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Cell-Mediated Effector Mechanisms in Aging Humans

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Abstract. Specific and nonspecific cell-mediated effector mechanisms have been simultaneously assayed in 15 aged humans. 8 were female and 7 male, including a 114-year-old male in remarkably good health. Proliferative response to alloantigens, the generation of T killer cells and the ability to express cell-mediated lympholysis as well as the presence of natural cell-mediated cytotoxicity against K562 tumor cell line and the capacity to mount an ADCC response to RhD+ human red blood cell sensitized with anti-D antisera, revealed that in the human aged, while T function significantly declines, nonspecific cell-mediated effector mechanisms are operative.

Introduction

The immunological changes which are linked to the process of aging have been the subject of recent research [1–5]; reports on abnormalities of cell- [6–10] and antibody-mediated immune responses [11–16] in both humans and mice, although often contradictory, suggest a decline in T cell function as a major hallmark of the immunopathology of aging. Very little is known about the status of the different cell-mediated effector mechanisms (CMI) in the aged and its possible influence in adaptation and survival in the last decades of life.

We have studied several parameters of cellular immune responses as a means to achieve more knowledge in clinical immunopathology [17–19]. We have now applied such an approach to the study of effector mechanisms in aging. Our results suggest that in the presence of a failure of T cell function, nonspecific CMI effector mechanisms may take over, and play a significant role during immunosenescence.

Material and Methods

Population Studied

15 healthy aged were included in the present investigation, after they fulfilled health criterias by a clinical protocol which included: physical examination, X-rays and routine laboratory as well as a special questionnaire designed to rule out immunological, infectious or tumor conditions. Their age ranged from 80 to 114 years; 8 were female (range 80–104) and 7 were males (80–114). They were receiving no therapy and lived in a public nursing home which belongs to the Venezuelan Ministry of Health.

Two types of controls were included; one group appropriately selected in our laboratory to set normal values for the immunological screening procedures and the other were 15 healthy subjects, ranging between 10 and 48 years, included as reference for some of the evaluated parameters.

Lymphocyte Preparations

Peripheral blood lymphocytes (PBL) were isolated on Ficoll-Hypaque gradients [20] and washed three times with RPMI 1640 medium (Microbiological Associates, Bethesda, Md.). The PBL were resuspended and adjusted to the desired concentration in RPMI 1640 supplemented with 25 m*M* Hepes buffer, 100 U/ml penicillin and 100 μg/ml streptomycin (Microbiological Association Bethesda, Md.) and 2% heat-inactivated pooled human serum (NHS). Monocyte contamination (acridine orange) in most of the experiments runs between 5 and 10%.

Standard Pool of Cryopreserved Lymphocytes

PBL used as stimulators in allogenic conditions were obtained from normal blood donors and processed following the methods of *Barclay* [21] and *Oldham* et al. [22]. The freezing medium consisted of RPMI 1640, 40% of NHS, 100 U/ml penicillin and 100 μg/ml streptomycin, 4% Hepes buffer (1 *M* pH 7.0), 10% dimethylsulphoxide (DMSO) and 10 U/ml of sodium heparin; both cells and freezing media were mixed slowly at 4°C and stored in 1-ml vials at −70°C. For further processing, the frozen cells were placed at 37°C and diluted 1:10 in appropriate medium; washed two times in RPMI 1640 and adjusted to desired concentration; cell viability determined by trypan blue was always greater than 98%.

Mixed Lymphocyte Culture (MLC)

MLC reactions were performed using the *Hartzman* [23] micro-method. Mitomycin C-treated (50 μg/ml for 30 min at 37 °C) cryopreserved cells were utilized as stimulators. They were added at a concentration of 10^5 cells/well to obtain a stimulator-responder ratio opf 2:1; the MLC response was performed in the presence or absence of autologous serum using U-bottom microtes II plates. The plates were covered by rigid lids and incubated in a humidified atmosphere at 37 °C with 5% CO_2. MLC cultures were termined on day 6, following a 12-hour pulse with 1 μCi of ^{3}H-thimidine (specific activity: 2 Ci/mmol; New England Nuclear, Boston Mass.). The harvesting process was carried out in a Mash II harvester. Proliferative responses were expressed as relative proliferation index (RPI) as described by *Dean* et al. [24]. RPI is the ratio between the net cpm of the studied subject and the net cpm of 3 or more controls assayed simultaneously. Cut-off values (established as the lower 10th percentile of normal RPI values) for alloantigens (based on 80 normal controls) was ≧ 0.66. This allowed us to define depressed or enhanced proliferative responses with accuracy [19]. All determinations were done in triplicate. The filter papers were place in a liquid scintillation spectrometer for final analysis.

Generation of T Cytotoxic Cells

Effector T cells were generated in vitro following the procedure of *Lightbody* [25]. Briefly, MLC was established using as responder cells PBL from aged persons or controls; mitomycin-treated cells from the control cryopreserved panel were used as stimulators. The cultures were set by triplicate in tubes with a ratio of 1.5×10^6 responder cells to 3×10^6 stimulators (1:2) in 2 ml of RPMI medium, with 20% of heat-inactivated NHS. The cultures were incubated for 5 days at 37 °C in 5% CO_2. 18 h prior to harvesting, 0.1 ml from each tube was labeled with 1 μCi of ^{3}H-thymidine; the cytotoxic effector cells were obtained from the tubes with the highest proliferative responses. In a similar fashion, target cells were cultured, collecting blast cells (stimulated with phytohemagglutinin) from the tubes with the highest proliferative responses.

Direct Cytotoxicity Assay

Cell-mediated lympholysis (CML) was performed following the technique of *Brunner* et al. [26]. Briefly, U-bottom microplates and an effector to target cell ratio of 100:1 were used. Target cells were labeled with sodium chromate (^{51}Cr, New England Nuclear); 5×10^3 target cells were added to the wells containing 5×10^6 effector cells (experimental release); maximal release was achieved by the lysis of 5×10^3 target cells in distilled water and spontaneous release was obtained with a similar amount of labeled target cells in RPMI medium, cultured with 10, 1 and 0.1% autologous serum from the studied subjects. The plates were incubated at 37 °C for 18 h. Prior to harvesting, the plates were centrifuged for 10 min at 2.500 rpm; 100 μl of the supernatant were taken from each well and counted in a liquid scintillation spectrometer (Packard, Chicago); the percentage of lysis was calculated by the following equation:

$$\%\ \text{lysis:}\ \frac{\text{Experimental release} - \text{spontaneous release}}{\text{Maximal release} - \text{spontaneous release}} \times 100$$

Values over 5% of lysis are considered as an index of positive killing since 50 normal (nonsensitized controls) showed values less than 2%.

Natural Cell-Mediated Cytotoxicity (NCMC)

NCMC activity of PBL, was explored using as targets the K 562 cell line (Kindly provided by Dr. *O. Stutman*), derived from chronic myeloid leukemia [27]; the target cells were adjusted to $3–5\times10^6$ cells in 0.5 ml of RPMI 1640 (supplemented with 10% fetal calf serum and 2% gluthamine) and labeled with 150 μCi of ^{51}Cr at 37 °C for 45 min in a 5% CO_2 atmosphere; they were washed three times and adjusted to 1×10^5 cells/ml; 0.1 ml of target cells were mixed with 0.1 ml of effector cells (10×10^6 cells/ml) in U-bottom microplates; the final effector-target cells ratio was 100:1; after a 4-hour incubation period, the plates were centrifuged at 900 rpm for 10 min. The final processing and calculations were performed as described in the CML assay; values over 20% lysis were considered as an intact NCMC.

Antibody-Dependent Cell Cytotoxicity (ADCC)

The ADCC activity of peripheral blood mononuclear cells in both aged subjects and in 33 normal controls was determined using RhD+ human erythrocytes as target cells, coated with specific IgG anti-D (Ortho Diagnostic, Inc.). 6×10^4 sensitized and ^{51}Cr-labelled erythrocytes were incubated at different ratio with effector cells in a 0.2-ml volume using U-bottom microtest II plates. Plates were incubated at 37 °C in a 5% CO_2 atmosphere for 18 h. Results were expressed as the number of mononuclear cells capable of lysing 50% of the sensitized erythrocytes (K); K values were calculated by the Von Krogh equation as modified by *Trinchieri* et al. [28]. The normal range, calculated as the 80th percentile in 33 controls was 7.8×10^4–7.8×10^5 mononuclear cells [19].

Statistical Analysis

Chi square and Student's t tests were applied when needed.

Results

15 carefully selected healthy aging humans were the subjects of the present investigation; proliferative responses to alloantigens, and CML expression were performed in all aged subjects, while NCMC and ADCC capacity were investigated in 11 and 5 aged subjects, respectively.

To explore the relationship between adequate proliferative responses to alloantigens and the capacity to generate CML effector cells, a comparison of the results for MLC and CML was made for both the controls and the aging group (table I); as can be seen, in the control group a normal CML capacity followed a normal proliferative response (mean percentage of lysis was 45%); in the aging group, only 7 out of 15 were able to respond to alloantigens with MLC and CML, with a mean percentage of lysis of 21%. Autologous serum did not modify the MLC and CML capacity in the aging group.

The presence of NCMC against the K562 cell line was explored; the aging group showed intact NCMC, when compared with 24 healthy blood donors (table II). Finally, in table III, the ability of 5 aging subjects to mount an ADCC response is shown and compared

Table I. Relationship between proliferative responses to alloantigens and CML capacity

Group	Mean MLC response ± 1 SD (RPI)	Mean CML response, % of lysis
Controls (n = 15)	0.99 ± 0.15* (0.81 – 1.20)	45
Aged (n = 15)	0.46 ± 0.15 (0.10 – 0.80)	21

* $p < 0.001$.

Table II. Natural cell-mediated cytotoxicity

	% of lysis
Controls (n = 24)	26.25 ± 9.10 (range: 10–44)
Aged (n = 11)	26.09 ± 3.3 (range: 22–31)

Table III. ADCC capacity of peripheral mononuclear cells

Aged	Age/Sex	K × 10^5
F.J.P.	114 M	0.68
B.B.M.	88 F	7.3
O.E.	90 M	7.3
U.S.	96 M	1.4
R.P.M.	88 M	1.7

K = Number of mononuclear cells capable of lysis of 50% of target cells; range of K values for 33 normal controls was 0.78–7.8 × 10^5 mononuclear cells.

Table IV. CMI Effector mechanisms in A 114-year-old subject

Parameters	Response
MLC	0.21
CML	0
NMCC	26%
ADCC	K = 0.68 × 10^5

to the values obtained in 33 controls; all aging subjects demonstrated an intact ADCC capacity.

Immunological Status of a 114-year-old Subject

F.J.P. is a healthy 114 year old; his immunological evaluation showed a significant reduction of proliferative response to alloantigens (RPI: 0.21), absent expression of CML (both in NHS and autologous serum), and an intact NCMC (26% of lysis) and ADCC capacity (K = 68 × 10^5 mononuclear cells; table IV).

Discussion

During the last few years, a considerable effort has been made to try and elucidate the nature and consequences of immunological changes in the process of aging.

In humans, the available evidence suggests a progressive decline of T cell functions in aging. Decrease in positive delayed-type hypersensitivity skin test, in the ability to become sensitized to DNCB, reduced numbers of E rosettes and low responses to T cell activators have been reported [6–8] supporting the notion of a T cell dysfunction in the aged. However, other T cell functions have received very little attention or have not been explored.

In the aged mouse, a similar hypothesis regarding a diminished T cell function has been advanced [7, 8, 10] with additional evidence in regards to impaired allogenic response [29] and in the ability to reject allogeneic or syngeneic tumor cells [6].

Recent investigations [9, 10, 30, 31] have indicated that ADCC capacity and macrophage reactions may be intact during aging.

We have further investigated several CMI effector mechanisms, in a prospective research protocol, carefully screening healthy aging humans, having the opportunity to evaluate within the selected group, a 114-year-old male currently in remarkably good health.

Our results offer concrete new evidence of a failure of T cell function during aging with a significative depressed proliferative response to alloantigens and an absent or diminished capacity to generate T killer cells and to express CML.

Nevertheless, the obtained results also indicated that during aging, nonspecific CMI effector mechanisms such as NCMC and ADCC are not only intact but operative. The use of the K562 cell line has been associated with the measurement of NCMC activity,

and *Stutman* et al. [32] have made similar observations in the aged mice. On the other hand, monocytes effector function may be evaluated using RhD+ human red blood cells sensitized with anti-D antisera [33, 34]. Further, recent evidence tend to suggest that whole monocyte population tend to increase with age [35].

The immunological status observed in the 114-year-old aged, further support the above-mentioned effector possibilities; while his in vitro T cell function was found to be abrogated, he showed adequate expression of NCMC and ADCC.

New efforts should be made in trying to elucidate whether the decline of T cell function during the process of aging also compromise T cell regulatory circuits which in turn would add valuable information on the physiology of the immune system.

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