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Immunological responses of a healthy elderly population

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SUMMARY

We measured *in vitro* mitogen responses, delayed hypersensitivity skin tests, circulating immune complexes (CIC) and three autoantibodies in 279 healthy and 24 chronically ill individuals over 65, and in young controls. The elderly individuals had previously undergone a complete medical examination and laboratory screening tests, and were on no medications. Compared to the results of 180 young controls tested concurrently, the healthy elderly individuals had significantly depressed PHA responses and skin test responses. In addition, CIC and autoantibodies were increased in the healthy elderly group compared to young controls. There was no difference in PHA or skin test responses between the healthy and chronically ill elderly subjects, suggesting that the major determinant of depressed cellular immunity in the elderly is age *per se* and not age-associated diseases. Within the elderly population, aged 65–94, there was a significant decrease in PHA response with age. Previously it has been reported that correlations exist between measurements of cellular immune response (mitogen response and skin testing) and manifestations of autoimmunity (CIC or autoantibodies) in elderly subjects. However, in this well characterized healthy elderly population we could not verify an association between the cellular immune response and either autoantibodies or immune complexes. In addition, there was no increased prevalence of autoantibodies in subjects with CIC.

INTRODUCTION

Ageing is associated with changes in immune function in both experimental animals and man. In short-lived animals such as mice, three age-related patterns of change in immunological function have been decribed. First there is decreased cellular immune function as measured *in vivo* by graft acceptance and *in vitro* by lymphocyte proliferative response to antigens or T cell mitogens (Kay, 1978). Second, there is an increase in autoantibody formation with age in some strains (Naor, Bonavida & Walford, 1976). Finally, B cell responses are depressed as shown by decreased titre and avidity of the antibody response to certain antigens (Goidl, Innes & Weksler, 1976).

The immunological changes with age in humans are not as well defined. Perhaps the major problem with human gerontological reseach is the separation of physiological changes of age from physiological changes that are secondary to diseases that frequently accompany ageing (Kimmel, 1974). This is particularly difficult when many medical school based researchers use 'normal' elderly subjects garnered from the medical and surgical wards or outpatient clinics. Since the average patient over 65 attending an outpatient clinic has multiple medical diagnoses and is taking a mean of

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four prescription medications (O'Malley *et al.*, 1980), these patients are not ideal for determining physiological changes that occur strictly as a function of age. Hess & Knapp (1978) have used these arguments, as well as others, to suggest that the well defined immunological changes with age found in a short-lived species like the mouse may have no human equivalents, and that the previously described immunological changes found in older humans may be the result of unrelated diseases rather than fundamental changes with age.

In this paper we will report the results of immunological testing on a population of 279 men and women over the age of 65. Prior to entry into this study, all subjects stated they were in good health and on no prescription medication. In addition each subject received a complete medical history and physical examination as well as routine screening laboratory tests. Only those subjects considered to be in good health after all of the above were included in this study.

Our results confirm previous studies showing decreased delayed hypersensitivity skin test responses, decreased mitogen responses, increased circulating autoantibodies and increased circulating immune complexes (CIC) in elderly subjects compared to young controls. Interestingly, we found no correlation between the cellular immune status (skin testing and mitogen response) and manifestations of autoimmunity (autoantibodies and CIC). Indeed, there was even a poor correlation between the results of skin testing and the results of mitogen stimulation in these subjects.

MATERIALS AND METHODS

Subjects. The subjects in this study were recruited from the Albuquerque, New Mexico area in early 1979 by a series of announcements over radio and television and by articles in local newspapers. Criteria for entry included: (1) age 65 or more, (2) no prescription medication or daily non-prescription medication and (3) no known serious medical diagnoses; e.g. subjects with cancer, aortic stenosis, diabetes or transient ischemic attacks were excluded while subjects with osteoarthritis or cataracts were included as long as they were not on medication. All subjects then underwent a complete medical history and physical examination as well as routine laboratory tests (stool guaiac, PAP test, complete blood count, urinalysis, and a 24-channel automated chemical analysis of serum). The medical history, physical examination and laboratory tests revealed 24 patients with serious, previously undiagnosed illnesses; so that we studied 279 'healthy' and 24 'chronically ill' subjects over 65. The age range was 65–94. All subjects were fully ambulatory and living independently. In general our subjects were well educated and financially comfortable. Eighty-five percent of the subjects had finished high school and 49% had finished college. The mean monthly income (in 1979) was $1186. There were 135 men and 162 women of mean age 71·9 and 71·6, respectively.

Delayed hypersensitivity skin testing. Subjects were given intradermal injections of four antigens: streptokinase–streptodornase, candida, trychophyton and mumps. Induration was measured in two axies at 72 hr. Skin tests on all subjects were placed and read by the same individual, and were graded on a five point scale: $1 =$ no induration or erythema; $2 = > 0 \leq 5$ mm induration; $3 = > 5 \leq 10$ mm induration; $4 = > 10 \leq 20$ mm induration; and $5 = > 20$ mm induration.

Mitogen testing. Venous blood was drawn into syringes containing preservative free heparin. Peripheral blood mononuclear cells (PBMC) were separated on Ficoll-Hypaque gradients, washed three times in media, and cultured at 10^5 cells in 200 μl media plus 20% fetal calf sera (FCS). Triplicate cultures were pulsed at 60 hr with 5×10^{-7}Ci ^{3}H-thymidine, harvested onto filter paper at 68 hr in a semiautomated cell harvester, and read in a scintillation counter. Mitogens and drugs were added directly to the cultures; an optimal (4 μg/ml) and suboptimal (1 μg/ml) concentration of phytohemagglutinin (PHA) were employed (Goodwin, Messner & Williams, 1979). Each time PBMC from one or more elderly subjects were tested, PBMC from a young control, age 20–40, were run in parallel.

Assay for autoantibodies. Serum samples were obtained from the subjects at the same time that blood was drawn for the lymphocyte cultures. The sera were frozen at -70°C in small aliquots prior to assay. Rheumatoid factor was determined using the latex fixation method. Antinuclear antibody

was determined using cryostat sections of mouse and rat liver with initial overlay of test sera beginning at a 1 : 10 dilution followed by fluoresceinated polyvalent rabbit anti-human Ig (Rothfield & Stollar, 1967).

Anti-lymphocyte antibodies were determined by the microcytotoxicity test (Terasaki, Mottironi & Barnett, 1970). Sera were incubated with a panel of PBMC from 11 donors for 30 min at 15°C, followed by addition of rabbit complement and an additional 3 hr incubation at 15°C. Cells were stained with 5% eosin, fixed with neutral formaldehyde, and read by phase contrast microscopy. More than 30% killing in > 50% of the target panel was recorded as positive.

CIC were determined by the ^{125}I-C1q solid phase assay as previously described (Tung *et al.*, 1978).

RESULTS

Autoantibodies in sera from healthy old people

The prevalence of antinuclear antibody, lymphocytotoxic antibody, rheumatoid factor and CIC is given in Table 1. Sera were not always available for these assays, so a different number of subjects received each test. There is a clear increase in the percentage of sera from old subjects positive for each test, compared with sera from young controls. Of interest, the fact that a given serum was positive in one of these tests did not increase the chance of it being positive in the remaining tests. Fifty percent of the elderly subjects were positive for at least one test, which is exactly the figure predicted if positivity for these tests does not cluster in a subgroup of the elderly. Delespesse *et al.* (1980) reported an association between the presence of CIC and the presence of one or more autoantibodies in 189 healthy subjects aged from 20 to > 70. We found no such association in our elderly population using a variety of statistical techniques.

Delayed hypersensitivity skin testing

Two hundred and twenty-eight of the 279 healthy elderly subjects underwent skin testing with four common antigens. Seventy-six subjects (33%) had no induration at 48 hr to any of the skin antigens. In contrast, all 30 of the young controls tested responded with at least > 5 mm induration to one or more of the skin test antigens.

Mitogen respone of PBMC from healthy old people

The mitogen response of the 279 healthy old people to an optimal and suboptimal concentration of PHA is presented in Figs 1 and 2, compared to the responses of 180 young controls tested in parallel.

Table 1. Autoantibodies and circulating immune complexes in healthy elderly subjects

	Old subjects		Young Controls	
Assay	positive/total tested	% positive	positive/total tested	% positive
Antinuclear Ab	50/278	18	4/98	4
Rheumatoid factor	38/278	14	4/98	4
Lymphocytotoxic Ab	27/278	10	3/93	3
Circulating immune complexes	43/197	22	5/100	5

Positive sera were defined as follows: for antinuclear Ab a titre of ≥ 1 : 10, for rheumatoid factor at titre of ≥ 1 : 20, for lymphocytotoxic Ab > 30% killing in > 50% of samples of peripheral blood lymphocytes from 11 normal donors, and for circulating immune complexes > 2 s.d. above the mean of values of a panel of normal control sera.

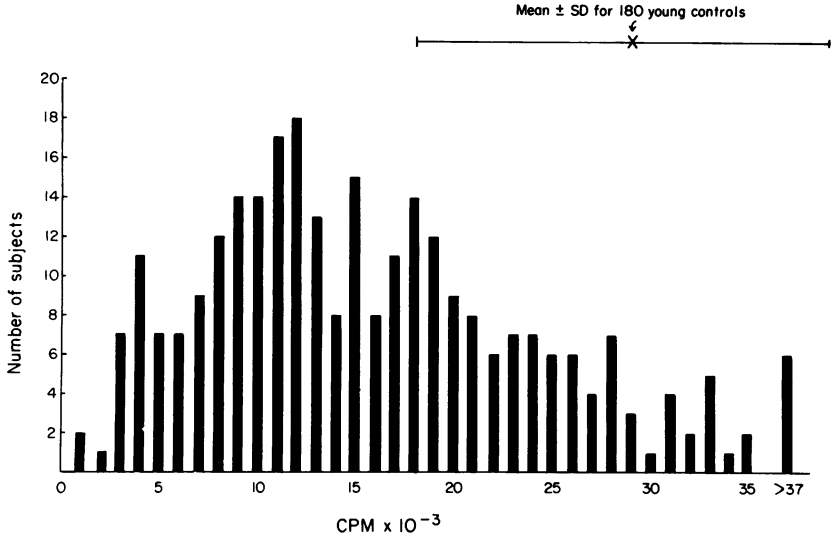

Fig. 1. Distribution of responses of lymphocytes from 279 healthy old people to a suboptimal concentration (4·0 g/ml) of PHA. The mean ±one standard deviation (SD) of the response of lymphocytes from 180 young controls run in parallel is shown for comparison.

The mean response of the elderly subjects is significantly less than the mean response of the young controls to both mitogen doses ($P < 0{\cdot}0001$ by Student's t-test for each PHA dose). At the 4·0 μg/ml PHA (optimal) dose the mean response of the 279 old people is 61% of the mean response of the 180 young controls, and is 55% at the suboptimal PHA dose (1·0 μg/ml). At the suboptimal PHA dose 185 of the old people (66%) had a response that was more than one. s.d. below the mean of the response of the young controls. Inspection of Figs 1 and 2 reveals that the distributions of PHA response are not quite normal, being skewed towards higher PHA responses. Thus, there is a

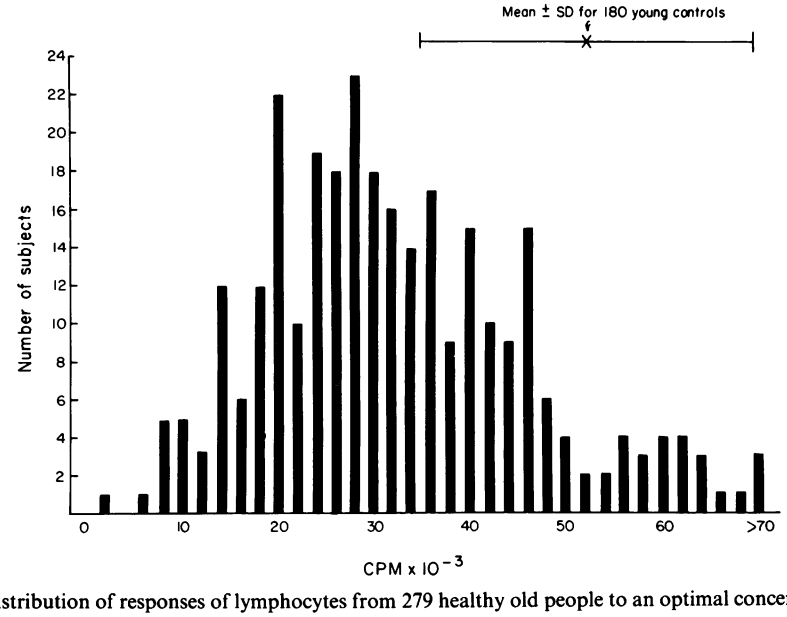

Fig. 2. Distribution of responses of lymphocytes from 279 healthy old people to an optimal concentration (4·0 g/ml) of PHA.

Table 2. Comparison of PHA and skin test responses among 'healthy' and 'chronically ill' old people*

	c.p.m.	
	Healthy (279)	Chronically ill (24)
PHA (1 g/ml)	15,947±560	17,364±1,655
PHA (4 g/ml)	31,797±873	34,394±2,774
	Sum of skin test scores	
	Healthy (228)	Chronically ill (24)
Skin tests	6·7±0·2	7·4±0·7

* Data given as mean ±s.e.m. None of the differences between the healthy and chronically ill groups were significant. The chronically ill group contained individuals with cancer, congestive heart failure, chronic obstructive pulmonary disease and cornonary artery disease. Most were on a variety of medications at the time of blood drawing.

population of healthy old people with PHA responses grouped about the mean of the PHA responses for the young controls, while the majority of healthy old people have depressed PHA responses.

When we compared the PHA and skin test responses of the 24 'chronically ill' old people to the responses of the 279 healthy old people we found no significant differences (Table 2). Thus age *per se*, and not accompanying illness, is the major determinant of depressed cellular immunity of this population. Within the healthy elderly group, there was a significant decrease in PHA response with increasing age (Fig. 3; $r=-0{\cdot}23$, $P<0{\cdot}0001$ for 4 μg/ml PHA and $r=-0{\cdot}22$, $P=<0{\cdot}001$ for 1 μg/ml PHA). Also, there was a non-significant trend for skin test scores to decrease with age ($r=-0{\cdot}13$, $P=0{\cdot}10$). There was no correlation between age and either presence or titre of CIC or the three autoantibodies within the healthy elderly subjects. The total white count of the elderly subjects tended to increase with age ($r=0{\cdot}15$, $P<0{\cdot}02$).

Next we looked for any sex differences in the results of the immunological testing. There was no difference in PHA skin test scores or frequency of autoantibodies between the 125 men and 142 women in our healthy old people group. We did find that the males had higher total white blood counts ($6{\cdot}06\times10^3$ versus $5{\cdot}74\times10^3$, $P<0{\cdot}05$ by Wilcoxin two-sample test), while the females had higher absolute lymphocyte counts ($2{\cdot}11\times10^3$ versus $1{\cdot}75\times10^3$, $P<0{\cdot}0001$ by Wilcoxin two-sample test). This difference remained after controlling for age.

Relationships between the immunological tests

There was a small but statistically significant correlation between mitogen response and total skin test score ($r=0{\cdot}18$, $P<0{\cdot}01$ for 1 μg/ml PHA and $r=0{\cdot}15$, $P<0{\cdot}05$ for 4 μg/ml PHA). These low coefficients of correlation imply that less than 2% of the variance in PHA reactivity can be explained by reactivity in the skin test scores. There was no correlation between skin test reactivity and either WBC or absolute lymphocyte count.

We re-examined 100 of the subjects 1 year after initial assessment in order to determine the reproducibility of the skin test responses and PHA responses in this group. The correlations between the initial and repeat PHA stimulation results ($r\geq0{\cdot}55$, $P<0{\cdot}001$) and between the initial and repeat skin test scores ($r=0{\cdot}72$, $P<0{\cdot}001$) were quite a bit higher than the initial correlation between skin test scores and PHA results. Thus the poor correlation between skin test and PHA results is not simply due to assay variability.

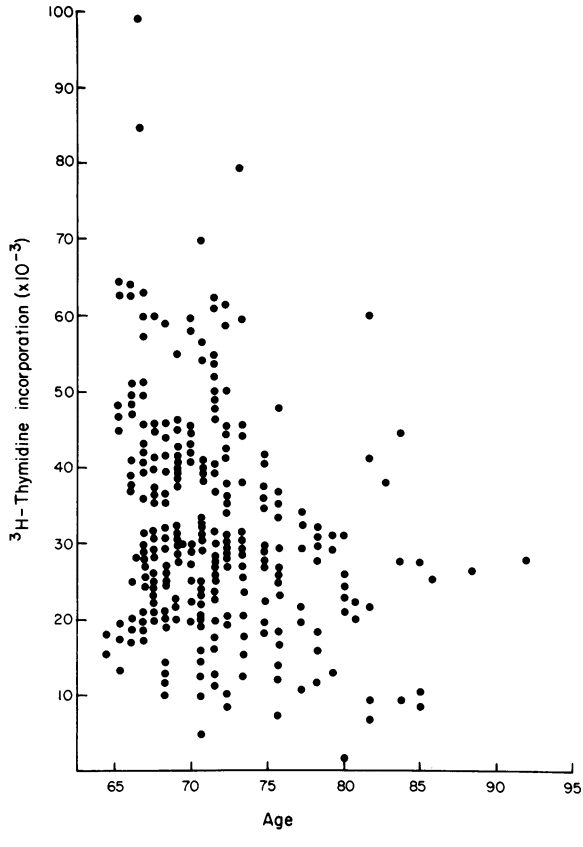

Fig. 3. Response to PHA (4·0 g/ml) in 279 healthy old people versus age of the subject. The PHA response in net c.p.m. of ^{3}H-thymidine incorporation decreases with increasing age of the subjects ($r = -0{\cdot}23$, $P < 0{\cdot}0001$). This coefficient of correlation, though highly significant because of the large number of subjects studied, is quite small, with only 5% of the variance in the PHA responses in this group due to the age differences of the subjects.

Hallgren *et al.* (1973) reported a negative association between the PHA response and the presence of autoantibodies in an elderly population. We found no association between the measures of T cell function (PHA response and delayed hypersensitivity skin testing) and the measures of autoimmunity (CIC, rheumatoid factor, antinuclear antibody and lymphocytotoxic antibody). For example, skin test reactivity and PHA responses do not differ in those subjects with or without detectable CIC.

Finally, we examined whether there was any correlation between cellular immune response and the amount of exercise our subjects received (Cassel, 1971). There was no correlation between PHA response and activity level ($r = 0{\cdot}01$, $P = 0{\cdot}9$), but there was a weak negative correlation between level of physical activity and skin test response ($r = -0{\cdot}16$, $P < 0{\cdot}05$).

DISCUSSION

Previous studies of changes in immune response with age in humans have produced conflicting results. For example many investigators have reported an increase in prevalence of various antoantibodies with age (Hallgren *et al.*, 1973; Cammarata, Rodnan & Fennell, 1967; Mackay, 1972). More recently, however, Pandey *et al.* (1979) found no association between antinuclear antibodies and age in a large population of healthy caucasian men and women. Similarly, various

authors have reported that the percentages of circulating T cells are raised (Hallgren *et al.*, 1978), depressed (Girard *et al.*, 1977), or unchanged (Kishimoto, Tomino & Inomata, 1979) in elderly humans.

Most workers have reported a decrease proliferative response of lymphocytes from elderly donors to antigens and to T cell mitogens (Hallgren *et al.*, 1978; Czlonkowska & Korlack, 1979; Roberts-Thompson *et al.*, 1974; Inkeles *et al.*, 1977; Goodwin & Messner, 1979). But even in this case the results are conflicting. Czlonkowska & Korlak (1979) reported decreased proliferative response (expressed as stimulation index of ^{3}H-thymidine incorporation) of lymphocytes from 30 subjects over 60 compared to young subjects to the mitogen PHA but not to Con A, PWM, candida or SKSD. Hallgren *et al.* (1978) found a significant stepwise decrease with age in response to several concentrations of PHA and one of two concentrations of Con A starting at 40. Portaro, Glick & Zighelboim (1978) studied 200 healthy, working subjects aged 21–70 and found no age-related decrease in response to several concentrations of PHA. The authors pointed out that their group of subjects was better characterized as the health status than previously reported subjects in which a decline in PHA response had been found. On the other hand, the oldest subjects in this study were 70, and the number of subjects in the oldest two categories (61–65 and 66–70) may have been too small (11 and 14, respectively) to recognized substantial differences in PHA responses. Ben-zwi *et al.* (1977) found that the proliferative response of lymphocytes from a poorly characterized group of individuals aged 60–92 years to several concentrations of PHA and Con A was not different from young controls if net c.p.m. were used as the measure. They pointed out that because ^{3}H-thymidine incorporation of unstimulated cultures of PBMC from their old subjects was larger than in young controls, the stimulation indices for the old subjects were lower.

While both the mitogen responses and the delayed hypersensitivity skin testing were depressed in this healthy elderly population, there was a very poor correlation between these two measures of cellular immune response. Many authors have postulated that a decrease in T cell function is the primary immunological change with age (Kay, 1979; Meredith & Walford, 1979). Thus the increase in autoantibodies is seen as secondary to a loss of T suppressor cell function, not to a specific B cell defect (Hallgren & Yunis, 1977; Gershon & Metzler, 1977). We found no correlation between either measure of cellular immunity and the measures of autoimmunity in this population, nor was there any degree of intercorrelation between the presence or titre of CIC or any one of the three autoantibodies that were measured. The presence of one autoantibody or of CIC did not increase the chance of an individual being positive for another autoantibody.

The young and elderly subjects studied in this present report were on no prescription or non-prescription medications except for an occasional laxative or analgesic. All were judged healthy after complete medical examinations and laboratory testing. In addition, these subjects have now been followed for another 2 years since the immunological evaluation was performed. They have all received two more annual comprehensive medical evaluations. Any subjects who developed serious illness during the 2 year follow-up were excluded from this analysis. Even with this rigorous characterization of our population, we still found a substantial age related decrease in cellular immune responses assessed by either *in vivo* (skin testing) or *in vitro* (PHA response) methods. In addition, we found no difference in PHA response between our healthy subjects and the subjects excluded from the initial analysis because of potentially serious medical illness. This would strongly suggest that, in our population, age itself and not age-associated diseases is the major determinant of depressed cellular immune responses.

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