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Restriction of immunoglobulin heterogeneity, autoimmunity and serum protein levels in aged people

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SUMMARY

Ninety-one sera of persons above 80 years of age were screened for autoantibody activity against lipoproteins (anti-LDL 7, anti-HDL 6 positive), for rheumatoid factor activity (Latex 14, Waaler-Rose 7 positive) and for antinuclear factors (11 positive). Among the sera with autoantibody activity 29% showed deviations of the normal kappa/lambda ratio of immunoglobulins, as opposed to 22% of the sera without detected autoantibody activity. In 3% of the sera an M component was detected. Determination of the α_1-acid glycoprotein, α_1-antitrypsin, haptoglobin, haemopexin, complement component C_3c and C_4, IgG, IgA and IgM levels showed significant increases in α-, and β-globulins as well as in IgG and IgA in sera of the aged persons as compared to a normal population between 20 and 60 years old. No significant difference was noted between the γ-globulin concentration in sera of aged persons with or without autoantibody activity. The evaluation of the relationship between serum protein levels and alterations of the kappa/lambda ratio indicated that the α- and the β-globulins were significantly raised in sera with altered kappa/lambda ratios, whereas, with the exception of M component containing sera the γ-globulin levels seemed not significantly affected by changes in this ratio.

INTRODUCTION

Ageing is associated with significant changes in activity of the immune system (see review by Walford, 1969; Makinodan, Perkins and Chen, 1971; Mackay, 1972). The ability to respond to exogeneous immunogenic stimuli as well as the cell-mediated immune functions become impaired with advancing age (Kishimoto, Tsuyuguchi & Yamamura, 1969; Mackinodan, Perkins & Chen, 1971; Waldorf, Willkens & Decker, 1968; Hallgren *et al.*, 1973; Weksler & Hutteroth, 1974; Foad *et al.*, 1974). Concomitant with the decline of immune responses to extrinsic antigens an increase in reactiveness to intrinsic components and an increase in the average immunoglobulin levels is apparent (Rowley, Buchanan & Mackay, 1968; Teague *et al.*, 1972; Haferkamp *et al.*, 1966; Kalff, 1970). In addition a relatively high incidence of monoclonal immunoglobulins (M components) has been reported in the old age (Hallen, 1963; Englisova *et al.*, 1968; Radl *et al.*, 1975).

The discrepancy between abundance of immunoglobulins and impaired defence against foreign antigens raises the possibility that the high immunoglobulin levels of aged people are due to immune responses against autologous antigens or alternatively that they are the consequence of some adjuvant effect associated with a non-specific stimulation of immunoglobulin secreting cell clones.

The present investigation was undertaken to explore the possible relationship between the occurrence of autoantibodies, the production of homogeneous immunoglobulins and the levels of some α- and β-globulins, as well as of the γ-globulins (IgG, IgA and IgM) in aged persons.

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MATERIALS AND METHODS

Plasma samples were collected from ninety-one subjects above 80 years of age. All samples were taken after a 12-hr fast. Normal sera were obtained from the hospital's staff and from orthopaedic patients aged between 20 and 60 years old.

Detection of rheumatoid factor. Rheumatoid factor activity was screened for by the latex fixation test of Singer & Plotz (1956) and by the sensitized sheep cell test of Waaler (1940) and Rose, Ragan, Pearce & Lipman (1948). Latex particles coated with human gammaglobulins were purchased from Hyland, sheep red blood cells sensitized with rabbit gammaglobulins from Denver-Wampole, Laboratory.

Detection of antinuclear factors. Antinuclear factor activity was tested by the immune-fluorescence technique, using a fluoresceinisothiocyanate-(FITC)-labelled antiserum to human immunoglobulins. Human lymphocytes were isolated from ascites by the use of a Ficoll gradient in the cytocentrifuge. The lymphocytes were fixed with a mixture of ethanol and acetic acid. The fixed lymphocytes were incubated with the sera to be tested for 30 min. After washing with phosphate-buffered saline (PBS) pH 7·8, they were incubated with the FITC-labelled antisera for 30 min and washed again with PBS for 15 min.

Detection of anti-lipoprotein activity. Sera were screened for anti-lipoprotein activity by the passive haemagglutination of human red blood cells (O, Rh negative), which had been coated with LDL or HDL respectively by means of bisdiazotized benzidine as previously described (Butler & Brunner, 1966). Negative controls were set up simultaneously with both an inactivated normal serum pool incubated with lipoprotein-coated human red blood cells and with the inactivated serum samples incubated with uncoated human red blood cells. Specificity against Ag factors (Butler, 1969) was tested by haemagglutination/inhibition studies using LDL of single donors with different Ag factors.

Preparation of lipoproteins. Lipoproteins of very low density (VLDL), low density (LDL) and high density (HDL) were isolated by repeated ultracentrifugation at different densities (Gidez, 1968).

Screening for deviations of the kappa/lambda ratio of immunoglobulin light chains. Deviations from the normal ratio were detected by the double line method according to Skvaril & Barandun (1973). This method takes advantage of the fact that a mixture of two antisera against kappa and lambda light chains gives two precipitin lines upon reaction with kappa and lambda immunoglobulins in the double diffusion technique in agar. The distance between these two lines varies with alteration of either the kappa or the lambda concentration in the serum. Obviously, the greatest deviations are observed in sera containing M components. M components additionally were screened for by agar-gel electrophoresis.

Quantitative determination of serum protein levels. The concentrations of immunoglobulins, α_1-antitrypsin, haptoglobin, haemopexin, C_3c and C_4 were determined by the radial immunodiffusion method according to Mancini, Carbonara & Heremans (1965) using 'Partigen^R' plates (Behringwerke/Marburg/Lahn).

Statistical evaluation. For statistical evaluation the Student's *t*-test was used.

RESULTS

The results obtained by screening ninety-one sera of individuals above 80 years of age are summarized in Table 1. Six sera (7%) reacted with human LDL and HDL, one displayed only anti-LDL activity. (Titres above 1:8 were considered positive only). The presence of isoantibodies against genetically determined polymorphisms of LDL (Ag-factors (reviewed by Butler, 1969)) could be excluded since the sera reacted with every human LDL of single donors with different Ag-specificities. Fourteen sera (15%) exhibited rheumatoid factor activity as demonstrated by the Latex fixation test. Five of these sera also reacted with the lipoprotein-coated red blood cells. In the Waaler–Rose test seven sera were positive for rheumatoid factors, two of these were also positive for anti-lipoprotein activity. Reactions with nuclear material from human lymphocytes were weakly positive in eleven neat sera (12%). When diluted 1:5 no

TABLE 1. Autoantibodies in persons above 80 years old

Autoantibody activity	No. of cases tested	No. of positive reactions	
		LDL	HDL
Lipoproteins	91	7 (8)	6 (7)
		Latex	Waaler–Rose
Rheumatoid factors	91	14 (15)	7 (8)
Antinuclear factors	91	11 (12)	

Figures in parentheses are percentages.

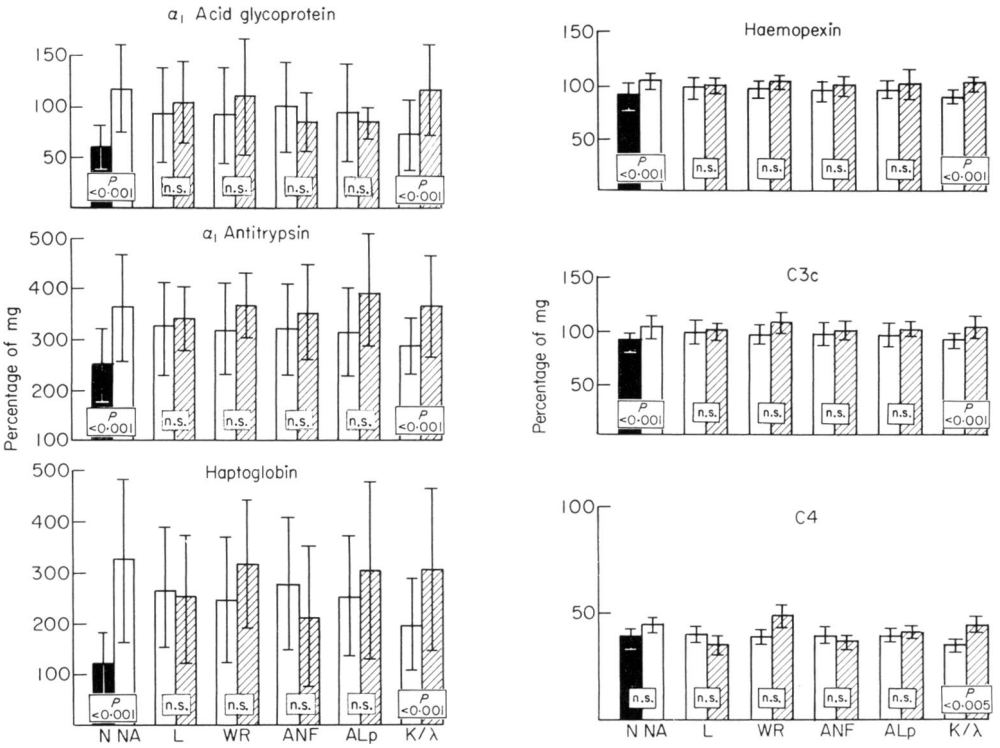

FIG. 1. α-globulin levels in ninety-one persons over 80 years of age. ■ N = Normal value. □ NA = 'Normal' aged n.s. = Not significant. ▨ Aged persons with autoantibodies. L = Latex positive; WR = Waaler-Rose positive; ANF = Antinuclear factor; ALp = Anti-lipoprotein autoantibody; κ/λ = Alterations of the kappa/lambda ratio of Ig light chains.

FIG. 2. β-globulin levels in ninety-one persons over 80 years of age. Symbols are the same as those used in Fig. 1.

clearly positive reaction was detectable. In control sera the frequency of autoantibody activities was below 1%.

The level of α-globulins (α_1-acid glycoprotein, α_1-antitrypsin, α_2-haptoglobin), β-globulins (haemopexin, C_3c, C_4) and γ-globulins (IgG, IgA, IgM) were compared with those in sera of 20–60-year-old persons. The α-globulin levels were significantly higher in sera of the aged (as compared to a well-matched normal population) (Fig. 1). Similar results were noticed with the β-globulins (Fig. 2). In the immunoglobulin fraction the IgG and IgA levels were significantly higher than normal values, whereas the concentration of IgM was not significantly different within the two groups, aged and normal (Fig. 3).

Alterations of the kappa/lambda ratio of the immunoglobulins were observed in 22% of the sera (M components excluded). In three of the ninety-one sera from persons above 80 years of age an M component was detected.

The following conclusions can be drawn from the analysis of these ninety-one sera: there is no apparent relationship between autoantibody activity and α-, β- or γ-globulin levels in the aged population (Figs 1, 2 and 3). Alterations of the kappa/lambda ratio are not significantly influenced by the levels of IgG, IgA or IgM, or by the total protein concentration (Fig. 3). The α- and the β-globulin levels, however, are significantly higher in sera exhibiting alterations of the kappa/lambda ratio than in sera with a normal ratio (Fig. 1, *2*). Alterations of the kappa/lambda ratio are significantly ($P<0{\cdot}05$) more frequent in sera with autoantibody activity (29%) than in sera without autoantibodies of the three specificities tested (22%) (Table 2).

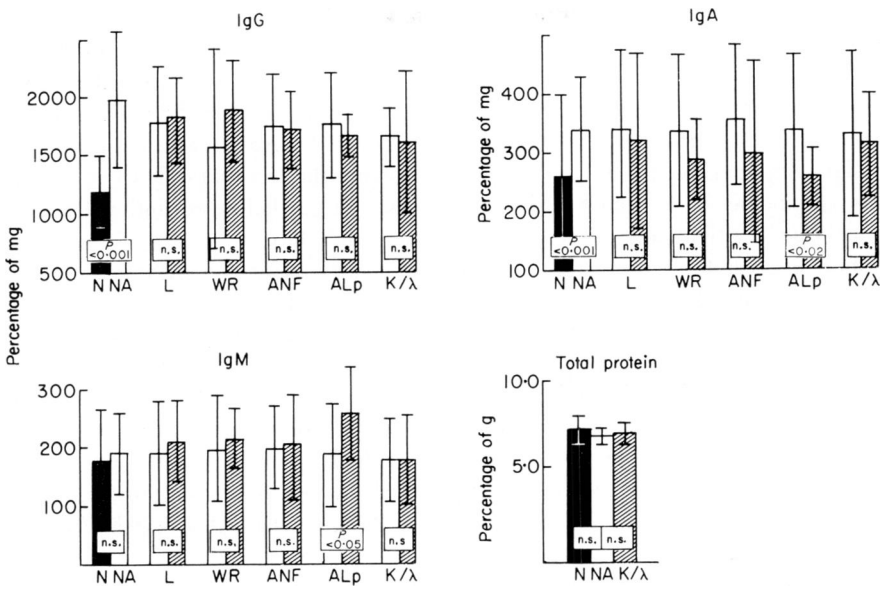

FIG. 3. γ-globulin and total protein levels in ninety-one persons over 80 years of age. Symbols are the same as those used in Fig. 1.

TABLE 2. Relationship between alteration of the kappa/lambda ratio and autoantibody activity

	No. of cases with normal κ/λ ratio	No. of cases with altered κ/λ ratio
Anti-lipoprotein	5 (71)	2 (29)
Latex positive	10 (71)	4 (29)
Waaler–Rose positive	5 (71)	2 (29)
Antinuclear factor	8 (73)	3 (27)
Without detected autoantibody activity	49 (78)	14 (22)

Figures in parentheses are percentages.

DISCUSSION

The relationship between autoantibody activity, immunoglobulin heterogeneity and the levels of the immunoglobulins (IgG, IgA, IgM) and of the predominant α- and β-globulins was studied in humans above 80 years of age. In agreement with previous reports an increased frequency of rheumatoid factor activity (Sachse & Poser, 1961; 1962; Heimer, Levin & Rudd, 1963; Cammarata *et al.*, 1964) and antinuclear factor activity (Cannat & Seligmann, 1965; Litwin & Singer, 1965; Rowley *et al.*, 1968), was observed. The same result applies for a new type of autoantibody activity, specific for the protein part of lipoproteins, which seems to be associated with the same types of diseases as rheumatoid factors are (Riesen & Noseda, 1975). A relationship between autoantibody activities and the restriction of the immunoglobulin heterogeneity, as evidenced by alterations of the kappa/lambda ratio (Skvaril & Barandun, 1973), is suggested by the finding that autoantibody containing sera more frequently exhibit restrictions in heterogeneity than sera without detectable autoantibodies. Since the kappa/lambda ratio was shown to be considerably constant in normal individuals of 20–60 years of age (Fahey, 1963; Skvaril *et al.*, 1975), it should provide a suitable indicator for a restricted immunoglobulin heterogeneity.

In some instances a deviation of this ratio probably reflects the emergence of an M component. Two possibilities could account for the observation of restrictions in heterogeneity in autoantibody containing sera. Either these could result from a specific immune response with restricted heterogeneity against autoantigens or the chronic stimulation by autoantigens could give non-specific rise to single cell clones. It is noteworthy that M components arising in long standing rheumatoid arthritis occasionally do not exhibit anti-IgG activity (Potter, 1971). This observation and the fact that only three of the numerous autoantibody specificities have been tested here may explain the failure to find a direct relationship between autoantibody activity and raised levels of immunoglobulins.

The question whether the increase of the γ-globulin concentration is a pathologic or a physiologic feature of ageing is rather difficult to evaluate because only a small percentage of people above 80 years of age may be considered 'clinically normal'. Longitudinal observations of serum immunoglobulin levels suggested a gradual increase in serum IgG and IgA in surviving older humans and a selective mortality of aged persons with relative immunodeficiency (Buckley, Buckley & Dorsey, 1974). In our survey thirteen of ninety-two persons were found normal with respect to clinical findings and serum-immunoelectrophoretic pattern. The IgG and IgA concentrations of these were significantly higher as compared to a normal population, whereas the IgM level was not significantly different. Similar data have been reported by other authors (Haferkamp *et al.*, 1966; Grundbacher & Schreffler, 1970; Kalff, 1970; Finger, Emmerling & Hof, 1973).

The levels of the α- and β-globulins were significantly higher in the aged persons than in a normal population: this observation is in agreement with previous findings (Gingold, 1958; Karel, Wilder & Beber, 1956). This increase seems to be closely associated with deviations from the normal kappa/lambda ratio of the immunoglobulins. In fact the concentrations of the α- and β-globulins in the sera of aged persons with normal kappa/lambda ratios were not significantly different from normal values. This suggests that the high levels of these globulins are not directly age-related. An increase of the α_1-acid glycoprotein and α-antitrypsin levels is commonly observed in association with acute or chronic inflammation. This gives support to the idea that the restricted heterogeneity of the immunoglobulins in some aged persons is related to an active stimulation.

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