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METABOLIC PROPERTIES OF HUMAN IgA SUBCLASSES

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SUMMARY

The metabolism of the two subclasses of IgA was investigated in fourteen turnover studies in individuals with normal immunoglobulin serum concentrations. Differences in the catabolism of radioiodinated IgA1 and IgA2 myeloma proteins were found. The average biologic half-life was 5·9 days for IgA1 and only 4·5 days for IgA2. Fractional catabolic rates were 24% of the intravascular pool per day for IgA1 and 34% for IgA2. The intravascular compartment contained approximately 55% of the total body pools of both IgA subclasses.

The mean IgA1 serum concentration in the individuals used for turnover studies was 2·3 mg/ml, the mean IgA2 level was 0·32 mg/ml. Synthetic rates of 24 mg/kg per day for IgA1 and 4·3 mg/kg per day for IgA2 were calculated. The low serum concentration of IgA2 results mainly from this low synthetic rate. The fractional catabolic rate of IgA2 is higher than that of IgA1. This further increases the difference between the serum concentrations of the two IgA subclasses.

Quantitative determinations of both IgA subclasses were made in twenty-four sera of young male adults. The mean concentrations were 1·81 mg/ml for IgA1 and 0·22 mg/ml for IgA2.

INTRODUCTION

Human IgA is subdivided into two antigenically distinct subclasses referred to as IgA1 and IgA2 (Feinstein & Franklin, 1966; Kunkel & Prendergast, 1966; Vaerman & Heremans, 1966). Both of them are present in serum and external secretions. IgA1 predominates in the serum (Vaerman, Heremans & Laurell, 1968; Fudenberg & Vyas, 1971; Grey *et al.*, 1968). In secretions, IgA2 may contribute up to 50% of the total IgA (Grey *et al.*, 1968). IgA of both subclasses occurs in monomeric and polymeric form. Most of the normal serum IgA is present as a monomer, whereas IgA in secretions is predominantly a dimer (Tomasi *et al.*, 1965). Previous metabolic studies were done with IgA isolated from normal

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and myeloma sera (Solomon & Tomasi, 1964; Nadorp *et al.*, 1972; Strober *et al.*, 1968) or from milk (Stiehm, Vaerman & Fudenberg, 1966). The metabolic behaviour of IgA from these various sources seems to be similar.

The IgA subclasses show important structural differences. The monomeric four chain structure of IgA1 molecules is similar to the other immunoglobulins. In IgA2 molecules, at least in the genetic variant $Am_2(+)$ common among Caucasians, the heavy–light chain disulphide bonds are absent (Grey *et al.*, 1968; Kunkel *et al.*, 1969; Jerry *et al.*, 1970). Instead, the disulphide bridges are localized between the two light chains and between the two heavy chains.

The present investigation was carried out in order to see whether these structural characteristics are correlated with metabolic differences. For this purpose, turnover studies with radiolabelled monoclonal IgA1 and IgA2 proteins were performed in seven individuals. Furthermore, serum concentrations of IgA1 and IgA2 were established in normal young adults.

MATERIALS AND METHODS

Preparation of the radioiodinated proteins

Two myeloma proteins were used for all turnover studies, one was an IgA1/L, the other an IgA2/K of the allotype $Am_2(+)$. The proteins were isolated from serum by zone electrophoresis followed by gel filtration on Sephadex G-200. Column fractions corresponding to the monomeric 7S IgA were used for labeling. Both preparations were found by immunoelectrophoresis to be contaminated only with traces of other serum proteins.

IgA1 was labelled with ^{125}I and IgA2 with ^{131}I by the iodine monochloride method (McFarlane, 1958). The preparations had an average of less than one atom of iodine per molecule of protein and contained less than 2% of non-precipitable radioactivity. Before use, they were tested for sterility and pyrogenicity.

Quantitative determinations of total IgA, IgA1 and IgA2

Antisera to the subclasses of IgA were raised in rabbits (anti-IgA2) and pigs (anti-IgA1). After extensive absorption with serum proteins, they were specific for the heavy chains of either IgA1 or IgA2. The serum concentrations of the two subclasses were determined by a solid phase radioimmunoassay as extensively described for the quantitative analysis of IgG subclasses (Morell *et al.*, 1972): Immunoadsorbents were prepared by coupling the globulin fractions of the antisera to bromoacetyl cellulose. Binding of radiolabelled isolated IgA1 or IgA2 to the immunoadsorbent was quantitatively inhibited by unlabeled antigen, either isolated IgA myeloma proteins or IgA of both subclasses present in sera. Subclass concentrations in the sera were determined graphically by comparing their binding inhibition with the linear part of the binding inhibition curve obtained with serial dilutions of isolated IgA myeloma proteins and the W.H.O. reference preparation 67/97. The concentrations of isolated myeloma proteins were determined by measurement of the optical density ($E^{1\%}_{280} = 13{\cdot}4$).

Quantitative determinations of the IgA subclasses in normal sera were performed in twenty-four sera of 20-year-old male adults. All sera were $Am_2(+)$. Am_2 allotypes in the isolated IgA2 myeloma protein and in the sera were kindly determined by Dr Erna van Loghem, Central Laboratory of the Netherlands Red Cross, Amsterdam.

As a control total IgA in the sera of the turnover patients was measured by radial immunodiffusion using the IDP SEVAC set (Prague, Czechoslovakia). The W.H.O. reference preparation 67/97 was used as a standard. This standard serum contains 95·3 IU or 1·3 mg of IgA/ml (Rowe, Anderson & Grab, 1970; Mancini *et al.*, 1965).

Patient selection

Seven male volunteers were selected for turnover studies. Their immunoglobulin levels were in the normal range. All subjects were hospitalized at the Tiefenau University Hospital in Berne with a variety of diseases. Their ages ranged from 19 to 60 years. None of them had signs of intestinal or renal protein loss.

Turnover study protocol

None of the patients were actually ill during the period of the study. Their serum immunoglobulin levels remained constant and they were thus assumed to be in a steady state concerning immunoglobulin metabolism. In order to block thyroidal uptake of radioactive iodine, all were given fifteen drops of Lugol's solution daily during the entire study period. Intravenous injections of both ^{125}I-IgA1 and ^{131}I-IgA2 were given simultaneously. The dose of radioactivity was in the order of 30 μCi. Ten minutes after injection, a blood sample was drawn for plasma volume determinations. Eight hours later and then daily for 13 days, additional blood samples were collected. Complete urine collections could only be obtained from three patients during the period of the study.

Plasma and urine samples were counted in an automatic gamma ray well-type scintillation counter with a thallium-activated sodium iodide crystal. A pulse height analyser allowed differentiation of the two isotopes in the samples.

Data analysis

The changes in the plasma radioactivity were plotted on semilogarithmic paper and the biologic half-life (T 1/2) of the labelled proteins was determined graphically from the final slope of the curves.

Total circulating and total body pools, the fraction of the intravascular pool catabolized daily (fractional catabolic rate, FCR) and the synthetic rate (turnover rate) were determined by multicompartmental analysis according to Matthews (1957). The fraction of the body pool remaining intravascularly was calculated according to Nosslin (1964). In addition, in the cases with complete urine collections, the whole body radioactivity was calculated by cumulative subtraction of the radioactivity excreted in the urine. Fractional catabolic rates of the intravascular pool were determined from the ratios between daily urine excretion of the isotopes and the plasma radioactivity of the corresponding day (Berson *et al.*, 1953). Results obtained by the different methods were in agreement.

RESULTS

IgA subclass levels in normal human sera

First, the concentrations of the two IgA subclasses were determined in the W.H.O. reference preparation 67/69. Standard binding inhibition curves were established in radioimmunoassays with five isolated IgA1 and three IgA2 myeloma proteins, all of them in the

monomeric form (7S). At a given protein concentration, however, the various proteins showed considerable differences in the binding inhibition capacity. Thus, for calibration of the W.H.O. reference serum, a selection of IgA myeloma proteins had to be made, a difficulty which was also encountered by Grey *et al.* (1968).

For total IgA in this standard serum, 1·3 mg/ml or 95·3 IU/ml, the value recommended by the W.H.O. Immunoglobulin Reference Centre in Lausanne was taken (Rowe, Anderson & Grab, 1970). Subclass concentrations in this reference serum were estimated to be 1·1 mg/ml for IgA1 (85%) and 0·2 mg/ml (15%) for IgA2.

In all subsequent determinations, serial dilutions of the W.H.O. reference serum were used to establish standard curves from which the IgA1 and IgA2 concentrations in the sera could be estimated.

The IgA subclass levels in the sera of twenty-four normal young male adults (20 yr old) are shown in Table 1.

TABLE 1. IgA subclass concentrations in twenty-four young male adults

	IgA1 (mg/ml)	%	IgA2 (mg/ml)	%	Total IgA (sum) (mg/ml)	%
Mean	1·18* ± 0·57	89	0·22 ± 0·08	11	2·03 ± 0·64	100
Range	1·12 – 2·90	86–93	0·12 – 0·47	7–14	1·28 – 3·37	100

* Mean value ± 1 standard deviation.

When the sum of the two subclasses was compared with total IgA determined directly in the radial immunodiffusion method, some disagreement was often noticed. In both methods, however, extreme values were consistently either high or low.

Metabolism of IgA subclasses

The turnover data obtained in the seven individuals are summarized in Table 2. The mean serum levels were somewhat higher than those established in the normal young adults (Table 1). In the average, 88% of the total IgA was IgA1 and 12% was IgA2.

The biological half-lives for IgA1 ranged from 5·3 days to 6·6 days with an average of 5·9 days. The survival of IgA2 was shorter with half-lives ranging from 4·1 to 5·0 days (mean: 4·5 ± 0·3 days).

Curves of the decrease of the radioactivity in the plasma are shown in Fig. 1. Each point represents the mean of the plasma radioactivity at a given time in all seven individuals. From the sixth day until the end of the study the points on the two curves differ by more than one standard deviation.

Table 2 demonstrates that for IgA2, fractional catabolic rates of the intravascular pool (FCR) were considerably higher than for IgA1. The mean values were 0·32 ± 0·04 compared to 0·24 ± 0·02.

Distribution in the body compartments was similar for both IgA subclasses: about 55% were found to be intravascular.

The calculated synthetic rates for IgA1 ranged from 18 to 26 mg/kg/day. The average

TABLE 2. Turnover of IgA subclasses

Name	Age yr	Plasma volume (ml/kg)	Serum concentration IgA1 (mg/ml)	Serum concentration IgA2 (mg/ml)	Total circulating pool IgA1 (mg/kg)	Total circulating pool IgA2 (mg/kg)	Total body pool IgA1 (mg/kg)	Total body pool IgA2 (mg/kg)	Intravascular IgA1 (%)	Intravascular IgA2 (%)	Survival T 1½ IgA1 (days)	Survival T 1½ IgA2 (days)	FCR * IgA1	FCR * IgA2	Synthetic rate IgA1 (mg/kg/day)	Synthetic rate IgA2 (mg/kg/day)
Ri	50	39	3·3	0·43	129	16·8	248	29·0	52	58	6·0	5·0	0·24	0·26	31	4·4
Sc	19	47	2·0	0·28	94	13·2	171	22·0	55	60	5·5	4·5	0·25	0·29	24	3·8
So	46	45	2·1	0·30	95	13·5	190	25·5	50	53	5·8	4·3	0·26	0·35	25	4·7
Hu	25	40	1·8	0·22	72	8·8	120	15·4	60	57	5·3	4·1	0·26	0·38	19	3·3
Lo	25	38	2·1	0·32	80	12·2	157	21·0	51	58	6·6	4·2	0·22	0·32	18	3·9
Ts	35	42	2·2	0·27	92	11·3	161	19·8	57	57	6·0	4·4	0·23	0·33	21	3·7
Ma	60	51	2·8	0·44	143	22·4	251	38·6	57	58	6·3	4·8	0·21	0·28	30	6·3
Means ±1 SD		43 ± 4	2·3 ± 0·5	0·32 ± 0·08	101 ± 26	14·0 ± 4·4	185 ± 49	24·5 ± 7·6	54·6 ± 3·7	57·3 ± 2·1	5·9 ± 0·5	4·5 ± 0·3	0·24 ± 0·02	0·32 ± 0·04	24 ± 5	4·3 ± 1·0

* FCR is the fraction of the intravascular pool catabolized per day.

value was 24±5 mg/kg/day. For IgA2, synthetic rates scattered between 3·3 and 6·3 mg/kg/day (Mean: 4·3±1·0 mg/kg/day).

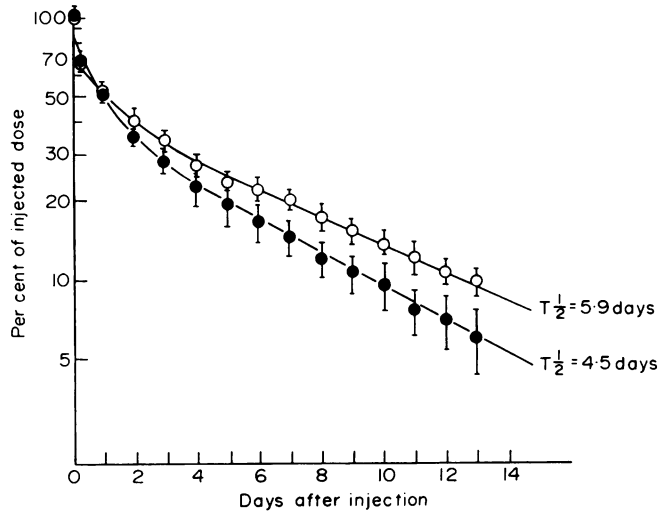

FIG. 1. Decrease of the radioactivity in the plasma following the injection of labelled (○) IgA1 and (●) IgA2 myeloma proteins. The points represent mean values for seven individuals. ± standard deviations are given in vertical bars.

DISCUSSION

Values reported in the literature for IgA serum concentrations have been summarized and discussed by Vaerman (1970) in connection with his own extensive study on 462 sera of healthy, 20–61-year-old, Caucasian blood donors. He found a mean IgA level of 2·63 mg/ml. Considerably higher or lower normal values, however, have also been reported. These discrepancies may in part be due to technical differences in the immunologic methods used for determinations, to subspecificities of the antisera or to different proportions of IgA monomers and polymers in standards. Biological factors like age, sex and race of the serum donors also have an influence on IgA levels (Vaerman, 1970). The mean concentration taken as the sum of the two subclasses in the sera of the seven subjects used for the turnover studies agrees well with Vaerman's value. In the sera of 20–40-year-old individuals, Vaerman found a mean IgA concentration of only 2·26 mg/ml (Vaerman, 1970) which is similar to the sum of the two subclasses reported here for the 20-year-old individuals, 2·03±0·64 mg/ml.

Quantitative data on the IgA subclass distribution in sera are scant. Of 402 monoclonal IgA proteins, 7% were typed as IgA2 by Vaerman (Vaerman, 1970). The same figure was obtained in our laboratories with 273 IgA myeloma sera (Skvaril, unpublished results). In a series of 165 IgA myeloma proteins, Jerry *et al.* (1970) found an incidence of 13% for IgA2. Thus, assuming that myeloma proteins reflect a random selection from immunoglobulin-producing cells or their precursors, IgA2 should contribute about 7–13% to the total IgA.

Previous estimates in normal sera were about 93% for IgA1 and 7% for IgA2 (Vaerman,

Heremans & Laurell, 1968; Fudenberg & Vyas, 1971). In one report (Grey *et al.*, 1968) on three sera from mothers shortly after delivery, the percentage of IgA2 was much higher, 20–36%. These three investigations were carried out with only one subclass specific antiserum, either anti-IgA1 or anti-IgA2. For the other subclass value, the difference to the total IgA concentration was taken. In the present study where both subclasses were determined directly in sera of young adults, IgA1 averaged 89% and IgA2 11%, with ranges from 86% to 93% and from 7% to 14%.

Metabolic data from turnover studies with intravenously injected radio-labelled IgA refer only to IgA which after synthesis enters the circulating pool and is catabolized in or close to the intravascular compartment. Total IgA synthesis in the body, including secretory IgA, the bulk of which is excreted, is higher than the 24–30 mg/kg/day reported here and elsewhere (Solomon & Tomasi, 1964; Strober *et al.*, 1968). Circulating IgA is not transferred into secretions at a scale exceeding the non-specific transudation observed with all plasma proteins (Tomasi *et al.*, 1965; Stiehm *et al.*, 1966; South *et al.*, 1966; Strober *et al.*, 1970; Butler *et al.*, 1967). The fractional catabolic rates of both IgA subclasses, therefore, are not significantly influenced by external loss.

In all seven individuals, IgA2 was catabolized faster than IgA1. A similar phenomenon also exists for the subclasses of IgG: IgG3 has a shorter survival and a higher fractional catabolic rate than the other subclasses (Spiegelberg, Fishkin & Grey, 1968; Morell *et al.*, 1970). Thus, within the immunoglobulin classes IgG and IgA, structural as well as metabolic variations are seen.

The serum concentration is an important factor in the control of the catabolic rate of IgG (Waldmann & Strober, 1969). No such concentration–catabolism relationship exists for IgA and the degradation rates in subjects with normal, low or elevated serum IgA levels are similar (Solomon & Tomasi, 1964; Strober *et al.*, 1968). A number of biologic activities of IgG, including the rate of catabolism, are determined by the Fc-piece of the molecules (Waldmann & Strober, 1969). For IgA molecules the portion responsible for catabolism is not known. Enzymatic cleavage of human IgA into biologically active fragments is extremely difficult (Grey, Abel & Zimmerman, 1971) and turnover experiments with appropriate fragments have not yet been done.

IgA1 comprises about 85% of the total synthesis of serum IgA (28·3 mg/kg/day), and IgA2 only 15%. The low serum levels for IgA2 are mainly due to this low synthetic rate. An additional factor which further tends to reduce IgA2 in the serum is its higher rate of catabolism.

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