Decreased In Vitro Humoral Immune Responses in Aged Humans

SAVITA G. PAHWA, RAJENDA N. PAHWA, and ROBERT A. GOOD, *Department of Immunobiology, Memorial Sloan-Kettering Cancer Center, New York 10021*

ABSTRACT Induction of antigen-specific and nonspecific (polyclonal) humoral immune responses in vitro was investigated in peripheral blood mononuclear cells of aged (65–85 yr) and young (20–30 yr) volunteers. In vitro immunization of lymphocytes with antigen (sheep erythrocytes) was performed in a recently described microculture system, and anti-sheep erythrocyte plaque forming cells were quantitated in a direct hemolytic plaque assay. Immunoglobulin secreting cells, induced polyclonally with pokeweed mitogen, were quantitated in a reverse hemolytic plaque assay. Significant depressions of antigen-specific as well as polyclonal responses were noted in relation to advancing age. Antigen-specific responses were more frequently depressed than polyclonal responses.

T cell mitogen concanavalin A (Con A) was used to amplify functions of autologous immunoregulatory T cells. Addition of 10 μg/ml Con A to lymphocytes of young donors at culture initiation resulted in activation of suppressor cells and abrogated antigen-specific responses. Delayed addition of Con A, on the other hand, enhanced responses, presumably because of activation of helper T cells. Similar manipulations of lymphocyte cultures from aged donors showed failure of Con A to suppress antigen-specific responses in approximately half of the responders. In many nonresponders, responses within normal range were elicited by the delayed addition of Con A to their lymphocyte cultures. Deviations beyond the range of expected responses were noted in 32.5% of the co-cultures between pokeweed mitogen stimulated young and aged cells. Our findings suggest that age-related deficiencies of B cell function are frequently associated with dysfunction of immunoregulatory T cells and are only occasionally due to intrinsic defects of B cells.

INTRODUCTION

The aging process is accompanied by decreased immunologic vigor, and is associated with an increased incidence of infections, autoimmunity, and malignancy (1). The age-associated decline in immune competence has been shown in experimental animals to encompass both cell-mediated and humoral immunity. Although deficiencies in T cell function in aged humans have been well established, B lymphocyte function has been less well characterized. Studies by Kisimoto et al. (2, 3) indicated that polyclonal immunoglobulin production after stimulation of B lymphocytes of aged volunteers with pokeweed mitogen was normal or increased. Investigations by Delfraissy et al. (4) however, have revealed marked deficits of specific antibody-forming capacity after in vitro sensitization of peripheral blood lymphocytes with antigen in aged humans.

In the present study we have used a newly developed microculture assay system (5, 6) to study specific antibody responses in vitro in aged humans. Simultaneously, we investigated the polyclonal induction of immunoglobulin-secreting cells (ISC)[1] in the same population utilizing pokeweed mitogen (PWM) as a stimulant. Responses of aged volunteers were compared with those of healthy young volunteers who were tested simultaneously. A great variability was noted in the responsiveness of aged volunteers to both antigen and PWM, and ranged from normal to markedly deficient responses. Deficits in antigen-specific antibody responses were observed more frequently than

This work was presented in part in 1980 at the 64th Annual Meeting of the Federation of American Societies for Experimental Biology at Anaheim, California.

Received for publication 28 July 1980 and in revised form 26 November 1980.

[1] *Abbreviations used in this paper*: Con A, concanavalin A; ISC, immunoglobulin-secreting cells; PBM, peripheral blood mononuclear cells; PFC, plaque-forming cells; PWM, pokeweed mitogen; SRBC, sheep erythrocytes.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/81/04/1094/09 $1.00*
Volume 67 April 1981 1094–1102

decreases in PWM-induced ISC. The cellular bases for the immunodeficiency of aging are discussed.

METHODS

Study population

Aged volunteers for this study consisted of individuals who responded to a request made on television for elderly blood donors. These volunteers, ranging in age from 65 to 85 yr, were ambulatory people in good health from middle and upper socioeconomic classes. A questionnaire was filled out for each individual providing details of medical history, use of prescription medications, and occupational hazards. Height and weight were recorded in each instance and physical examination, complete blood count, screening profile of blood chemistries, and urinalysis were performed in most instances. At the time of study, aged volunteers were well within 10% of desired body weight,[2] normotensive, in good health, and free of intercurrent illnesses. 13 volunteers were on the prescription medications for treatment of: hypertension (4), arthritis (4), sleeplessness, anxiety, or depression (3), hypothyroidism (1), and angina pectoris (1). Six others admitted taking aspirin occasionally. Healthy laboratory personnel (20–30 yr) served as young controls. A few individuals in the intermediate age group (31–55 yr) were also tested. Males and females in the study group were equally distributed in the seventh and eighth decades of life. In the ninth decade there were two males and no females.

Isolation of cells

20 ml of heparinized venous blood was obtained from young and aged volunteers. Peripheral blood mononuclear cells (PBM) were isolated from the blood by standard Ficoll-Hypaque centrifugation.

Cell cultures

Cultures of PBM were established to generate antigen (SRBC)-specific plaque-forming cells (PFC) and also for induction of PWM-induced nonspecific (polyclonal) ISC cells.

Sensitization with antigen. Lymphocyte cultures were performed as described (5, 6). Briefly, 0.1-ml vol consisting of serial dilutions of PBM ranging from 5 to 0.15×10^6 cells/ml were plated in flat bottom microtiter plates (Costar Data Packaging, Cambridge, Mass. No. 3596). RPMI 1640 medium was supplemented with penicillin, streptomycin, glutamine, pyruvate, nonessential amino acids, and 5% fetal calf serum (Microbiological Associates, Walkersville, Md., lot 94055) as described by Mishell and Dutton (7). The medium also contained 50 μM 2-mercaptoethanol and 10% pooled human blood group A serum. Care was taken not to use batches of human sera that tended to produce pseudo plaques (8). SRBC-absorbed sera were not used in our experiments because of the possibility of dissolved antigen obscuring antigen-negative controls. Cultures were innoculated with 5×10^6 SRBC as the sensitizing antigen and 0.003% heat-killed *staphylococcus aureus* of Cowan strain I (American type culture collection, Rockville, Md., No. 1285), as a B cell mitogen (9). Supernatant fluid of lipopolysaccharide-stimulated adherent human PBM cells, produced as described (10), was added as a source of Interleukin-I in a final concentration of 10%. Cultures were fed daily with 10 μl of nutritional cocktail (7).

PWM stimulation. Cultures consisting of 5×10^6 PBM in 1 ml of medium RPMI 1640 supplemented with antibiotics and 15% fetal calf serum were performed in 12×75-mm plastic tubes (Falcon Labware, Div. Becton Dickinson, Oxnard, Calif., No. 2003). PBM were stimulated with pokeweed mitogen (GIBCO, Grant Island Biological Company, Grand Island, N. Y. No. 981518) in a final strenth of 10 μ/ml.

All cultures were incubated for 6–7 d in 5% CO_2 at 37°C with 100% humidity.

PFC assays

At termination of cultures, cells were washed twice in medium containing 5% fetal calf serum. Antigen-stimulated cultures were tested for anti-SRBC PFC in a modified (7) Jerne hemolytic plaque assay (11). PMW-stimulated cultures were assayed for ISC in a reverse plaque assay, modified from the method of Gronowicz et al. (12) using protein A-coated SRBC as targets, and rabbit anti-human immunoglobulin as a developing serum. Results of anti-SRBC PFC and of ISC were expressed as actual or log responses per 10^6 and 10^4 cultured cells, respectively.

Investigation of immunoregulatory T cells

Addition of concanavalin A (Con A) to antigen-stimulated cultures. The T cell mitogen Con A was added to antigen-stimulated cultures at a final concentration of 10 μg/ml. Cultures received Con A either at culture initiation (day 0) or after a delay of 48 h. The rationale for timed addition of Con A with respect to activation of helper and suppressor T cells has been described (5) and is discussed in subsequent sections. Addition of allogeneic irradiated T cells (2,000 rads) at culture initiation, with delayed addition of Con A was sometimes used as a means of providing exogenous help (5) to PBM cultures.

Co-culture with allogeneic cells in PWM-stimulated cultures. PBM cells from young individuals were co-cultured with allogeneic PBM from young and old individuals in a 1:1 ratio keeping the final cell concentration and volume constant at 0.5×10^6 cells/ml. The observed response was expressed as a percentage of the expected, with the latter being calculated as a mean of the individual ISC responses of the two volunteers whose cells were being co-cultured.

Serum immunoglobulins

Levels of immunoglobulin (Ig)G, IgA, and IgM were determined by radial immunodiffusion on five random serum samples from volunteers who manifested poor ISC responses to PWM, as well as on five samples from those who were normal responders in this assay system.

Statistical methods

Results of aged and young groups were subjected to linear regression analyses and the significance of the slopes was determined (Figs. 1 and 4). In addition, significance of differences between responses of young and aged individuals was determined by the Student's t test.

[2] Metropolitan Life Insurance Company (1959).

RESULTS

Antigen-specific PFC responses

PBM of young and aged volunteers were sensitized in vitro with SRBC, and anti-SRBC PFC were determined. Peak anti-SRBC responses were most often obtained at cell densities of 2.5–5.0 × 10^5/culture in both, young as well as aged volunteers. Fig. 1 depicts peak PFC responses of individuals of various ages on a logarithmic scale. Individuals varied widely in their ability to generate antigen-specific PFC, and the variability was more evident in the aged population than in the young. In comparison to young volunteers, deficient PFC (<50 PFC/10^6 cultured cells, or log < 1.7) responses were observed in 21 out of 47 (44.7%) of the aged individuals; in 10 of them responses were <log 1.0. Neither the intake of aspirin nor prescription medicines, nor the gender of the donor appeared to influence antigen-specific responses. Males and females were equally distributed in the poor responder group, and incidence of poor responses in donors taking medications of any sort was similar (6 of 16 or 38%) to that observed for the group as a whole. The decline in anti-SRBC PFC responses with advancing age was statistically significant, P value of the slope of the regression line being <0.0001. Mean log PFC response (±SE) of aged volunteers (1.79±0.14) was also significantly less ($P < 0.0001$) than the mean log PFC response of young volunteers (2.5±0.11). Anti-SRBC PFC in antigen-free cultures amounted to <10% of those seen in antigen-stimulated cultures in both young and aged individuals (data not shown).

Addition of Con A to cultures

Appropriately timed addition of the T cell mitogen Con A has previously been shown to effectively amplify functions of helper and suppressor T cells in antigen-stimulated cultures (5). This mitogen was therefore used to modulate immunoregulatory T cell function in the aged population.

Early addition of Con A. Addition of Con A (10 μg/ml) to young PBM at culture initiation regularly resulted in suppression of their PFC responses (Fig. 2). This effect has been attributed to the activation of suppressor T cells. In PBM cultures of older people, two patterns of responses were seen: in half of the aged, anti-SRBC PFC responses were suppressed by Con A, but in the remaining half the responses were enhanced. The enhancement of anti-SRBC PFC response in aged PBM cultures most likely resulted from the preferential activation of helper T cells in these individuals.

Delayed addition of Con A. Addition of Con A 48–72 h after initiation of culture uniformly enhanced anti-SRBC PFC responses in both young and old volunteers (Fig. 3). This effect of Con A has been attributed to activation of helper T cells that are functioning at a time when B cells are still responsive to helper influences while being relatively resistant to suppressor influences (5). The majority of the previously poorly responsive aged PBM could, with delayed addition of Con A, mount an anti-SRBC PFC response in the same order of magnitude as their younger counterparts. In a few aged individuals, (Table I) Con A-induced enhancement of anti-SRBC PFC was rather small. Addition of exogenous helper T cells in the form of irradiated T cells from young donors followed by the delayed addition of Con A to PBM cultures resulted in enhancement of PFC responses in three (1–3) of six poor responders.

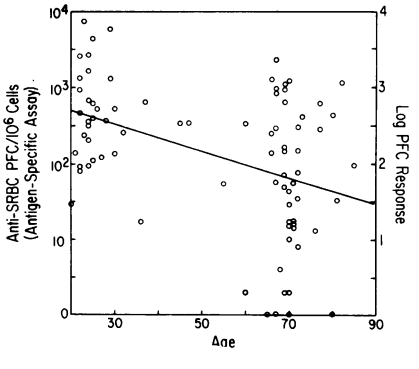

FIGURE 1 Antigen-specific, anti-SRBC PFC responses of PBM cultures from young and aged donors. Anti-SRBC PFC responses are plotted on a log scale. Slope of responses in relation to advancing age is statistically significant ($P < 0.0001$).

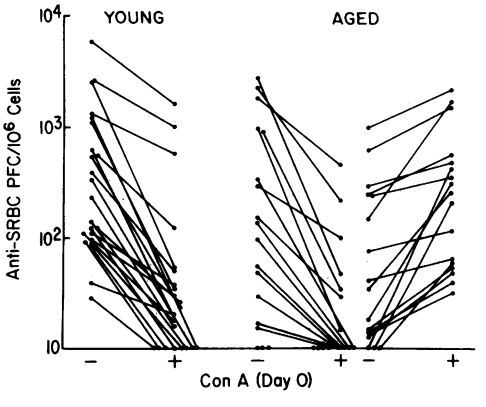

FIGURE 2 Addition of Con A, 10 μg/ml at culture initiation to antigen-stimulated PBM cultures of young and aged donors. Results depict anti-SRBC responses of cells cultured in absence (−) or presence (+) of Con A. In contrast to PBM cultures from young donors where Con A added on day 0 regularly suppressed PFC responses, aged donors' PBM fell into two groups, one that was suppressed and another that was enhanced after addition of Con A.

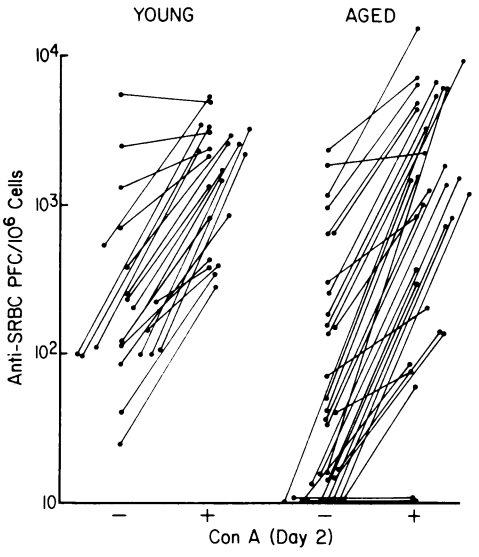

FIGURE 3 Peak anti-SRBC PFC responses of PBM cultures from young and aged donors cultured in the absence or presence of 10 μg/ml Con A added 48 h after culture initiation.

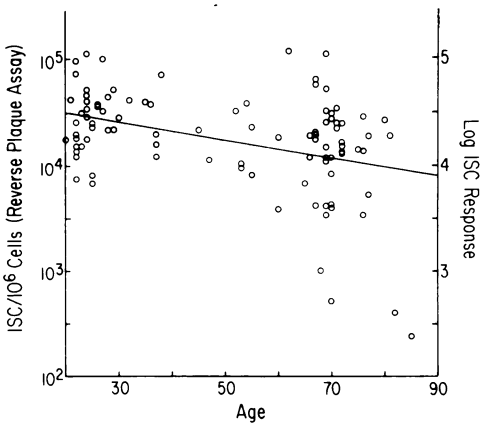

FIGURE 4 PWM-induced ISC in cultures of young and aged PBM donors. Slope of regression line of ISC responses on age is statistically significant ($P < 0.0001$).

PWM stimulation

Generation of ISC. PBM from aged and young volunteers were cultured in the presence or absence of PWM for 7 d and the ISC generated were quantitated in a reverse plaque assay. The results of ISC responses in relation to age are shown in Fig. 4. A great variability in responsiveness was apparent in both young and old individuals, more so in the latter. While a large proportion of the aged individuals had responses comparable to the young, decreased responses of <6,000 ISC/10^6 cultured cells (<log 3.8, or values below the range observed in young PBM) were noted in 12 out of 50 (24%) of the aged individuals. A third of the latter manifested severely depressed responses of <1,000 (log 3) ISC/10^6 cells. while intake of medications did not appear to influence the ISC responses, it was observed that of the 12 poor responders, 10 were male. Regression analysis of results shown in Fig. 4 revealed that the age-associated decline in ISC was significant with the P value of the slope of <0.0001. Mean log ISC response (±SE) of the aged (4.1±0.08) was also significantly less ($P < 0.001$) than that of young volunteers (4.41±0.04). Background ISC responses in the absence of PWM were comparable in the two groups (data not shown). It should be noted that although the polyclonal ISC responses showed a statistically significant decline with aging, the responses of several aged volunteers were adequate.

TABLE I
Provision of "Help" to Aged Poor Responder Cultures in Antigen-specific Assay System

Aged donor	Anti-SRBC PFC/10^6 cells: PBM alone*	PBM + Con A d_2‡	PBM + Tx + Con A d_2§
1	0	88	332
2	4	61	665
3	44	50	324
4	0	0	0
5	14	19	25
6	97	90	92
Young donors response±SE	805±277	2,279±389	

* PBM cultured in several cell densities (see Methods) and immunized with antigen. Results depict peak anti-SRBC PFC response.

‡ PFC response of PBM cultures that, in addition to antigen, received 10 μg/ml Con A added 48 h after culture initiation. d_2, day 2.

§ PFC response of PBM cultures with irradiated (2,000 rads) allogeneic T cells (Tx) added at culture initiation and 10 μg/ml Con A added 48 h later (d_2).

Co-culture between aged and young PBM

PBM from young individuals were co-cultured with allogeneic PBM from aged and young volunteers in a 1:1 ratio. ISC responses obtained were expressed as a percentage of responses expected. The overall spread of values obtained in allogeneic co-cultures was much greater in young × old co-cultures as compared to young × young co-cultures (Fig. 5). Responses observed in young × old co-cultures were enhanced in 5 and suppressed in 9 of 43 such co-cultures. When suppression was correlated with ISC responses, 5 of 9 individuals who showed suppression were noted to be poor responders; conversely, of 11 poor responders so tested, abnormal suppression was noted in 5. Thus

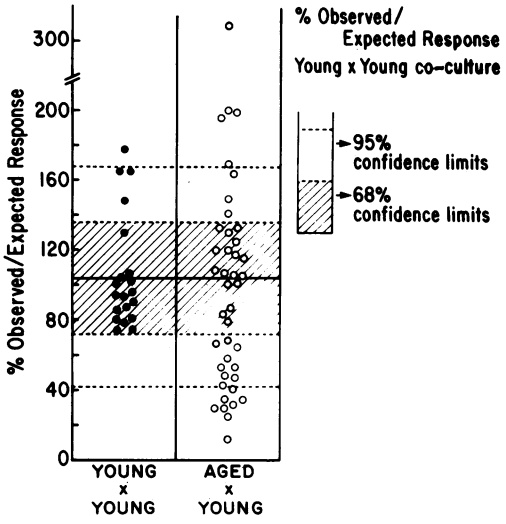

FIGURE 5 Co-cultures of PBM from young and aged donors. Cells from each pair of donors were co-cultured in a 1:1 ratio. Percent observed/expected response was calculated as described in Results.

there did not appear to be a direct correlation between decreased ISC responses and suppression in such co-cultures.

Serum immunoglobulins

Immunoglobulins were determined in sera from five people each belonging to the PWM-induced normal ISC responder group and the poor ISC responder group. Results are shown in Table II. Abnormalities were more pronounced in the poorly responsive group; IgG was below the lower limit of normal in four, IgA was markedly elevated in two, and IgM was elevated in two serum samples. In the normal responder group, except for two samples that showed elevated IgM, no other abnormalities were noted.

Comparison of polyclonal and antigen-specific responses

In comparing responses of 42 aged individuals who were tested simultaneously in the two assay systems, it was observed that five had depressed responses to both antigen and PWM; responses only to PWM were depressed in 6 and only to antigen in 15 of the aged volunteers. Delayed addition of Con A to antigen-stimulated PBM cultures could enhance PFC responses even in individuals whose PBM suppressed ISC in PWM-stimulated allogeneic co-cultures; early addition of Con A suppressed their antigen-specific responses.

TABLE II
Serum Immunoglobulins of Aged Volunteers

	Serum immunoglobulins		
	IgG	IgA	IgM
		mg/dl	
Nonresponders*			
1	446 (↓)‡	760 (↑)	160
2	700 (↓)	121	264
3	777 (↓)	407	302 (↑)
4	653 (↓)	345	281
5	1,909 (↑)	690 (↑)	399 (↑)
Responders*			
1	924	220	248
2	1,748	334	242
3	1,600	211	191
4	956	289	358 (↑)
5	832	300	298 (↑)
Normal range	800–1,800	90–450	60–280

* Nonresponder and responder designates those aged volunteers who were abnormal or normal when their PBM were tested for generation of ISC in response to PWM stimulation.
‡ Results above or below the adult range are designated with arrows in parenthesis pointing up or down.

DISCUSSION

In this study we have performed simultaneous analyses of polyclonally triggered and antigen-specific antibody responses in vitro in aging human volunteers. Our results show that aging may be associated with depression of antigen-specific antibody responses and/or nonspecific immunoglobulin secretion, with defects of the former being more frequent than those of the latter.

Generation of antigen-specific antibody responses in vitro has not been possible in humans until recently. A major problem in this context has been the control of suppressor cells which inhibit the induction of antigen-specific responses. In the culture system used for the present study (5, 6) a broad range of cell concentrations is used to select for a cell density at which there is an optimal balance of helper T cells and precursor B cells. Furthermore, helper and suppressor cell activity can be preferentially stimulated by appropriately timed addition of the T cell mitogen con A to the cultures. Suppressor influences are effective only if present during the early stages of B cell activation. Once B cell activation is already well under way, suppressor influences fail to be effective, whereas helper effects can still readily enhance B cell responses. This reasoning forms the basis for the use of a T cell mitogen to amplify helper and suppressor cell effects. Early addition of Con A can abrogate B cell responses by activation of

suppressor T cells. With delayed addition of Con A, however, helper effects predominate.

Using this assay system, a significant age-associated decline in the antigen-specific antibody responses was noted in aged human subjects. One mechanism whereby B cells respond poorly to T cell-dependent stimuli might be due to a lack of adequate help provided by T cells. Clear evidence exists for helper T cell defects in aging mice (13–16). In humans, studies performed by Delfraissy et al. (4) have shown that T cells from aged individuals fail to help B cells from young volunteers to produce antibody response to antigenic stimulation in vitro. In the present study, we attempted first to activate autologous helper T cells of aged individuals by delayed addition of Con A to PBM cultures. By doing so, we observed that many old people who had initially manifested poor anti-SRBC responses could now respond in normal or near normal range. The poor response in this group could thus be attributed to a functional deficiency of T helper cells which could, in most instances, be overcome by the delayed addition of Con A to the cultures. This deficiency of helper T cell function may have been qualitative rather than quantitative. Alternatively, a lack of helper T cells (actual paucity or outnumbering by excessive suppressor cells) may have been compensated for by their activation with Con A. In a small percentage of the aged, delayed addition of Con A failed to reconstitute anti-SRBC PFC responses. Provision of exogenous helper T cells could successfully induce antigen-specific antibody responses in some of them. The remaining individuals failed to respond to antigen despite provision of "optimal" help. Thus, in the majority of the poor responders, a lack of B cell response to specific immunization in vitro could be attributed to deficiencies in the function of helper T cells. Recently, poor antibody formation in aging humans in response to in vivo immunization with tetanus toxoid has also been attributed to helper T cell defects (17).

Simultaneous analysis of PWM-induced polyclonal stimulation of ISC in PBM of aged individuals showed normal or decreased responses. The downward slope of ISC in relation to advancing age was statistically significant. Earlier, in a comparison of mean responses of young and aged humans, secreted IgG has been reported to be normal (18) or increased with normal IgA and IgM (2) in supernatant fluids of PWM-stimulated PBM cultures of aged donors. Methods used for quantifying B cell responses and for making comparisons between young and old were different in these studies as compared with ours. We have quantitated total rather than isotype-specific ISC, and have quantified ISC at termination of culture as opposed to determinations of immunoglobulin that accumulates in culture supernatant fluids during the entire culture period. Changes might be present in immunoglobulin production per cell which would not be accounted for in a plaque assay. Our study deals with many more subjects than either of those mentioned above, and even in our study many individuals responded normally, and deficiencies of polyclonal responses were less frequent than those of antigen-specific responses. Analysis of the results by linear regression however brought into focus a highly significant decline in log ISC responses with aging.

It is of interest that in random serum samples of normal and poor responders in this assay system, IgG was found to be frequently depressed in poor responders but IgA and IgM were normal or elevated. Normal responders all had normal levels of serum IgG. It is possible that a poor ISC response indicated a concommitant or impending IgG deficiency in the aged individuals. In testing peripheral blood lymphocytes, however, one is testing a rather restricted lymphocyte pool, and thus no definite conclusions can be made about the biologic significance of poor polyclonal ISC responses. Suffice it to say that the abnormality was present in a statistically significant number of the aged population tested here. Also of interest is the observation that of 12 poor responders, 10 were male. Autoantibodies, on the other hand, are more commonly observed in females. Whether an existing deficiency of polyclonal responses bears any relation (presumably an inverse one) to development of autoantibodies remains to be determined.

The complexity of aging becomes apparent in the paradoxical observations that have been made during investigation of immunologic functions. Suppressor cell activity for example, has been found to be increased (15, 16, 19–23) or decreased (24–26) with aging. These observations suggest that a variety of immunoregulatory imbalances may be associated with aging, and that their expression may be closely linked to the stage of aging, as well as to the genetic makeup of the animal or individual being tested.

Spontaneous suppressor cells have been identified in the lymphoid systems of old mice (15, 16, 19–21) and inhibition of T cell-dependent as well as of T cell-independent antibody responses has been observed. Suppressor cells in aged mice have been shown to belong to T cell lineage (16, 22) and also to be of non-T cell origin (21). In our studies, evidence for increased suppressor activity was sought for in an allogeneic co-culture system with PWM stimulation. Deviations beyond the normal range of expected responses were observed in several instances (14 of 43, [32%]) when aged PBM were co-cultured with young PBM. Excessive suppressor activity, which was observed in co-cultures of 20% aged PBM did not correlate directly with

poor ISC responses. Approximately half the individuals whose cells showed excessive suppression in co-culture responded poorly. Of all poor responders tested in this manner, again approximately half manifested excessive suppression. Enhancement beyond expected responses was observed in a few instances. These findings reflect functional immunoregulatory imbalances in peripheral blood lymphocytes of aged individuals. Determination of T cell surface antigen phenotypes using monoclonal antibodies should prove revealing in this context. In the assay systems used here, it is not clear whether the suppressor cells were spontaneous or activated by PWM, which is a T as well as a B cell mitogen (27). The presence of suppressor cells could either result in immunodeficiency and/or serve to perpetuate an already existing immunodeficient state in some instances.

Cellular mechanisms involved in the breakdown of immune surveillance resulting in uncontrolled activity of autoreactive B cells are not clearly understood. Aging mice as well as aging humans tend to develop autoantibodies with or without overt disease manifestations. Excessive production of auto antiidiotype antibody (28) and antibody to modified self antigens (26) has been describe in aging mice. The latter defect has been attributed to lack of suppressor T cells. In humans, studies of suppressor cell function in aging have utilized the T cell mitogen Con A to activate suppressor cells. In T cell proliferative assays, investigations of Con A-induced suppressor T cells has yielded somewhat conflicting results. Using a T cell proliferative assay Hallgren and Yunis (24) observed deficiencies in Con A-induced suppressor cell activity. Antel et al. (23) however, using a somewhat different experimental protocol, described increased Con A-induced suppressor cell activity in aging humans. In the system developed by Delfraissy et al. (4) for quantifying specific antibody responses, deficiencies were observed in the ability of Con A-activated aged lymphocytes to suppress antibody responses of allogeneic lymphocytes. In agreement with the latter findings, we observed that in the autologous system used by us for activating suppressor cells, doses of Con A that regularly suppressed antibody responses in the young failed to do so in a significant proportion of the aged. In fact, in the aged population, this dose of Con A often enhanced antibody-producing cells, most likely resulting from a preferential activation of helper cells in the absence of activable suppressor cells. Whether deficiencies of Con-A induced suppressor cells were qualitative or quantitative needs further investigation. Recent studies of Kishimoto et al. (3) using the PWM system argue in favor of the former possibility.

Besides the existence of immunoregulatory disturbances, a decline in the functional capacity of B lymphocytes has also been shown to occur in aging mice. In the present study, although abnormalities in B cell function for the most part could be attributed to deranged immunoregulatory mechanisms, defects of B cells per se were not ruled out. Defects of B cells may vary in intensity and may include, for example, an increased requirement for helper T cells, increased sensitivity to suppressor cells, and decreased capacity of B cells to undergo terminal differentiation or to produce immunoglobulin. Failure of induction of specific antibody-producing cells was in fact observed in a few aged individuals despite provision of optimal help. It is not known whether in this study population any deficiencies existed in proportions or absolute numbers of circulating B lymphocytes. Conflicting reports exist showing normal (18, 29) or decreased (30) B cell numbers in aging humans. Decreased numbers of B cells oculd conceivably be present in some individuals and thereby play a significant role in producing the observed abnormalities of immunoglobulin and/or antibody production. In the majority of aged individuals, however, existing B cells in peripheral blood could be induced to function adequately under appropriate culture conditions.

Although statistically significant differences between responses of old and young volunteers were observed in both PWM and antigen-specific culture systems, abnormalities were more pronounced in the latter. B cell responses to antigen and to nonspecific stimuli did not go hand in hand. Whereas 12% of aged individuals had depressed responses to both stimuli, some others had depressed responses only to PWM or only to SRBC. These findings may reflect differences in subpopulations of B cells that might respond to one or the other stimulus. Responding B cells may differ for example in their maturation stage or in the cellular requirements for immunoglobulin or antibody production. Our recent studies have, in fact, suggested that mechanisms involved in specific and nonspecific activation of human B cells may be different.[3]

We have not dealt here with the influence of monocytes on the humoral responses of aging individuals. Direct evidence for age-associated defects in monocyte function has not been documented. The antigen-specific assay system used in the present study overcomes existing monocyte deficits in the responding population by the supplementation of cultures with monocyte-derived factors. Polyclonal B cell responses are relatively less dependent on monocytes in our hands (unpublished observations) and in those of some others (31, 32). Adherent cells have, however, been shown in some situations to be able to inhibit humoral

[3] Manuscript submitted for publication.

immune responses (33, 34). This issue will be given consideration in future studies.

The present study indicates that all the immunologic abnormalities of humoral immunity that have been described in the aged and/or autoimmune prone mice, (viz. decreased B cell responses, increased spontaneous suppressor cells, decreased inducible suppressor cells, decreased spontaneous helper cells, and intrinsic B cell defects) may be observed in aging humans. Of interest is the observation that a considerable number of the aged individuals manifested no abnormality whatsoever in vitro, illustrating the variability of immunologic vigor in an aged human population. The variability of the humoral immune responses in vitro and of immunoregulatory imbalances suggests that aging humans comprise a heterogeneous group, and that certain individuals may be genetically predisposed to develop one or more of the immunologic aberrations that are associated with aging.

ACKNOWLEDGMENTS

We are indebted to Dr. H. T. Thaler of the Biostatistics Laboratory for statistical analysis; Dr. R. Bhalla for serum immunoglobulin determinations; Dr. M. Hoffmann for helpful discussions; and to Mss Edith Espiritu and Rebecca Moshief for expert technical assistance. We thank Dr. M. Weksler, Dr. G. Siskind, and Miss J. Innes of Cornell University Medical Center for providing blood samples and medical information of aged volunteers.

This project was supported by grants from the National Institutes of Health AG 00541, CA 17404, Al 11843, NS 11457, CA 08748, CA 19267, and the Zelda Radow Weintraub Fund.

REFERENCES

1. Makinodan, T., M. L. Heidrich, and A. A. Nordin. 1975. Immunodeficiency and autoimmunity in aging. *In* Immunodeficiency in Man and Animals. D. Bergsma, editor. Sinauer Associates, Inc., Sunderland, Mass. 193–198.
2. Kishimoto, S., S. Tomino, K. Inomata, S. Kotegawa, T. Saito, M. Kuroki, H. Mitsuya, and S. Hisamitu. 1978. Age-related changes in the subsets and functions of human T lymphocytes. *J. Immunol.* **121:** 1773–2780.
3. Kishimoto, S., S. Tomino, H. Mitsuya, and H. Fujiwara. 1979. Age-related changes in suppressor functions of human T cells. *J. Immunol.* **23:** 1586–1593.
4. Delfraissy, J. F., P. Galanaud, J. Dormont and C. Wallon. 1980. Age-related impairment of the in vitro antibody response in the human. *Clin. Exp. Immunol.* **39:** 208–214.
5. Hoffmann, M. K. 1980. Antigen specific induction and regulation of antibody synthesis in culture of human peripheral blood mononuclear cells. *Proc. Natl. Acad. Sci. (U. S. A.)* **77:** 1139–1143.
6. Pahwa, S., R. A. Good, and M. K. Hoffmann. Role of mitogens and antigens in antibody production by human B lymphocytes. *J. Clin. Immunol.* In press.
7. Mishell, F. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126:** 423–442.
8. Muchmore, A. V., I. Koski, N. Dooley, and R. M. Blease. 1976. Artificial plaque formation in vitro and in vivo due to passive transfer to specific antibody. *J. Immunol.* **116:** 1016–1023.
9. Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein-A antibody adsorbent parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* **115:** 1617–1624.
10. Finelt, M., and M. K. Hoffmann. 1979. A human monocyte function test: release of B cell differentiation. *Clin. Immunol. Immunopathol.* **12:** 281–288.
11. Jerne, N. K., and A. A. Nordin. 1963. Plaque forming in agar by single antibody producing cells. *Science (Wash., D. C.).* **140:** 405–407.
12. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* **6:** 588–590.
13. Price, G. B., and T. Makinodan. 1972. Immunologic deficiencies in senescence. I. Characterization of intrinsic deficiencies. *J. Immunol.* **108:** 403–412.
14. Heidrich, M. L., and T. Makinodan. 1973. Presence of impairment of humoral immunity in nonadherent spleen cells of old mice. *J. Immunol.* **111:** 1502–1506.
15. Goidl, E. A., J. B. Innes, and M. D. Weksler, 1976. Immunological studies of aging. II. Loss of IgG and high avidity plaque forming cells and increased suppressor cell activity in aging mice. *J. Exp. Med.* **144:** 1037–1048.
16. Callard, R. E., and A. Basten. 1978. Immune function in aged mice. IV. Loss of T cell and B cell function in thymus dependent antibody responses. *Eur. J. Immunol.* **8:** 552–556.
17. Kishimoto, S., S. Tomino, H. Mitsuya, H. Fujiwara, and H. Tsuda. 1980. Age-related decline in the in vitro and in vivo synthesis of anti-tetanus toxoid antibody in humans. *J. Immunol.* **125:** 2347–2352.
18. Barrett, D. J., S. Stenmark, D. W. Wara, and A. J. Ammann. 1980. *Clin. Immunol. Immunopathol.* **17:** 203–211.
19. Segre, D., and M. Segre. 1976. Humoral immunity in aged mice. II. Increased suppressor T cell activity in immunologically deficient old mice. *J. Immunol.* **116:** 735–738.
20. Makinodan, T., J. Albright, P. Good, and M. L. Heidrick. 1976. Reduced humoral immune activity in long lived old mice. An approach to elucidating its mechanisms. *Immunology.* **31:** 903–911.
21. Singhal, S. K., J. C. Roder, and A. K. Duwe. 1978. Suppressor cells in immunosenescence. *Fed. Proc.* **37:** 1245–1252.
22. Callard, R. E., B. F. De St. Groth, A. Basten, and I. F. C. McKenzie. 1980. Immune function in aged mice. V. Role of suppressor cells. *J. Immunol.* **124:** 52–58.
23. Antel, J. P., M. Weinrich, and B. G. W. Arnason. 1978. Circulting suppressor cells in man as a function of age. *Clin. Immunol. Immunopathol.* **9:** 134–141.
24. Hallgren, H. M., and E. J. Yunis. 1977. Suppressor lymphocytes in young and aged humans. *J. Immunol.* **118:** 2004–2008.
25. Cantor, H., and R. K. Gershon. 1979. Immunological circuits. Cellular composition. *Fed. Proc.* **38:** 2058–2064.
26. Naor, D., B. Bonavida, R. A. Robinson, I. N. Shibata, D. E. Percy, D. Chia, and E. V. Barnett. 1976. Immune response of New Zealand mice to trinitrophyenylated syngeneic mouse red cells. *Eur. J. Immunol.* **6:** 783–789.
27. Waxdal, M. J., S. F. Nilsson, and T. Y. Basham. 1976. Heterogeneity of the pokeweed mitogen and the respond-

ing lymphocytes. *In* Mitogens and Immunology. J. J. Oppenheim and D. L. Rosenstreich, editors. Academic Press, Inc., New York. 161–172.
28. Schrater, A. F., E. A. Goidl, G. J. Thorbecke, and G. N. Siskind. 1979. Production of auto-anti-idiotypic antibody during the normal immune response to TNP-Ficoll I. Occurrence in AKR/J and BALB/c mice of Hapten-Augmentable anti-TNP plaque forming cells and their acceterated appearance in recipients of immune spleen cells. *J. Exp. Med.* **150:** 138–153.
29. Gupta, S., and R. A Good. 1979. Subpopulations of human T lymphocytes. X. Alterations in T, B, third population cells, and T cells with receptors for immunoglobulin M ($T\mu$) or G ($T\gamma$) in aging humans. *J. Immunol.* **122:** 1214–1219.
30. Cobleigh, M. A., D. P. Braun, and J. E. Harris. 1980. Age-dependent changes in human peripheral blood B cells and T-cell subsets: correlation with mitogen responsiveness. *Clin. Immunol. Immunopathol.* **15:** 162–174.
31. Fauci, A. S., K. R. Pratt, and G. Whalen. 1976. Activation of human B lymphocyte. II. Cellular interactions in the PFC response of human tonsillar and peripheral blood B lymphocytes to polyclonal activation by pokeweed mitogen. *J. Immunol.* **117:** 2100–2104.
32. Saxon, A., R. H. Stevens, and R. F. Ashman. 1977. Regulation of immunoglobulin production in human peripheral blood leukocytes: cellular interactions. *J. Immunol.* **118:** 1812–1879.
33. Waldmann, T. A., R. M. Blaese, S. Broder, and R. S. Krakauer. 1978. Disorder of suppressor immunoregulatory cells in the pathogenesis of immunodeficiency and autoimmunity. *Ann.Intern. Med.* **88:** 226–238.
34. Katz, P., and A. S. Fauci. 1978. Inhibition of polyclonal B-cell activation by suppressor monocytes in patients with sarcoidosis. *Clin. Exp. Immunol.* **32:** 554–562.