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IMMUNOGLOBULIN PATTERNS IN HUMANS OVER 95 YEARS OF AGE

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SUMMARY

Immunoglobulin patterns were investigated in seventy-three volunteers older than 95 years. An idiopathic paraproteinaemia was found in 19% of the cases. A restriction of heterogeneity and an imbalance in the κ/λ ratio of the immunoglobulins was seen in a number of other sera. Determinations of immunoglobulin levels in sera of individuals without paraproteinaemia showed an increase in IgA and IgG. The quantitations of the IgG subclasses demonstrated that an increase in the IgG1 and IgG3 subclasses is responsible for the elevated level of the IgG. The variation in the immunoglobulin levels increased significantly with age for IgM and for the three major IgG subclasses. No abnormalities were found in the urine or in the mixed saliva. These results indicate that selective changes in the extent of the antibody–immunoglobulin repertoire characterize the immunoglobulin pattern of ageing man.

INTRODUCTION

Immunological studies in man have revealed that both the cellular and the humoral immune responses decline with advancing age. When studying immunoglobulin levels in sera of aged people. one would accordingly expect a decrease. This was actually found only for IgG and IgM in the age groups between 30 and 60 years by Buckley & Dorsey (1970). Four other groups observed. in contrast. an increase in IgG and IgA during ageing. while IgM was hardly changed (Haferkamp *et al.*, 1966: Grundbacher & Schreffler. 1970: Kalff, 1970: Finger. Emmerling & Hof. 1973). In a more recent longitudinal study. Buckley. Buckley & Dorsey (1974) found that about two-thirds of the ageing subjects studied revealed increasing levels of IgG and IgA, while in a smaller portion of subjects the immunoglobulin levels decreased. No data are available from these studies on the presence of homogeneous immunoglobulins (H-Ig)—'idiopathic paraproteins'—in sera of the individuals studied. This is a phenomenon of ageing which has been repeatedly described by other investigators (see review by Waldenstrom. 1973: add by Zawadzki & Edwards. 1972). Our study was performed in order to determine whether the changes in the immunoglobulin levels during

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ageing involve all immunoglobulins with their subpopulations, or whether there are selective changes only. Special attention was paid to the presence of idiopathic paraproteins.

MATERIALS AND METHODS

Sera, urine and mixed saliva from seventy-three volunteers older than 95 years and living in homes for the aged in The Hague were made available for the investigation. This group constitutes 35% of the total population over 95 years of age living in The Hague. The available information on the social–economic level and the physical health indicates no substantial difference between our group of volunteers and others living at home. These aged volunteers—twenty males and fifty-three females-did not suffer from any overt disease. On physical examination, no enlarged lymph nodes or hepatosplenomegaly were found. Autopsy was performed in nine of the thirty-four individuals who died within 18 months after examination. Two of them had a paraproteinaemia, but no signs of lymphoreticular malignancy were found. Sera from eight\-nine healthy blood donors between 41 and 65 years of age were obtained from the Blood Bank of the University Hospital in Leiden and used for the comparative immunoglobulin determinations. The other two groups of young adults who were used for the same purpose have been previously described (Radl *et al.*, **1970:** Morell *et al.*, 1972).

Serum protein concentrations were determined by the biuret method. Quantitation of the IgG, IgA, IgM and IgD classes was performed by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965), modified by Voormolen-Kalova, Van Den Berg & Radl **(1974).** The levels of the four IgG subclasses were determined by a radioimmunoassay (Morell **et *al.*, 1972).** Paraproteins **(H-Ig)** were revealed by agar electrophoresis according to Wieme (1959) and classified by immunoelectrophoresis and immunoselection (Radl, 1970). An assessment of the κ/λ ratio of the immunoglobulins was performed by the 'double line' immunodiffusion technique (Skvaril & Barandun, **1973)** and by immunoselection (Radl, **1970).** Salivary immunoglobulins were tested by the double radial immunodiffusion method using monospecific antisera against IgG, IgA, κ and λ light chains. and against the secretory component. **All** of these antisera were raised in rabbi. d made monospecific by adsorptions on immunoadsorbents as described previously (Radl *et al.*, 1974).

RESULTS

In fourteen of the seventy-three serum samples (19%), an M-component was revealed by agar electrophoresis. Classification by immunoelectrophoresis and immunoselection showed that six of these paraproteins belonged to the IgG class, four to the IgA, and four to the IgM class. In eight cases, the light chain of the paraprotein was of the κ type; in six cases, of the λ type. The concentration **of** the paraprotein never exceeded 1·5 g/100 ml. In addition, the gamma-globulins of ten other cases showed irregularities in the density of the gamma region in agar electrophoresis, indicating a restriction of heterogeneity of the immunoglobulins. However, these changes were too subtle to be further classified. Sera from forty-seven individuals without paraproteinaemia were assessed for the κ/λ Ig ratio. An imbalance in the κ λ ratio was found in nineteen cases (40%). In sixteen cases (34%), this imbalance was in favour of immunoglobulins containing the λ light chains; in three cases (6%) immunoglobulins of the κ light chain type were in excess, as compared with sera from normal young adults.

The Concentration of IgG, IgA and IgM immunoglobulins was determined in sera of individuals without paraproteinaemia and expressed in i.u. (Rowe, Anderson & Grab, 1970). and in mg/ml calculated from i.u. (Rowe. Grab & Anderson, 1972). The values were compared with those of three other groups of healthy young adults and older blood donors (Table 1). Results are given for both males and females together because there was no significant difference between sexes for any immunoglobulins and age groups; the only

TABLE 1. Concentrations of IgG, IgA and IgM in human sera during ageing

Age (years)	n	IgG geometric mean value (range ± 1·96 s.d.)		Difference*	IgA geometric mean value (range ± 1·96 s.d.)		Difference*	IgM geometric mean value (range ± 1·96 s.d.)		Difference*
		mg/ml	i.u.		mg/ml	i.u.		mg/ml	i.u.	
20–30	23	10·86 (7·20–16·37)	135 (90–204)	$P<0·02$	1·93 (1·18–3·23)	136 (84–227)	$P<0·01$		125 (72–215)	n.s.
41–50	40	11·09 (7·13–17·23)	133 (89–214)	$P<0·02$	1·45 (0·63–3·36)	102 (44–237)	$P<0·001$		134 (61–293)	n.s.
51–65	49	11·60 (7·66–17·57)	141 (95–219)	n.s.	1·65 (0·76–3·56)	124 (57–269)	$P<0·001$		140 (72–272)	$P<0·01$
>95	59	12·48 (7·64–20·40)	153 (95–253)		2·54 (1·16–5·54)	179 (82–390)			124 (40–383)	

n.s. = not significant.

* Significance of difference between the given age group and the over 95 years group

TABLE 2. Concentration of IgD in sera of humans over 95 years of age

	Males— geometric mean value (range ± 1.96 s.d.)	Females— geometric mean value (range? 1.96 s.d.)	Total— geometric mean value (range ± 1.96 s.d.)
n	20	53	73
mg/100 ml	1·7 (0·6–4·7)	1·8 (0·6–5·1)	1.8 (0·6–5·0)
i.u.	12 (5–33)	13 (4–36)	13 (5–35)

TABLE 3. Comparison of IgG subclass levels between young adults and aged persons

	Young adults— geometric mean value (range? 1.96 s.d.) (mg/ml)	Aged persons (>95)— geometric mean value (range? 1.96 s.d.) (mg/ml)	Significance of difference between the two groups
n	108	57	
IgG1	6.4 (3·8–10·7)	7.3 (3·4–15·4)	P< 0.02
IgG2	3.0 (1·5–6·0)	3.3 (1·2–9·4)	n.s.
IgG3	0.5 (0·2–1·3)	0·8 (0·2–3·1)	P< 0·001
IgG4	0.3 (0·04–2·1)	0.3 (0·04–3·5)	n.s.

n.s. = not significant.

exception was the finding of higher IgM levels in females ($P<0·05$) in the group of 51–65 years of age. The IgG and, especially, the IgA levels in the over 95 years group show a significant increase as compared to the three other groups. The IgM concentration does not much differ, showing a significant decrease only in comparison with the group of 51-65 years. The levels of IgD were determined in sera of all individuals of the over 95 years group and the are shown in Table 2.

The concentrations of the four IgG subclasses are presented in Table 3. Here again, no difference between sexes was found. When comparing values of the individual IgG subclasses of the old people with those of the young adults, a significant increase in IgG1 and IgG3 concentration was found in the former group.

The question as to whether the presence of a paraprotein can influence the levels of immunoglobulins of the other classes was studied by comparing the immunoglobulin levels in the individuals without a paraproteinaemia with the corresponding immunoglobulins in

TABLE 4. Comparison of the immunoglobulin levels in the aged group between persons without and with a paraproteinaemia

Paraproteinaemia	IgG geometric mean value (range ± 1·96 s.d.)		Difference*	IgA geometric mean value (range ± 1·96 s.d.)		Difference*	IgM geometric mean value (range ± 1·96 s.d.)		Difference*
	mg/ml	i.u.		mg/ml	i.u.		mg/ml	i.u.	
Absent	12·48 (7·64–20·40)	155 (95–253)	$P<0·05$	2·54 (1·16–5·54)	179 (89–390)	$P<0·01$	1·05 (0·34–3·24)	124 (40–383)	n.s.
	n = 59			n = 59			n = 59		
Present	10·27 (5·45–19·37)	128 (68–241)		1·65 (0·44–6·18)	116 (31–434)		0·82 (0·33–2·06)	97 (39–243)	
	n = 8			n = 10			n = 10		

n.s. = not significant.

* Significance of difference between the two groups.

the individuals with a paraproteinaemia (Table 4). The class of immunoglobulins to which the paraprotein belonged was excluded from the calculations. The mean values for all three major classes were lower in the group of individuals with a paraproteinaemia. but the difference was significant only for IgG and IgA.

The data given in Table I indicate that the variability in the immunoglobulin levels increases with age. However. a statistical comparison of variance showed a significant difference only for IgM between the over 95 group and the two groups comprising the individuals under 50 years of age. For IgG and IgA. no significant difference was found. By contrast. when IgG subclasses were analysed. the three major subclasses showed a significant difference in variance between the old and the young.

No Bence-Jones protein was detecred in the urine samples of the aged. Examination of the mixed saliva showed no deficiency in any of the immunoglobulins or in the secretor? component.

DISCUSSION

Our findings of a high incidence of paraproteinaemia at an advanced age are in agreement with the data of Englisova *et al.* (1968). The question as to whether these paraproteinaemias can be classified as idiopathic in all cases. is difficult to answer. All detailed examinations necessary to exclude myeloma, Waldenström's macroglobulinaemia or other malignancies of the lymphoreticular system could not be performed. However, on the basis of the relatively low and steady levels of the paraproteins, the absence of Bence-Jones proteinuria. normal clinical examination, and negative autopsy findings in some of the deceased subjects, we are inclined to believe that most of the paraproteinaemias in our group could be designated as idiopathic.

The results of the determinations of immunoglobulin levels in sera of individuals without paraproteinaemia show the same increasing trend for IgA and IgG during ageing as found in the unselected groups reported previously (Haferkamp *et al.*, 1966: Grundbacher & Schreffler. 1970: Kalff, 1970; Finger *et al.*, 1973). The quantification of the IgG subclasses showed that an increase in the IgG1 and IgG3 subclasses is responsible for the elevated level of the IgG.

In evaluating our data, the problem of selection should be mentioned first. In the absence of a longitudinal study, there is an implied selection for survival. We think, however, that we have excluded a significant bias within the age group studied. With these restrictions in mind, we can summarize our findings as follows. The overall immunoglobulin levels in era of ageing people do not show a decrease; on the contrary, there is a trend towards increasing levels of IgA and IgG, while IgM remains at about normal levels. This would not support the idea of a decrease in the immune capacities during ageing. However, when taking into account the other parameters studied such as the increasing variability in IgM and in the subpopulations of IgG, an imbalance in the κ/λ ratio and the restricted heterogeneity of the immunoglobulins expressed *ad maximum* in the appearance of 'idiopathic paraproteins', we can Speculate that not the quantity but the quality, i.e. the extent of the antibody repertoire, is altered during ageing. The immunoglobulin patterns in sera primarily reflect the functions of the B-cell immune system. but also indirectly those of the T-cell system. In order to clarify the mechanisms involved in the changes in the immune system during ageing. more sophisticated techniques and more detailed studies covering larger areas of the

immune system network will be necessary. In this respect also, more possibilities may be offered by using a suitable experimental animal model which shows similar phenomena as those observed in ageing man (Radl & Hollander, 1974).

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