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Cell-mediated immunity in an ageing population

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SUMMARY

Eight hundred and eighty patients hospitalized in a geriatric hospital were routinely tested with 2, 10, 30 and 100 i.u. tuberculin. Among those, fifty-four patients were selected on the basis of negative skin tests and absence of evident disease interfering with the function of the immune apparatus. A battery of tests analysing cell-mediated immunity was applied to those fifty-four patients. It appears that elderly patients having a negative test to 100 i.u. tuberculin show very infrequent sensitization to three other thymus-dependant antigens.

The capacity of this selected population to become sensitized to DNCB is poor (20%). Furthermore they exhibit a low per cent of peripheral blood T cells (36%) and a poor capacity to respond *in vitro* to mitogens such as PHA. Testing the *in vitro* response to a battery of antigens demonstrates a good correlation with the results of the skin tests. Finally the leucocytes of 25% of this selected population failed to produce LIF *in vitro* in the presence of PHA. These results suggest not only an absolute decrease in the population of circulating T lymphocytes in those elderly humans; but very likely, at least in some cases, a functional impairment of T cells.

INTRODUCTION

As in other biologic systems immune function also undergoes profound changes during the life cycle of an individual organism. To understand how the ageing process affects the immune system, large quantitative and functional studies among individuals of different ages are needed.

Responsiveness to thymus-dependent antigens, originally contacted in early life, is said to decrease with advancing age (Walford, 1968; Nossal, 1962). On the other hand, little information is available concerning the capacity of aged subjects to deal with newly encountered antigens (Cammarata, Rodmen & Fennell, 1967).

According to several reports there is a decrease in the absolute number of T lymphocytes in older people (Valtuena *et al.*, 1973; Carosella, Mochanko & Braun, 1974), and, possibly, an increase in the population of B cells which may account for the augmented level of serum immunoglobulins (Ippoliti *et al.*, 1974). Though several groups of diseases might be responsible, at least partly, for the development of immune dysfunction in old age (Aisenberg, 1962), it is more difficult to understand the genesis of depression of cell-mediated immunity in apparently healthy aged subjects.

This study aimed to analyse the immune function of elderly humans selected from among patients hospitalized in a geriatric hospital or attending an out-patient clinic for minor physical discomfort.

MATERIALS AND METHODS

Selection of patients. 880 patients hospitalized in a geriatric hospital or attending an out-patient clinic were included in the first phase of the study. They all were intradermally skin tested with 2, 10, 30 and 100 i.u. of PPD (Tuberculin Berna, Berne, Switzerland). 604 patients were discarded because of the presence of a clear-cut positive skin test. A second group of 209 subjects having a negative skin test to tuberculin was also excluded from the study because of the presence of patho-

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logical conditions which could interfere with the function of the immune apparatus (58% of cancer, 18% acute infections including tuberculosis, 16% autoimmune diseases and some other debilitating clinical conditions). The remaining group of sixty-seven tuberculin negative subjects was further investigated.

Immunologic investigations. (1) *Skin tests.* The sixty-seven selected patients were intradermally skin tested with a battery of four antigens: tuberculin (PPD 100 i.u.); Streptokinase-Streptodornase (Varidase-Lederle) 250 u; *Candida albicans* (Institut Pasteur, Paris), 0·1 ml of a 1/500 sol.; *Aspergillus* mix (Institut Pasteur, Paris), 0·1 ml of a 1/500 sol. Thirteen of these sixty-seven subjects having more than one single positive test were also excluded from the study.

(2) *Primary immune response to DNCB.* Dinitrochlorobenzene (Fluka, Buchs, Switzerland) was used for sensitization. It was prepared in 10% acetone solution. A ring of 2·0 cm was painted on the right forearm and covered with a plastic bandage for 10 days. Three weeks later, a 0·1% solution of DNCB was applied over a similar area of the opposite forearm. The results were read 3 and 6 days later, and estimated from 0 to ++++ according to the size of the local induration and redness.

(3) *Dosage of serum immunoglobulins.* Immunoglobulins G, A and M were determined quantitatively by means of Immunoplates (Hyland, Los Angeles, U.S.A.). Serum IgE levels were measured by a standardized radioimmunoassay, Phadebas (Pharmacia, Uppsala, Sweden).

(4) *Lymphocyte culture technique.* Lymphomonocytic cells, separated on a layer of Ficoll-Isopaque (Böyum, 1968), were cultured in microplates Falcon (Falcon, Oxnard, Calif., U.S.A.). TC 199 medium (Difco Lab., Detroit, U.S.A.) enriched with 10% normal human pooled AB serum was used. The following stimulatory agents were used: PHA-P 1:50 (Difco Lab., Detroit, U.S.A.); PPD 0·5 u/10^6 cells (Staaten Serum Institute, Copenhagen, Denmark); *Candida albicans* 1:100, 0·1 ml/10^6 cells (Antigènes Mérieux, Lyon, France); Streptokinase-Streptodornase (Varidase-Lederle) 250 u/10^6 cells; and *Aspergillus* (Institut Pasteur, Paris) 1:1000, 0·1 ml/10^6 cells. Following 4 days incubation at 37°C in a CO_2/air atmosphere, ^{3}H-thymidine 1·0 mμCi/10^6 cells (NEN Chemical, Dreieichenhain, Germany) was added. Cells were harvested with the aid of a semi-automatic cell-harvester (Skatron, Bergen, Norway) and the activity measured on a Packard scintillation counter.

(5) *E-rosette binding cells.* Washed SRBC were adjusted to a 0·5% suspension. A volume of 0·25 ml (10^6) lymphocytes were mixed with 0·25 ml SRBC and incubated at 37°C for 10 min. The cell suspension was incubated at 4°C for 3 hr. One drop of the cell suspension was mounted on a glass slide and covered with a coverslip. 200 lymphocytes were counted and all cells binding more than three SRBC were considered as T cells.

(6) *Ig-bearing cells.* 100 μl of polyvalent rabbit anti-human immunoglobulin serum 1/10 (Institut Pasteur, Paris) were added to lymphocytes (0·25 ml or 10^6 cells). Following a 1 hr incubation at 4°C, the cells were washed three times in cold Hanks's solution. 50 μl of fluorescent sheep anti-rabbit serum (Institut Pasteur, Paris) was added. After a 1 hr incubation the cells were washed and layered on glass slides. Presence of membrane fluorescence was determined using a Wild fluorescence microscope.

(7) *Production and assay for lymphokine activity.* Unseparated lymphomonocytes were cultured for 48 hr at 37°C in TC 199 plus 1% human serum albumin (10^7 cells/vial) both in the presence or the absence of 50 g PPD, 1·0 ml of 1% *Candida albicans* extract or PHA. Following centrifugation and dialysis the supernatant was used for LIF assay. This assay was done on whole blood leucocytes or Ficoll-Isopaque separated human lymphomonocytic cells from PPD and *Candida* negative donors according to a recently published procedure (Girard & Cuevas, 1976).

RESULTS

Skin tests

The results of the skin test with a battery of four antigens are illustrated in Table 1. Among the sixty-seven patients of the group over 65 years, two patients were shown to have positive tests to three antigens, three to two antigens and eight to one isolated antigen. Only the patients showing negative tests to all four antigens or only one positive test were further investigated.

TABLE 1. Results of skin tests to various antigens, among subjects of different age groups

Age group (No. of patients)	No. of positive tests			
	Tuberculin	*Candida albicans*	SK–SD	*Aspergillus*
15–25 (46)	26	19	43	15
25–40 (56)	53	41	67	34
40–55 (53)	56	49	64	41
55–65 (51)	44	34	48	43
>65 (67)	0	8	9	3

In the same table, results representing the per cent of positive skin tests among different groups of healthy people are shown for comparison with an unselected younger population.

Primary immune response to DNCB

These results, as shown on Table 2, demonstrate that only two of forty-six patients failed to become sensitized to DNCB among healthy subjects aged 20–40, eleven among the forty-eight subjects of age 40–60, and thirty-four among the forty-four elderly subjects (>65 years).

TABLE 2. Primary immune response to DNCB among different age groups

Age group (No. of patients)	No. of tests				
	Negative	+	++	+++	++++
>65 (44)	34	1	4	2	3
20–40 (46)	2	—	10	22	12
40–60 (48)	11	5	14	26	3

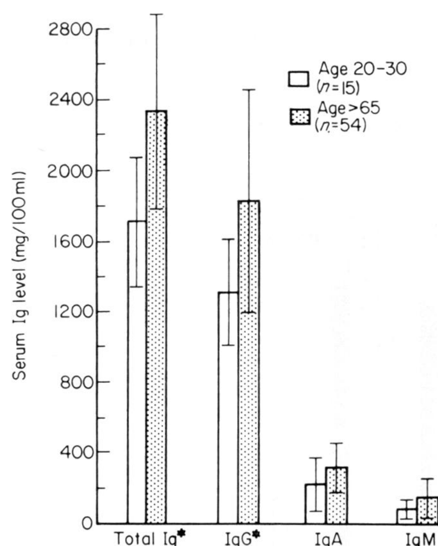

FIG. 1.

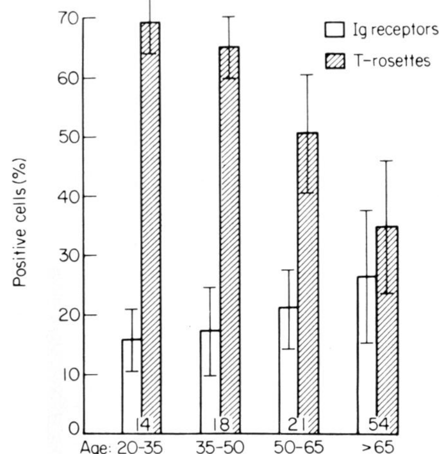

FIG. 2.

FIG. 1. Total serum Ig and IgG, IgM and IgA levels ± s.d. in patients over 65 years and a 20–30-years-old control group.

FIG. 2. Determination of percentage of E-rosette forming cells and Ig-bearing lymphocytes ± SD in different age categories.

Serum Ig levels

The measurements of serum Ig levels are shown as mean values on Fig. 1. The results are compared to those obtained in a group of younger healthy subjects (age 20–30). The average values for older people are somewhat higher for total Ig and IgG when compared to that of the younger group; however, the difference is not statistically significant. The values for serum IgE (not shown in the Figure) are 167 units ±86 *vs* 126±63 for younger subjects. Here also the difference is not statistically significant.

Estimation of peripheral blood T and B lymphocytes

These results are shown in Fig. 2. They illustrate analysed specimens taken from different age classes. The mean values for T cells are highest among the group of healthy subjects are 20–35. A statistically

significant decrease is observed among healthy subjects who belong to the 50–65 age group. The average value is even lower among patients over 65, with, however, a large dispersion of the results. In the latter group, absolute values were calculated in most cases, and the following T cell counts were obtained: 850 ± 430 per mm^3, which value represents a true T lymphopenia when compared with 20–35 year group (1740 ± 680).

B lymphocytes show a progressive percentage increase with advancing age, with also an absolute increase per unit/vol. In patients over 65 years we found 685 ± 380/mm^3 *vs* 505 ± 160 for the younger group aged 20–35.

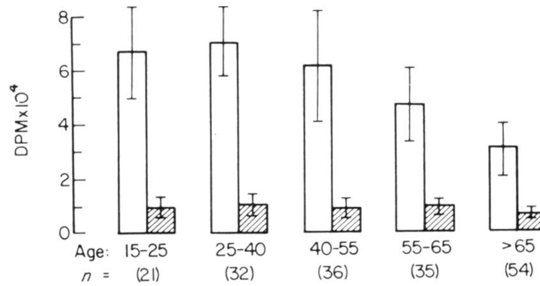

Fig. 3. Peripheral blood lymphocytes response to PHA *in vitro* (open columns) and spontaneous DNA synthesis (hatched columns) ± s.d. in different age categories.

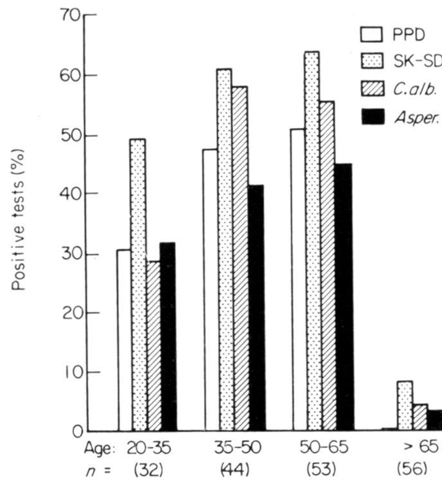

Fig. 4. *In vitro* response of peripheral blood lymphocytes to four thymus-dependent antigens in different age categories.

In vitro lymphocyte stimulation

The results as obtained with PHA stimulation of peripheral blood lymphomononuclear cells are shown in Fig. 3, which shows a progressive decrease in the lymphocyte responsiveness to PHA from the age of 40. The average values as obtained in the group of aged people are statistically significantly decreased when compared to all four groups of younger subjects. On the other hand, there is no significant difference in DNA synthesis of unstimulated cells among the five age groups.

Fig. 4 illustrates the results obtained following *in vitro* stimulation of peripheral blood lymphocytes with the same antigens used for the skin tests.

Among people over 65 years, there is a fair correlation between *in vitro* results and skin tests (Aisenberg, 1962; Böyum, 1968, Ippoliti *et al.*, 1974). This comparison cannot be done for the other groups, as the partition in classes of age was not exactly the same for skin test and *in vitro* stimulation of lympho-

cytes. However, these results illustrate the progressive development of immunity against cell-mediated antigens from age 20–65 among healthy adults.

Leucocyte migration inhibitory factor

The capacity to produce LIF among young healthy adults (age 20–40) and our group of old subjects (age >65) was studied using PHA, tuberculin (PPD) and *Candida albicans* as stimulatory agents. All the subjects selected as controls exhibited positive skin tests to the two antigens in concentrations mentioned earlier.

Table 3 illustrates the results. It shows that among positive controls there is a good agreement between the results of skin tests and the capacity to produce LIF. A good correlation was also found when comparing the results of skin tests and the capacity of LIF production among patients over 65. In that group, thirteen LIF tests were positive corresponding in eight cases to positive skin tests to the related antigen.

The table also shows that 25% of the old patients' cell populations failed to produce LIF in the presence of PHA (10^7 lymphomonocytes/test).

TABLE 3. Production of leucocyte migration inhibitory factor to PHA, PPD and *Candida albicans* by cells from young healthy adults and old subjects

Stimulatory agents	Age group 20–40	Age group >65
PHA	46/49	38/54
PPD	40/42	5/54
Candida albicans	42/42	8/54

DISCUSSION

The results reported here confirm findings reported by others (Gross, 1965; Waldorf, Wilkers & Dorker, 1968; Valtuena *et al.*, 1973; Carosella *et al.*, 1974), and bring some new information concerning the immunologic phenomena observed in old age.

As previously shown, among others by Miller & Jones (1973), there is a good correlation of lymphocyte transformation in the presence of specific antigen and the corresponding skin test sensitivity.

Among our fifty-four patients having all negative skin tests to 100 i.u. tuberculin, only four exhibited a clear-cut lymphocyte stimulation in the presence of this antigen. What is particularly striking, however, is the low frequency of positive skin tests to other thymus-dependent antigens in this group of patients. This is an additional argument for the presence of a true deficit in cell-mediated immunity in this population. To this impaired secondary immune response corresponds a low capacity to build a primary response to DNCB. The magnitude of this response (20%) is in the range that was observed in elderly patients with cancer by Gross (1965).

According to data reported in the literature in elderly subjects, there is an increase in serum levels of immunoglobulins and a high frequency of autoantibodies (Cammarata *et al.*, 1967, Wilkins, 1967; Valtuena *et al.*, 1973; Hallgreen *et al.*, 1973). Our data confirm the augmented values of serum immunoglobulins. However, the difference *v.* a younger control group is statistically significative only for total Ig, but for none of isolated Ig classes including IgE. To this increased Ig synthetic capacity corresponds an augmented per cent of B lymphocytes with advancing age. A significant difference is observed when comparing subjects over 65 and a 20–40 aged group. Similar findings were also reported by Augener *et al.* (1964).

Although the basis for the rosetting ability of human T lymphocytes is by no means clear, this assay is very useful for studying cell-mediated immunity in man. In confirmation of other reports (Carosella *et al.*, 1974; Smith, Evans & Steel, 1974), our data demonstrate an age related drop in the per cent of peripheral blood T lymphocytes. This change may be due to a real drop in circulating T cells or to a

change in their functional state. When calculating the absolute values for T cells, a true decrease becomes evident.

However, following addition of individual T and B lymphocytes, average values of 24±16 null cells could be found among our patients. By using more cell markers it perhaps would have been possible to decrease to some extent this observed value.

In spite of this, our data suggest the possibility of a functional disturbance responsible for the high values of null cells in old subjects.

Considering the diminished content of peripheral blood lymphocytes in T cells in old subjects, a reduced stimulation rate to PHA *in vitro* is to be expected and was indeed observed; thus confirming other reports (Waldorf *et al.*, 1968; Hallgreen *et al.*, 1973; Roberts-Thompson *et al.*, 1974). A surprising finding was the decreased stimulability by PHA of lymphocytes from patients of the age group 50–65. This contrasts with the finding of a progressively augmented response *in vitro* to various cell-mediated antigens from age group 20–30 to age group 50–65 among healthy subjects; whereas response decreases with advancing age, there is still a vigorous capacity to maintain a secondary response to these antigens.

Studies on the production of lymphokines demonstrate a good correlation between the cutaneous response to antigens and lymphocyte capacity to produce LIF *in vitro*. A rather good correlation could also be shown among the elderly subjects. However, in five cases a discrepancy became apparent, i.e. LIF production was present among five subjects exhibiting negative skin tests to the corresponding antigens.

Finally, a finding relevant to the hypothesis of a possible functional deficit of lymphocytes, is the study on the production of LIF in the presence of PHA. Indeed 25% of our patients failed to produce this lymphokine in the presence of this mitogen. When looking at individual results, no relationships could be found between this finding and especially low values for absolute T-cell counts.

Genetical factors are thought to modulate the capacity of the immune functions and to some extent condition ageing processes within the immune apparatus (Nossal, 1962; Burnet, 1970; Yunis & Greenberg, 1974). Recent works from our laboratory (Joris & Girard, 1975; Girard, Cuevas & Fernandez, 1976, submitted for publication) demonstrate that a large percentage of the old patients studied respond by increasing their PHA response, their absolute T-cell counts and their cutaneous response to antigens, following immunostimulation with tuberculin BCG, Levamisole or bacterial hydrolysate. These findings suggest that genetic factors are only partially operative in regulating ageing processes of the immune apparatus. Several environmental factors might play a major role in explaining the immune deficit observed among our elderly subjects. Nutritional factors (Walstenholme & O'Connor, 1967), vitamin deficiency (Axelrod, 1971), especially folic acid (Das & Hoffbraud, 1970), could eventually be considered 'responsible' for some of the observed deficits. As a matter of fact, six among those patients were shown to have a marked folic acid deficit. Following adequate treatment, five of those six patients recovered a normal cell-mediated immune function.

According to Roberts-Thompson *et al.* (1974), hyporesponsiveness to cell-mediated immune testing might represent, in very old people, a bad prognostic sign. The mortality in their study was significantly higher, over a 2 years' period, than that of comparable people exhibiting normal immune responses.

Investigations of the immune status should be applied more routinely in geriatric populations as there result sometimes indications for beneficial therapeutic measures.

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REFERENCES

AISENBERG, A.C. (1962) Studies on delayed hypersensitivity in Hodgkin's disease. *J. clin. Invest.* **41**, 1964.

AUGENER, W., COHNEN, G., REUTER, A. & BRITTIGER, G. (1964) Peripheral blood T and B lymphocytes in aged humans. *Lancet*, **i**, 1164.

AXELROD, A.E. (1971) Immune processes in vitamin deficiency states. *Amer. J. clin. Nutr.* **24**, 265.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *J. clin. Lab. Invest.* **21**, Suppl. 97, 77.

BURNET, F.M. (1970) An immunological approach to aging. *Lancet*, **ii**, 368.

CAMMARATA, R.J., RODMEN, J.P. & FENNELL, R.H. (1967) Serum antiglobulin and anti-nuclear factors in the aged. *J. Amer. med. Ass.* **199**, 455.

CAROSELLA, E.D., MOCHANKO, K. & BRAUN, M. (1974)

Rosettes-forming T cells in human peripheral blood at different ages cell. *Immunology*, **12**, 323.

DAS, K.C. & HOFFBRAUD, A.V. (1970) Studies of folate uptake by phytohemagglutinin stimulated lymphocytes. *Brit. J. Haemat.* **19**, 203.

GIRARD, J.P. & CUEVAS, M. (1976) Diagnosis of drug hypersensitivity application of a new procedure to test MIF and LIF. *Ann. clin. Res.* **8**, 74.

GIRARD, J.P., CUEVAS, M. & FERNANDES, B. (1976) Depression of cell-mediated immunity in an aging population. Tentative immuno-stimulation with BCG, levamisole and bacterial hydrolysate. *Allergol. Immunopath.* (In press).

GROSS, L. (1965) Immunological defect in aged population and its relationship to cancer. *Cancer*, **18**, 201.

HALLGREEN, H.M., BUCKLEY, C.E., GILBERSSEN, G.A. & YUNIS, S.Y. (1973) Lymphocytes phytohemagglutinin responsiveness, immunoglobulins and autoantibodies in aging humans. *J. Immunol.* **111**, 1101.

IPPOLITI, G., MARININ, G., CASIROLA, G. & INVONIZZI, R. (1974) T lymphocytes and immunoglobulins in the aged. *Lancet*, **ii**, 953.

JORIS, F. & GIRARD, J.P. (1975) Immune reponse in aged and young subjects, following administration of large doses of tuberculin. *Int. Arch. Allergy*, **48**, 584.

MILLER, S.D. & JONES, H.E. (1973) Correlation of lymphocyte transformation with tuberculin skin-test sensitivity. *Amer. Rev. resp. Dis.* **107**, 530.

NOSSAL, G.J.V. (1962) Cellular genetics of immune response. In: *Advances in Immunology*, Vol. 2. Academic Press, New York.

ROBERTS-THOMPSON, I.C., WHITTINGHAM, S., YOUNGCHAIYAD, U. & MACKAY, I.R. (1974) Aging, immune response and mortality. *Lancet*, **ii**, 368.

SMITH, M.A., EVANS, J. & STEEL, C.M. (1974) Age related variation in proportion of circulating T cells. *Lancet*, **ii**, 922.

VALTUENA, J.P., GUILABERT, M.J.G., DEL POZO PEREZ, M.A., DEL POZO CRESPO, F. & VELASCO, A. (1973) Auto-immunity, immunoglobulins and T lymphocyte population in old age. *Biomedicine*, **19**, 301.

WALDORF, D.S., WILKERS, R.F. & DORKER, J.L. (1968) Impaired delayed hypersensitivity in an aging population. *J. Amer. med. Ass.* **203**, 111.

WALFORD, R.L. (1968) *The Immunologic Theories of Aging*. Munksgaard, Copenhagen.

WALSTENHOLME, G.E.W. & O'CONNOR, M. (1967) *Nutrition and Infection*. Little Brown & Co., Boston.

WILKINS, R.F. (1967) The significance of antinuclear factors in older person. *Ann. rheum. Dis.* **26**, 306.

YUNIS, E.J. & GREENBERG, L.J. (1974) Immunopathology of aging. *Fed. Proc.* **33**, 2017.