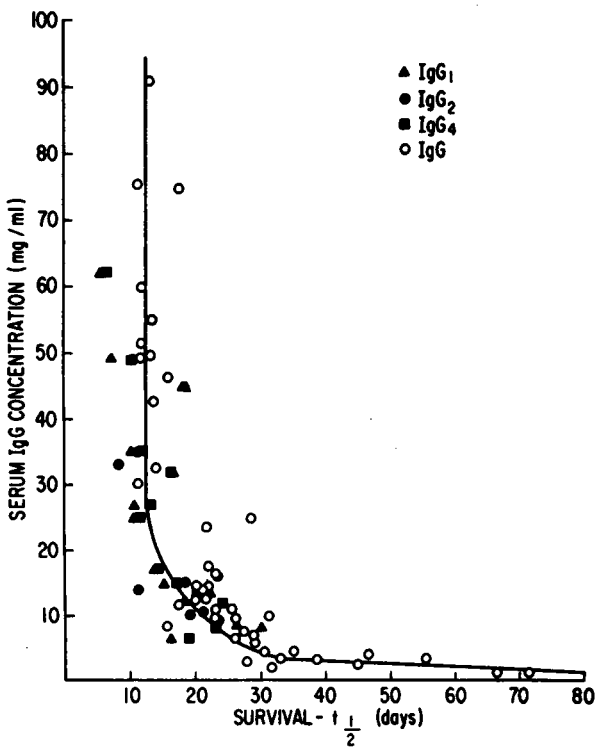

FIGURE 2 The relationship between serum IgG concentration and survival ($t_{\frac{1}{2}}$) of IgG, IgG_1, IgG_2, and IgG_4 proteins.

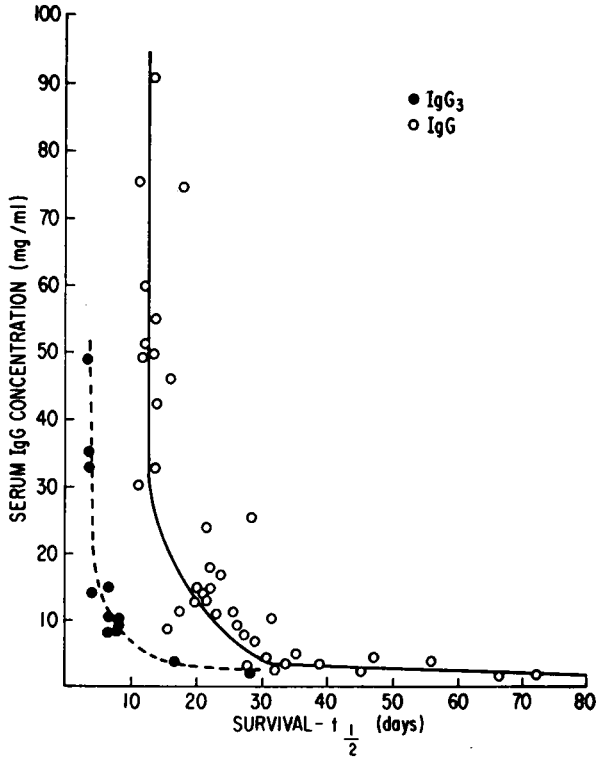

FIGURE 3 The relationship between serum IgG concentration and survival ($t_{\frac{1}{2}}$) of IgG and IgG_3 proteins.

IgG_3 was catabolized two to three times more rapidly than the other subclasses. G_3, however, is known to be a labile molecule (16). It may be fragmented upon serum storage and is very rapidly destroyed by proteolytic enzymes. It was necessary, therefore, to prove that the apparent short survival was not due to molecular degradation occurring during the isolation and radiolabeling of the myeloma protein. This possibility was tested by biosynthetically labeling a G_3-myeloma protein, thus avoiding the need to isolate and radiolabel the protein before injecting it for turnover study. In this procedure, isolation of the G_3 protein occurs after metabolism has taken place and any damage occurring during isolation does not affect the results. A patient with a G_3-myeloma protein was chosen for this study, since the low level of G_3 in normal serum makes it very difficult to isolate normal serum G_3 free of other serum proteins. Results of this study clearly show that the in vivo and in vitro labeled G_3 are metabolized similarly (Fig. 1) and indicate that the short survival and high catabolic rate of G_3 is a property of the native molecule. Since IgG subclasses differ from one another in the primary amino acid sequence of their Fc-fragments

(17, 18), and since it has been shown that survival of the IgG Fc-fragment is similar to that of the whole molecule, while survival of Fab-fragment is very short (2), it can be concluded that the unique metabolic properties of IgG_3 are determined by its Fc-fragment.

The normal serum concentration of G_3 is low, averaging in this study about 0.7 mg/ml, and G_3 accounts for only 7% of the total serum IgG. Calculated synthetic rates are also low, and the low serum G_3 concentration apparently results from the combination of a low synthetic rate and a high fractional catabolic rate. The synthetic rate of G_1 is about seven times that of G_3. The reason for this difference appears to be that in each individual, many more cells secrete G_1 than secrete G_3 protein. This conclusion is supported by fluorescent antibody studies of human spleen cells which show that of the cells containing IgG, only about 10% contain G_3 (19). It was not possible to calculate synthetic rates of G_2 and G_4 because methods for determining serum concentrations of these two subclasses were not available.

In IgG-myeloma patients the concentration of nonmyeloma IgG is usually decreased (20, 21). The present data indicate that this low level of nonmyeloma IgG is due to a combination of reduced synthesis and increased fractional catabolic rate. IgM and IgA serum concentrations are reduced in most cases of IgG-myeloma (20, 21). The survivals of IgA and IgM are normal in myeloma patients, while synthetic rates are profoundly reduced, to less than 15% of normal for IgM (22). The low levels of IgM and IgA are, therefore, caused solely by decreased synthesis.

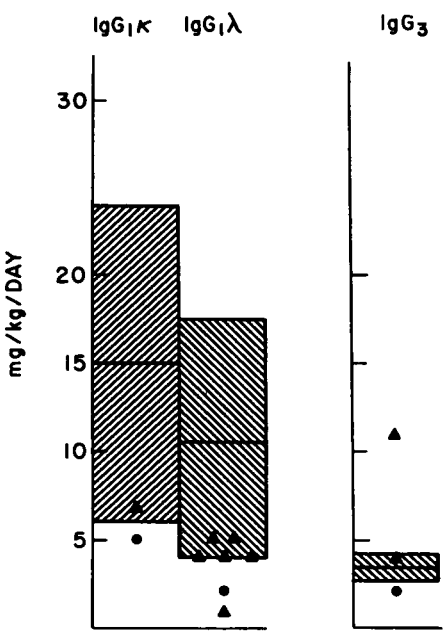

FIGURE 4 Synthesis of $IgG_1\kappa$, and $IgG_1\lambda$ and IgG_3 in patients with G_1 myeloma protein (▲) or G_2 myeloma protein (●). Synthetic rates of "nonmyeloma" G_1 in myeloma patients are generally low when compared with those of patients with normal IgG serum concentrations (cross hatched areas).

The serum concentration of IgG is an important factor in the control of human IgG catabolic rates. High serum levels of IgG are associated with high fractional catabolic rates (short IgG survivals), while low levels of IgG, as in humans with hypogammaglobulinemia, are associated with low catabolic rates and prolonged survival. The relationship between concentration and catabolism is specific for IgG molecules. The survival of IgG is not affected by the concentration of other serum proteins, including the other immunoglobulins (15, 23). The concentration-catabolism relationship must reflect the nature of the mechanism whereby IgG molecules are catabolized. It was of interest, therefore, to determine whether all subclasses participated in this phenomenon, especially since the catabolic rate of G_3 differs so markedly from that of the others. Representative proteins of each of the four subclasses were studied in patients with a range of serum levels of IgG. In general, catabolic rates were proportional to serum IgG concentration. Patients with G_1, G_2, or G_3 myeloma proteins were included in the study and it can be concluded, at least for these three subclasses, that an elevation of one subclass shortens the survival of all subclasses. Patients with elevated serum G_4 concentrations were not available. The concentration-catabolism relationships for isolated G_1, G_2, and G_4 proteins were very similar to that of IgG over the range investigated (Fig. 2). G_3 proteins showed a similar relationship, although the curve is displaced from that produced by IgG (Fig. 3). Two G_3 metabolic studies were performed in patients with hypogammaglobulinemia. The survival of G_3 in these patients was two to three times longer than in recipients with normal IgG concentrations, and seven times longer than in a patient with multiple myeloma and an IgG serum concentration of 30 mg/ml (Fig. 3).

Murine serum also contains definable IgG subclasses whose metabolic properties have been studied. The results are quite parallel to those found in man. One of the murine subclasses, γ_{2b} (γGH) has a shorter survival and higher fractional catabolic rate than the other subclasses (24). In mouse, as in man, a concentration-catabolism relationship has been observed; mice with high serum levels of IgG have high fractional catabolic rates, while those with low serum levels have low catabolic rates and long survival (23, 25).

The mechanism by which IgG is metabolically degraded is unknown. Brambell, Hemmings, and Morris (26) have proposed a hypothetical mechanism for catabolism which postulates a saturable protection system for IgG. IgG molecules are presumed to pass randomly

from the intravascular compartment to a closed compartment, such as a pinocytotic vacuole. Within the vacuole are a limited number of IgG specific receptor sites, to which a fraction of the trapped IgG molecules become attached. Proteolytic enzymes are released into the vacuole. Those molecules attached to receptor sites are protected, and subsequently released into the circulation, while unattached molecules are degraded.

The concentration-catabolism relationship demonstrated for a majority of the patients in the present study is consistent with this hypothesis and expands it by indicating that there are no subclass specific protective receptor sites. All four subclasses compete for the same receptors. A myeloma protein of any of the subclasses therefore would, by the overwhelming number of its molecules, occupy almost all the protective sites, leaving the molecules of the other subclasses exposed to catabolism at a high rate. The hypothesis must be modified, however, to account for the facts that G_3 participates in the concentration-catabolism relationship, but has a much higher catabolic rate. This indicates that although G_3 proteins share metabolic pathways with the other subclasses, they also are acted upon by other catabolic mechanisms. The location of catabolic sites and the details of both the catabolic mechanisms proposed by Brambell and the additional ones suggested by these studies are unknown at this time.

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