CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 35, 234–244 (1985)

Discrepancy in the Expression of Autoantibodies in Healthy Aged Individuals[1]

FRANCO SILVESTRIS,[2] WILLIAM ANDERSON, JAMES S. GOODWIN, AND RALPH C. WILLIAMS, JR.

Departments of Medicine and Cell Biology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Sera from 50 healthy old subjects and from 51 young controls were tested by ELISA assays for a panel of autoantibodies, including IgM RF, anti-DNA, anti-F(ab')$_2$, anti-thyroglobulin, anti-human albumin, anti-human hemoglobin, anti-secretory component from human IgA, and anti-gliadin. *In vitro* production of anti-DNA as well as anti-F(ab')$_2$ antibodies were measured after stimulation of PBMC by pokeweed mitogen (PWM) in 12 healthy elderly subjects and 11 young controls. Sera from elderly donors contained threefold higher amounts of IgM RF than young controls ($P < 0.001$). On the contrary, the levels of anti-DNA as well as anti-F(ab')$_2$ antibodies were similar in both groups ($P < 0.3$ for the two determinations). Anti-DNA and anti-F(ab')$_2$ antibodies were also measured in supernates of PWM-stimulated glass nonadherent PBMC cultures from both old and young healthy donors without finding any significant difference between the two groups. Additional ELISA tests were also performed in both elderly and young control sera to detect antibodies against six other different antigens. No significant difference was found in the percentages of positive sera between the two groups. This discrepancy in production of IgM RF compared to other autoantibodies in healthy elderly subjects does not provide support for a general increase of autoantibodies with aging. The biological significance of an increase in IgM RF with aging remains to be determined.

INTRODUCTION

Extensive studies during the past several years report the general decline of immune functions in both experimental aged animals and elderly people as the final effect of progressive senescence of both cellular and humoral immune mechanisms. The production of autoantibodies such as anti-γ-globulin, anti-nuclear factors, and anti-thyroglobulin—directed against the most common self antigens—have been reported to increase with aging (1–5). A higher incidence of these autoantibodies in old individuals when compared to young people may be the result of qualitative abnormalities in the helper/suppressor immunoregulatory mechanisms associated with aging (6, 7). Altered proportions in the subsets and function of T lymphocytes with increases in T-helper subpopulations may implement age-induced mechanisms resulting in increased production of autoantibodies (8–10). The autoantibody most frequently reported to increase with age is the IgM rheumatoid factor (RF). Previous reports describe an increase in serum levels of IgM RF in asymptomatic old subjects when compared to young people, as well

[1] Supported in part by Grants AMAI 13690, AMAI 13814, and AG 01245 from the National Institutes of Health and in part by a Center Grant from the Arthritis Foundation.

[2] Dr. Silvestris was the recipient of a grant from the Italian Research Council.

0090-1229/85 $1.50

as higher *in vitro* production of IgM RF by lymphocytes from aged individuals in cultures with and without pokeweed mitogen (PWM) stimulation (11–13). However, *in vitro* functional analyses of cellular subsets from both old and young subjects and their allogeneic combinations show that B cells from old donors produce significantly less RF *in vitro* than B cells from young controls. Moreover, the increased production of IgM RF *in vitro* from old subjects may be dependent upon helper activity of OKT4(+) T-cell subsets derived from old donors (14). Previous studies have also shown that there is no significant *in vitro* difference between young and aged normal subjects in the production of total IgG and IgM by peripheral blood lymphocytes (15). These data suggest that the cellular basis of the production of IgM RF may be different from the mechanisms governing production of other antibodies. In order to analyze possible differences in the production of autoantibodies between old and young donors, *in vivo* production of eight different autoantibodies was studied in healthy aged and young donors. In addition, *in vitro* inductions of anti-DNA and anti-$F(ab')_2$ antibodies by PWM-stimulated lymphocytes were assayed using peripheral blood mononuclear cells from both old and young people.

Since our findings indicate that among all autoantibodies tested only the IgM RF seems to increase with aging, it seems possible that the IgM RF involves a biological function which may be different from the role of other common autoantibodies. The real function of IgM RF still remains unclear. Its dissimilarity to the behavior of other autoantibodies during aging might indicate that the IgM RF could be involved in biologic phenomena somehow unique to the aging process.

MATERIALS AND METHODS

Donors. Two groups were studied: 51 healthy old donors (25 men and 26 women), aged over 65 (mean 73.9 ± 5.4); and 50 young subjects (26 men and 24 women), ages 20–27 (mean 25.2 ± 2.1). We selected the following criteria to include subjects in this study: (1) age 65 or more; (2) no prescription medication or ingeston of any daily nonprescription medication; (3) no known serious medical diagnoses; and (4) no relatives with any kind of autoimmune diseases. This last criterion was strictly adhered to throughout the study. Patients having first degree relatives with rheumatoid arthritis, systemic lupus, mixed connective tissue disease, Sjögren's syndrome, scleroderma, Graves disease, chronic thyroiditis, or multiple sclerosis resulted in exclusion from the study. The presence of autoantibodies as well as the prevalence of various immunological aberrations in unaffected relatives of patients with autoimmune disease has previously been well documented (16–18). Medical history, physical examination, and laboratory tests performed on all subjects studied revealed no medical problems. The subjects selected for this study were obtained from an original larger number of donors (89 old individuals and 63 young controls), but the number was restricted on the basis of the above criteria in order to obtain selected, homogeneous groups of healthy subjects.

IgM RF ELISA assay. A method previously described (19, 20) was used with slight modification. Briefly, microtiter 96-well flatbottom polyvinylchloride plates (Dynatech, Alexandria, Va.) were incubated overnight at 4°C with human IgG

purified by DEAE chromatography from Cohn Fraction II. After incubation, the plates were washed three times with 5% calf serum (CS) in phosphate-buffered saline (PBS) and then incubated for 1 hr at room temperature with the same solution in order to block any subsequent, nonspecific binding. Known standard concentrations of IgM RF obtained from a patient with mixed cryoglobulinemia were used in the test in order to obtain a titration standard curve for the evaluation of the amount of IgM RF in each serum tested. Serum samples, diluted 1:40, were added in triplicate on the plates. After 3 hr incubation at room temperature (RT), the plates were washed three times and goat horseradish peroxidase (HRPO) anti-human IgM antiserum (Tago, Burlingame, Calif.), previously absorbed on affinity columns with human IgA and IgG, was added to the wells at a 1:1000 dilution. After 1 hr incubation at RT, the plates were washed three times with PBS and stained for 20 min by the ABTS (2.2 Azino-di-[3 ethyl-benzthiazoline sulfoneate]) staining solution (Boehringer Mannheim Biomedicals, Indianapolis, Ind.). Finally, the plates were read at 405λ in an "ELISA-spectrophotometer" (Dynatech). Optical absorbance of each IgM RF determination of all the sera were converted into microgram amounts by evaluating all the determinations on the standard curve. Each determination was done in triplicate. The mean of the triplicate for each serum was the relative amount of IgM RF in the sera.

Anti-ssDNA antibody assay. The previously adopted ELISA method for the detection of anti-DNA antibodies (21, 22) was performed with slight modifications. Microtiter polyvinylchloride plates, previously activated with poly-L-lysine (Sigma, St. Louis, Mo.), were incubated overnight with heat-denaturated calf thymus DNA (Sigma) at the concentration of 1 μg/ml in 5% CS. Sera diluted 1:40 in 5% CS were added to the plates. After 3 hr incubation at RT, the goat peroxidase-conjugated anti-human immunoglobulin antiserum was diluted 1:1500 in identical 5% CS solution. After incubation for 1 hr at RT, the ABTS staining solution was added to the wells. The subsequent reading in the ELISA-spectrophotometer gave the final values of the test. Also in this case each sample determination was performed in triplicate and the absorbance of each determination was evaluated on a standard titration curve obtained in the same assay by correlating the optical density of 12 known amounts of affinity-purified anti-DNA antibodies. The arithmetic mean of the three determinations for each serum was considered the relative amount in micrograms per milliliters of anti-DNA antibodies.

Anti-F(ab')$_2$ antibody assay. ELISA assay for the detection and the measurement of the anti-F(ab')$_2$ antibodies was performed as previously described (22, 23). Test serum samples diluted 1:40 were incubated on the plates previously activated by F(ab')$_2$ derived from pepsin digested normal IgG. The 5 hr incubation at RT was followed by the addition of goat peroxidase conjugated γ-chain specific anti-human Fc fragment antiserum (Cappel Labs, Cochranville, Pa.), previously absorbed with normal F(ab')$_2$ and diluted 1:3000 in 5% CS. The test was completed as in the previously described tests. Also, as above in the other ELISA determinations, each determination was performed in triplicate and the relative values were evaluated on a standard titration curve obtained in each test with

known concentrations of affinity-purified anti-F(ab')$_2$ antibodies. The amounts in micrograms per milliliters of anti-F(ab')$_2$ antibodies obtained in this way were therefore considered as levels of antibodies relative to the known concentrations of anti-F(ab')$_2$ used as standards in the microtitration curve.

ELISA for other autoantibodies. Additional ELISA determinations were performed to evaluate titers of serum autoantibodies against other common antigens such as human albumin, human hemoglobin, human thyroglobulin, gliadin, and secretory component from human IgA. In addition, antibodies to LHCN (*Limulus* hemocyanin) were determined as a neutral control. All these antigens (albumin, hemoglobulin, gliadin, LHCN [Sigma], thyroglobulin [Mann Res. Lab., New York, N.Y.] and secretory piece [24]), were absorbed on microtitration plates by incubation of 10 μg/ml of antigen in 0.05 *M* $NaHCO_3$, pH 9.6, for 18 hr. After extensive washing of the wells with PBS–1%FCS, a 100-μl aliquot of the human sera, or doubling dilutions of the sera, was added to individual wells. The wells were then filled with 200 μl of PBS–1%FCS. Following 2.5 hr incubation at room temperature, the wells were emptied, washed, and refilled with 300 μl of a rabbit anti-human Ig-β-galactosidase conjugate optimally diluted in PBS–1%FCS. Following a second 2.5-hr incubation, the wells were again washed and refilled with 300 μl *p*-nitrophenyl-β-D-galactopyranoside (Sigma). The substrate was prepared by dissolving 0.2 mg/ml of reagent in PBS buffer. After an appropriate incubation period, the optical density at 410 nm was determined with the microplate reader. Titers reported represented the last dilution of sera which gave an optical density reading above background.

Rabbit anti-human Ig conjugate. For ELISA assays, the antibody enzyme conjugate was prepared as follows. Human IgG was purified from sera by DEAE-cellulose and Protein A chromatography. Human IgA and IgM were obtained from the sera of myeloma patients. These three protein preparations were used to immunize rabbits. The rabbit antiserum was purified by affinity chromatography on CM-BioGel A (BioRad Laboratories, Richmond, Calif.) coupled to the human proteins. The enzyme-labeled rabbit antibody was prepared by the procedure of Avremas (25).

Purification of anti-DNA and anti-F(ab')$_2$ antibodies. Affinity chromatography using Sepharose 4B was used to purify both anti-DNA and anti-F(ab')$_2$ antibodies. Heat denaturated and homogenized calf thymus DNA was used as antigen. Plasma from a patient with active systemic lupus erythematosus was the source of the anti-DNA antibodies. Fractions eluted with acidic buffer (glycine HCl 0.1 *M*, pH 1.8) were collected and concentrated approximately 100-fold by negative pressure ultrafiltration. High levels of anti-DNA activity were demonstrated in these eluted fractions in subsequent ELISA tests. DNase digestion of these fractions, using DNase coupled to Sepharose 4B, was performed to ensure that no free DNA was present in column eluates. A similar procedure was used to purify anti-F(ab')$_2$ antibodies. F(ab')$_2$ fragment derived from normal human pepsin-digested IgG was coupled to Sepharose 4B. Relevant amounts of anti-F(ab')$_2$ antibodies were removed in this way from the serum of a normal donor, which in a previous anti-F(ab')$_2$ antibody assay showed high serum level of antibody.

Immunoglobulins and their fragments. Normal IgG from allogeneic donors was

isolated from Cohn Fraction II (Sigma) by DEAE-cellulose chromatography using 0.015 *M* phosphate buffer, pH 6.3. Nisonoff's method using 2% pepsin digestion of whole IgG in 0.1 *M* acetate buffer was followed to obtain human $F(ab')_2$ fragment (26). Pepsin-digested IgG was passed through a Sephadex G-200 column in neutral buffer. The immunodiffusion analysis of the first filtration peak showed no residual reactivity using anti-Fc antiserum.

Cell cultures. Peripheral blood samples were collected from both healthy old donors and young controls. Peripheral blood mononuclear cells (PBMC) were obtained from Ficoll–Hypaque gradients and then washed three times in Hanks' balanced salt solution. Glass-adherent cells were removed by incubation of the cellular suspensions on plastic petri dishes in RPMI 1640 with 10% fetal calf serum (FCS). Non-glass adherent mononuclear cells were then resuspended to 1×10^6 cells/ml in complete medium (RPMI 1640 supplemented with 2 m*M*/ml L-glutamine, 200 UI/ml penicillin, 100 μg/ml streptomycin, and 10% FCS). PWM (Grand Island Biological Co., Grand Island, N.Y.) was added to the cellular suspensions at the final concentration 1:100. The cells were cultured in 12 × 75-mm plastic culture tubes (Falcon, 2054, Becton–Dickinson) at 37°C, 5% CO_2 atmosphere in a humidified incubator. Fresh medium was replaced in the tubes twices every week. Finally, after 14 days the supernates were collected and frozen at −25°C for several days until the day of the ELISA test for the detection and the measurement of anti-DNA and anti-$F(ab')_2$ antibodies.

Statistical analysis. Analysis of variances were performed by using χ^2 test, Fisher test, and Students *t* test.

RESULTS

The first set of experiments was performed to verify the difference in the titers of IgM RF in the sera of young and old people. Results are shown in Fig. 1. As previously noted, a characteristic difference in titers of IgM RF emerged when young and old healthy donors were compared (14). As can be seen in the figure, sera from elderly people contained threefold higher levels of IgM RF than the young controls. The geometric mean of the titers of IgM RF in sera from young donors was 4.82 compared with 13.27 μg/ml in old individuals. This difference between the two groups was highly significant ($P < 0.001$).

The same 50 sera from old subjects and 51 from young controls were tested for anti-DNA and anti-$F(ab')_2$ antibodies. Anti-DNA and anti-$F(ab')_2$ antibodies were compared individually in each group of males or females in both the elderly and the young people groups. As shown in Fig. 2, the levels of anti-DNA antibodies were very similar in all four groups. When values were compared in each of the four groups on the basis of sex or age, no significant differences were found ($P > 0.2$ in all the evaluations). Also anti-$F(ab')_2$ levels were not significantly different in the two groups tested. All statistical analyses performed between the two groups (old vs young), and within each group on the variance of the sex (males vs females) and the age, showed no relevant difference ($P > 0.2$ in all the evaluations performed).

The next set of experiments examined the possible differences in *in vitro* pro-

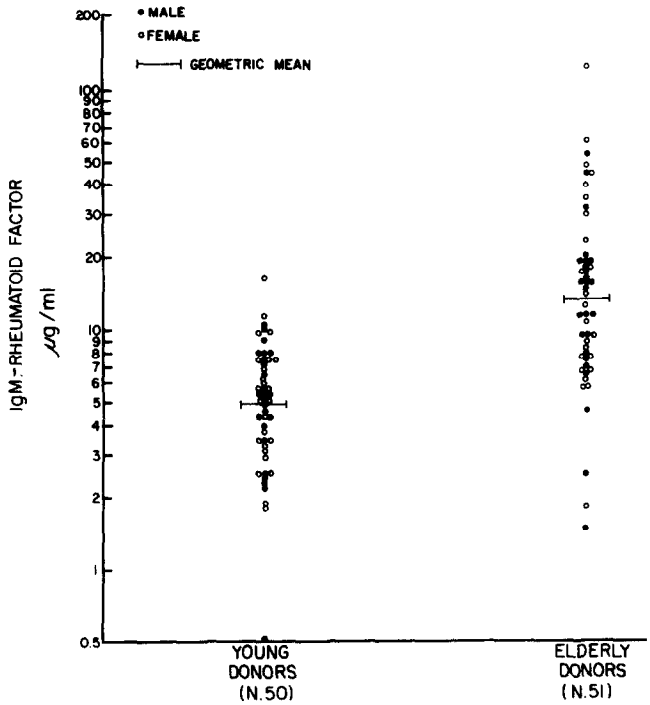

FIG. 1. Comparative titers of IgM RF as measured by ELISA assay in sera from young and old healthy donors.

duction among old and young people of two different autoantibodies such as anti-DNA and anti-F(ab')$_2$. Twelve old subjects and 11 young individuals were studied for anti-DNA and anti-F(ab')$_2$ autoantibody production *in vitro* after PWM stimulation of nonadherent PBMC. The results of these experiments are illustrated in Fig. 3. The production of anti-DNA antibodies from the nonadherent PBMC derived from asymptomatic old subjects was virtually negative in 9 of 12 supernates tested. In fact, in only 3 supernates were we able to detect anti-DNA antibodies by ELISA assay at concentrations of 1.9, 4.8, and 78 μg/ml. All other supernates were entirely negative. Similarly, in supernates derived from young controls, we were able to detect the production of anti-DNA antibody in 3 of 11 samples. In these instances, amounts of antibody measured in the three positive supernates (2.4, 2.8, 32.5 μg/ml) were slightly lower than in the old group. The remaining 8 supernates showed no detectable amounts in the ELISA assay of anti-DNA antibody. Statistical analysis of the production of anti-DNA antibody by the PBMC derived from old donors 7.06 (±6.43) μg/ml (mean ± SEM), compared to the production of the same antibody by the PBMC from young donors as 3.4 (±2.89) μg/ml, showed no significant difference ($P > 0.5$). Similarly, there was no difference in anti-F(ab')$_2$ antibodies produced by old and young donors' PBMC. Amounts of anti-F(ab')$_2$ antibody produced were 0.85 ± 0.5 μg/ml in the old group and 1.27 ± 0.8 μg/ml in the young controls ($P > 0.5$).

Finally, a set of experiments was performed to determine possible differences

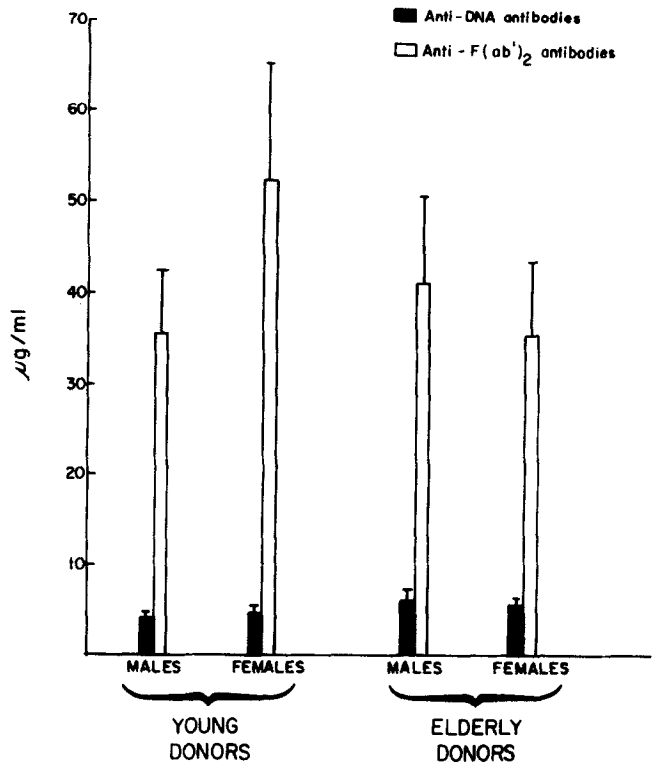

FIG. 2. Anti-DNA and anti-F(ab')$_2$ antibodies were measured by ELISA tests in sera obtained from young and old healthy donors. The levels of these autoantibodies are expressed as mean ± SEM. No significant difference was noted between the two groups ($P > 0.2$).

in proportions of other autoantibodies against other common antigens in the sera between the two groups. ELISA tests were completed to detect autoantibodies in 50 sera from old donors and as control in 51 sera derived from young subjects against thyroglobulin, human albumin, LHCN, secretory component derived from human IgA, human hemoglobulin, and gliadin. In order to increase the specificity of these tests, we considered positive only the samples which showed absorbance higher than 180λ at 1/64 as final dilution of the sera. As can be seen in Fig. 4, there was no difference between the two groups in the percentages of positive sera containing autoantibodies to thyroglobulin, human albumin, LHCN, and secretory component. A slight difference was present in the anti-human hemoglobin ELISA assay ($P = 0.2315$) and between the percentages of positive sera containing anti-gliadin antibodies ($P = 0.3894$). In Table 1 the results of ELISA autoantibody assays in all comparative tests between the two groups of asymptomatic old subjects and young control sera are summarized.

DISCUSSION

The results presented in this paper represent an attempt to provide additional data relating autoimmune phenomena and aging. The original purpose of our study

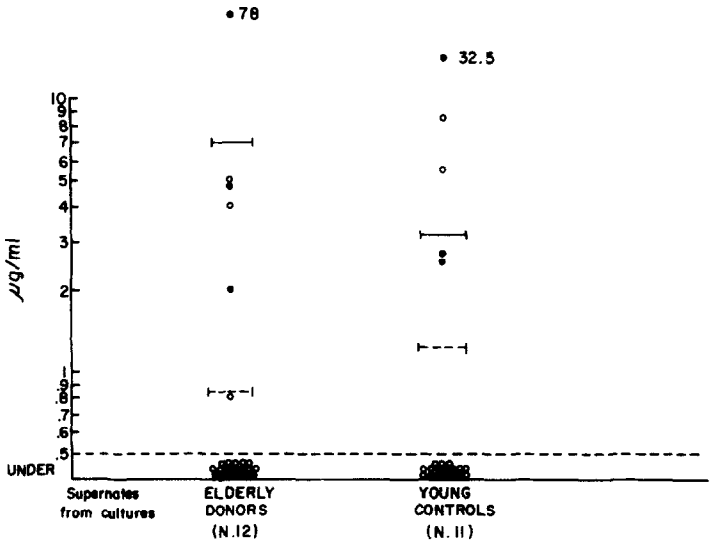

FIG. 3. Comparative profiles of *in vitro* production of anti-DNA (●) and anti-F(ab')$_2$ (○) antibodies by PWM-stimulated glass nonadherent cells obtained from 12 old subjects and 11 young controls. Supernates were tested after 14 days of culture. In the production of both the autoantibodies, the difference is still not significant ($P > 0.5$).

was to define the increase in anti-DNA antibody production by elderly asymptomatic donors as an expression of the enhancement of senescence of the immune system. With the exception of the difference in production of IgM RF in elderly people when compared to young donors, the results obtained in the other autoantibody assays in both old and young donors were for us absolutely unexpected.

Several previous reports have shown an increased prevalence of autoantibodies in elderly people (1–3). Other studies described a sex-dependent relationship between age and increased prevalence of anti-gastric and anti-thyroglobulin antibodies but no association between age and anti-nuclear antibodies in a large population of asymptomatic Caucasian subjects (4). In another report, a large panel of autoantibodies including anti-nuclear antibodies was studied with the final result of a rather uniform increase of all autoantibodies tested in aged people (27). Similar conflicting results have been reported in the analysis of some cellular components of the immune system. In fact, different authors report that in aged people, the percentages of circulating T cells are higher than in young controls (28), decreased (29), or unchanged (30). Other authors describe the immunological alterations related to aging as involving both cellular and humoral components of the immune system (31).

Previous reports have included data on the production *in vitro* of IgM RF by PBMC obtained from aged subjects using a T-dependent B-cell mitogen (PWM) (14) or a T-independent B-cell mitogen such as Epstein–Barr virus (29), with the final effect of a similar increased production *in vitro* of this autoantibody when compared to young controls. It is possible that these conflicting results may have

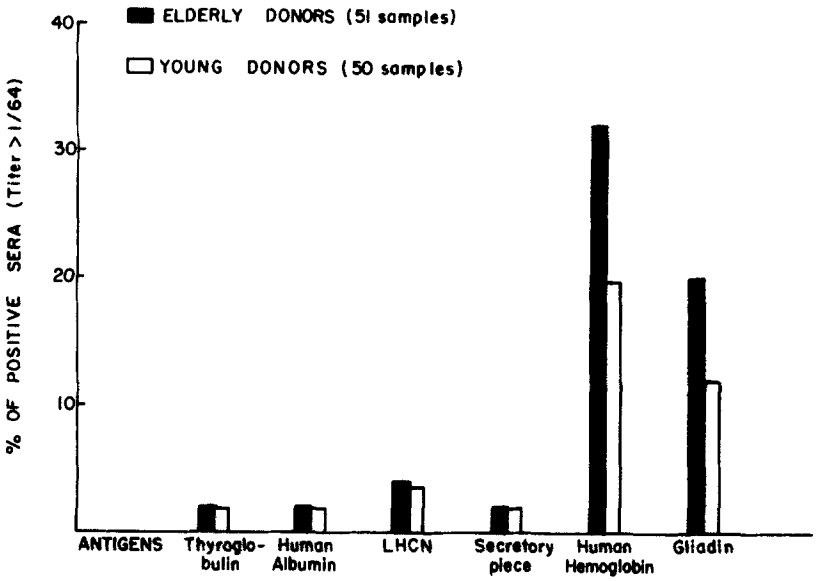

FIG. 4. Sera from old and young donors were tested against six different antigens. The percentages of positive sera for each group are compared. Minor differences are noted between the groups using human hemoglobin or gliadin as antigen.

resulted from analysis of populations of elderly subjects which, in many cases, have not been entirely normal, disease free, or homogeneous. A common statement in all the previous reports noted describes the selected old donor population as having no diseases or medications known to affect the immune system. It is clear that most medications have not been studied for their effect on immunologic function. Our own old donor population was rigorously selected and we would like to emphasize that old donors with relatives with any kind of autoimmune disease were specifically excluded from this study. In our study, a complete dis-

TABLE 1

COMPARATIVE STATISTICAL DIFFERENCES EMERGED BY ELISA ASSAYS FOR VARIOUS AUTOANTIBODIES, BETWEEN THE TWO GROUPS STUDIED

Autoantibodies	Difference in the two groups (P)
IgM-rheumatoid factor	0.0007
Anti-DNA antibodies	0.395
Anti-F(ab')$_2$ antibodies	0.384
Anti-thyroglobulin abs	>0.6
Anti-human albumin abs	>0.6
Anti-secretory piece abs	>0.6
Anti-human hemoglobulin abs	0.231
Anti-gliadin abs	0.389
Anti-LHCN abs[a]	>0.6

[a] Anti-hemocyanin included as an additional control antibody system.

sociation in the *in vivo* production of IgM RF from old donors was found when compared to levels of seven other different autoantibodies assayed in both elderly and young subjects. Equal *in vitro* production of anti-DNA and anti-$F(ab')_2$ antibodies was recorded from 12 old donors and 11 young controls. This paradoxical dissociation of the IgM RF from the other levels of autoantibodies in aged people could suggest a different significance to IgM RF which may be distinct from the other autoantibodies assayed.

Allogeneic combinations between subjects of B and T cells derived from young and old donors showed that the production of IgM RF *in vitro* was strictly related to activation of OKT4(+) cells from old subjects (15). The increased helper activity in the T-cell population of aged individuals would appear to be responsible for this increased response. However, in our experiments, the same increased helper activity in old subjects seems to be unable to increase the production both *in vivo* or *in vitro* of the other autoantibodies tested. Our data would indicate that in very healthy elderly people the increased production of IgM RF may not be an expression of the general increase of autoimmune phenomena, but the elevations of this autoantibody might be involved in other as yet undefined biological mechanisms.

Several recent reports describe increased production of IgM RF in healthy donors after immunization of these subjects by tetanus toxoid (33). The IgM RF producing B cells after stimulation by tetanus toxoid were described as "low frequency autoreactive B cells." Other authors report that IgM RF produced *in vitro* by activated B cells is more reactive with surface IgG than with monomeric IgG in solution (34). Other studies demonstrate activation of the complement system induced by IgM RF (35). Although the specificity of IgM RF is still directed against the Fc portion of IgG, our results and those obtained by other workers suggest that the IgM RF is involved in the enhancement of binding of low-affinity IgG antibodies against their antigen, with the hypothetical final effect of overall increased antigen binding. It is possible that the production of IgM RF by autoreactive B cells represents a first line defense against bacterial or viral infections, until specific antibody production is activated by antigen-specific B cells. From this point of view, the discrepancy of the levels of IgM RF in elderly people compared to the other autoantibodies suggests that IgM RF is not an expression of increased levels of autoantibody in general, but rather a humoral immune response which may have some intrinsic protective capacity.

REFERENCES

1. Cammarata R. J., Rodnan, G. P., and Fennel, R. H., *J. Amer. Med. Assoc.* **199**, 455, 1967.
2. Goidl, E. A., Michelis, H. A., Siskind, G. W., and Weksler, M. E., *Clin. Exp. Immunol.* **44**, 24, 1981.
3. Hallgren, H. M., Buckley, C. E., III, Gilbertsen, V. A., and Yunis, E. J., *J. Immunol.* **111**, 1101, 1973.
4. Pandey, J. P., Fudenberg, H. H., Ainsworth, S. K., and Loadholt, C. B., *Mech. Ageing Dev.* **10**, 399, 1979.
5. Valtuena, J. P., Gonzales Guilabert, M. I., Del Pozo Perez, M. A., Del Pozo Crespo, F., and Velasco Alonso, R., *Biomedicine* **19**, 301, 1973.
6. Weksler, M. E., and Hutteroth, T. H., *J. Clin. Invest.* **53**, 99, 1974.

7. Hallgren, H. M., and Yunis, E. J., *J. Immunol.* **118**, 2004, 1977.
8. Kishimoto, S., Tomino, S., Mitsuya, H., and Fujiwara, H., *J. Immunol.* **123**, 1586, 1979.
9. Kishimoto, S., Tomino, S., Inomata, K., Kotegawa, S., Saito, T., Kuroki, M., Mitsuya, H., and Hisamitsu, S., *J. Immunol.* **121**, 1773, 1978.
10. Gupta, S., and Good, R. A., *J. Immunol.* **122**, 1214, 1979.
11. Koopman, W. J., and Schrohenloher, R. E., *J. Immunol.* **125**, 934, 1980.
12. Weiner, H. L., Scribner, D. J., Schocket, A. L., and Moorehead, J. W., *Clin. Immunol. Immunopathol.* **9**, 356, 1978.
13. Barret, D. J., Stenmark, S., Wara, D. W., and Amman, A. J., *Clin. Immunol. Immunopathol.* **17**, 203, 1980.
14. Rodriguez, M. A., Ceuppens, J. L., and Goodwin, J. S., *J. Immunol.* **128**, 2422, 1982.
15. Ceuppens, J. L., and Goodwin, J. S., *J. Immunol.* **128**, 2429, 1982.
16. Holman, H., and Deicher, H. R., *Arthritis Rheum.* **3**, 244, 1960.
17. Solheim, B. G., and Larsen, R. A., *Acta Med. Scand. Suppl.* **543**, 43, 1972.
18. Searles, R. P., Husby, G., and Messner, R. P., *Acta Pathol. Microbiol. Scand. Sec. C*, **85**, 463, 1977.
19. Gripenberg, M., Wafin, F., Isomaki, H., and Linder, E., *J. Immunol. Methods* **31**, 109, 1979.
20. Silvestris, F., Goodwin, J. S., and Williams, R. C., *Clin. Rheumatol.*, in press, 1985.
21. Eaton, R. B., Schneider, G., and Schur, P. H., *Arthritis Rheum.* **26**, 52, 1983.
22. Silvestris, F., Bankhurst, A. D., Searles, R. P., and Williams, R. C., Jr., *Arthritis Rheum.*, **27**, 1387, 1984.
23. Silvestris, F., Searles, R. P., Bankhurst, A. D., and Williams, R. C., Jr., *Clin Exp. Immunol.*, in press.
24. Anderson, W. L., and Tomasi, T. B., *Arch. Biochem. Biophys.* **182**, 705, 1977.
25. Avremas, S., *Immunochemistry* **6**, 43, 1969.
26. Nisonoff, A., Wissler, F. C., Lipman, L. N., and Woernley, D. L., *Arch. Biochem. Biophys.* **89**, 230, 1960.
27. Delespesse, G., Gausset, P. H., Sarfati, M., Dubi-Rucquoy, M., Debisschop, M. J., and Van Haelst, L., *Clin. Exp. Immunol.* **40**, 96, 1980.
28. Hallgren, H. M. Kersey, J. H., Dubuey, D. P., and Yunis, E. J., *Clin. Immunol. Immunopathol.* **10**, 65, 1978.
29. Girard, J. P., Paychere, M., Cuevas, M., and Fernandes, B., *Clin. Exp. Immunol.*, **27**, 85, 1977.
30. Kishimoto, S., Tomino, S., and Inomata, K., *J. Immunol.* **121**, 1773, 1979.
31. Portaro, J. K., Glick, G. I., and Zighelboim, J., *Clin. Immunol. Immunopathol.* **11**, 339, 1978.
32. Fong, S. C., Tsoukas, C. D., Frincke, L. A., Lawrance, S., Holbrook, T. L., Vaughan, J. H., and Carson, D. A., *J. Immunol.* **126**, 910, 1981.
33. Welch, M. J., Fong, S., Vaughan, J., and Carson, D., *Clin. Exp. Immunol.* **51**, 299, 1983.
34. Tanimoto, K., Cooper, N. R., Johnson, J. S., and Vaughan, J. H., *J. Clin. Invest.* **55**, 437, 1975.
35. Sabharwal, U. H., Vaughan, J. H., Fong, S., Bennet, P. H., Carson, D. A., and Curd, J. G., *Arthritis Rheum.* **25**, 161, 1982.

Received August 8, 1984; accepted with revision December 21, 1984.