Immunological Studies of Aging

DECREASED PRODUCTION OF AND RESPONSE TO T CELL GROWTH FACTOR BY LYMPHOCYTES FROM AGED HUMANS

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A B S T R A C T   Human lymphocytes from elderly and young donors were cultured with phytohemagglutinin (PHA) or concanavalin A. Cultures from old donors produced less T cell growth factor (TCGF) and incorporated less tritiated thymidine (^{3}H-Tdr) than did similar cultures from young donors in the presence of either mitogen. Furthermore, the response of lymphocytes from elderly donors to TCGF was impaired. Thus, PHA-activated T cells from aged donors showed no increase tritiated thymidine incorporation when incubated with exogenous human TCGF. In contrast, addition of exogenous human TCGF to PHA-activated peripheral blood leukocytes from younger individuals increased tritiated thymidine incorporation by 30–50%. The impaired response to TCGF was associated with decreased binding of TCGF by PHA-activated cells from old donors. TCGF production or responsiveness was not associated with the presence of "suppressor" activity in elderly T cell preparations. These studies suggest a possible molecular mechanism for the impaired proliferative response of elderly human T cells. These data lend support to the hypothesis that defects in the capacity to either produce or respond to TCGF may be a fundamental cause of immune deficiency.

INTRODUCTION

Lymphocytes from human donors >65 yr of age incorporate less thymidine when stimulated in culture with plant lectins than do lymphocytes from young individuals (1). The reason for the impaired proliferation of T cells from old people has not been defined. The relative and absolute number of T and B cells are not sig-nificantly altered with age although changes in the T-gamma and T-mu subpopulations have recently been reported (2). A detailed analysis (3) of the cellular basis of the impaired proliferative response of T cells from aged individuals suggested that two factors contributed to the proliferative defects: (*a*) a reduction in the number of mitogen-responsive T cells in the blood of elderly persons and (*b*) a failure of the mitogen responsive cells to proliferate normally in culture.

Recent studies have revealed that mitogen-induced proliferation of murine and human T lymphocytes in culture is driven by T cell growth factor (TCCF).[1] The proliferation of lymphocytes depends on their capacity to produce and respond to TCGF (4–6). Mitogens stimulate a subset of mature T cells (in collaboration with adherent mononuclear cells) to produce and release TCGF. A second subset of T lymphocytes rendered TCGF-responsive by the mitogen, binds TCGF and proliferatives (6, 7). The progeny of the TCGF-responsive T cells remain TCGF sensitive, allowing for their exponential in vitro culture simply by the continuous addition of TCGF (4, 8). Little evidence to date suggests that TCGF-producer T cells proliferate in response to TCGF.

In this report we present evidence that phytohemagglutinin (PHA)- and concanavalin A (Con A)-stimulated lymphocytes from elderly people produce less TCGF than do lymphocytes from young people cultured in an identical fashion. Furthermore, we found that lymphocytes from elderly people are less responsive to TCGF. These findings may explain, at least in part, the impaired proliferative response of lymphocytes from aged humans.

[1] *Abbreviations used in this paper*: Con A, concanavalin A; ^{3}H-Tdr, tritiated thymidine; PBL, peripheral blood leukocytes; PHA, phytohemagglutinin; TCGF, T cell growth factor.

METHODS

Assays for TCGF production and mitogen-induced proliferation. Mononuclear leukocytes (PBL) from elderly (67–81 yr) and young (21–30 yr) donors were isolated from peripheral blood samples by ficoll-hypaque density sedimentation using procedures previously described (4). Separated cells (1×10^6 cells/ml) were resuspended in Click's medium (supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum, 25 μM/ml HEPES buffer, 16 μM/ml NaHCO$_3$, 50 U/ml penicillin, 50 μg/ml streptomycin and 300 μg/ml fresh L-glutamine) and stimulated in replicate 200-μl cultures (No. 3096 flat-bottom microtiter plates, Costar Data Packaging, Cambridge, Mass.) with either (*a*) tissue culture medium, (*b*) Con A (10 μg/ml), (*c*) PHA (1% by volume, Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) or (*d*) purified human TCGF to which a mitogenic dose of PHA had been added. Human TCGF was produced from 48-h tissue culture medium conditioned by the PHA (1%) and alloantigen stimulation of PBL using procedures detailed elsewhere (4). Purified TCGF used in these studies was prepared by sequential 85% ammonium sulfate precipitation, Sephadex G-100 gel filtration chromatography, and diethylaminoethyl cellulose ion exchange chromatography using previously detailed methods (9, 10). After various time periods (see Results) 100 μl of supernatant fluid from stimulated cultures was removed and tested for the presence of TCGF activity. To assess cellular proliferation the identical cultures were pulsed for 4 h with tritiated thymidine (^{3}H-Tdr, 0.5 μCi/well, 20 Ci/mM, New England Nuclear, Boston, Mass.) and harvested onto glass fiber filter strips with the aid of a multiple automated sample harvester. Incorporated thymidine was counted by liquid scintillation and cellular proliferation was expressed in terms of mean cpm±1 SD of triplicate cultures. In experiments designed to determine whether poor TCGF production and proliferation exhibited by lymphocytes from elderly patients was the result of overabundance of suppressor cells, populations of both old and young cells were mixed 1:1 (final concentration 10^6 cells/ml) before testing.

TCGF microassay. Supernatant fluids harvested from cultures of both resting and stimulated PBL were tested for TCGF activity by their ability to sustain the proliferation of murine cytotoxic T cells harvested from long-term TCGF-dependent cultures (CTLL) (8). Using methods previously described (11), 3,000 CTLL cells were cultured for 24 h in 200-μl vol of supplemented Click's medium in the presence of a log$_2$ dilution series of a given putative TCGF-containing sample. After a subsequent 4-h pulse with ^{3}H-Tdr (0.5 μCi/well) it is routinely observed that only CTLL cells cultured in the presence of TCGF incorporate ^{3}H-Tdr in a dose-dependent manner (11). Cells cultured in the absence of TCGF are >95% trypan blue positive and incorporate only scintillant control levels of ^{3}H-Tdr. TCGF activity was quantified by means of probit analysis (11) of incorporation data which used as a 1-U/ml standard, a preparation of TCGF generated by the 48-h Con A stimulation of rat splenocytes (10^6/ml).

Quantitative absorption trials. The ability of a particular cell population to respond to a TCGF stimulation is dependent on the acquisition of cell surface TCGF receptors (5–7, 12, 13). Receptor number and/or affinity of lymphocyte populations can be determined either by their ability to bind radiolabeled purified TCGF (13) or by their ability to absorb purified TCGF from solution (6, 7, 12, 13).

Relative abilities of both old and young PHA-activated PBL to bind TCGF were examined in quantitative absorption trials. 95–100% viable blasts harvested from 48-h PHA-stimulated cultures (separated via ficoll-hypaque sedimentation) were washed extensively and adjusted to various cell concen-

trations ranging from 3×10^5 to 3×10^7 cells/ml in a given amount of purified human TCGF. After a 4-h incubation at either 37° or 4°C, cells were centrifuged (300 g for 10 min) and the supernatant fluid was tested for remnant TCGF activity. Absorption capacity determined at multiple cell concentrations was expressed in terms of the percentage of TCGF activity remaining after warm (37°) or cold (4°C) absorption.

RESULTS

TCGF production by lymphocytes from old and young donors. To determine whether the impaired T lymphocyte proliferation of elderly individuals was related to a defect in TCGF production, PBL from 10 old (67–81 yr) and 7 young (21–30 yr) donors were studied in five separate experiments. In each study, young and old lymphocytes were cultured in microwells with mitogenic doses of either Con A or PHA. After 48 h of culture half of the micro-well supernatant fluid (0.1 ml) was removed and the TCGF activity determined. Replicate cultures were incubated for an additional 24 h, after which they were pulsed for 4 h with ^{3}H-Tdr to determine mitogen-induced cellular proliferation. The results of these experiments are presented in Table I. Lymphocytes from elderly donors incorporated significantly less ($P < 0.001$) ^{3}H-Tdr than did lymphocytes from younger subjects. Similarly, supernatant fluids from elderly lymphocyte cultures incubated for 48 h with mitogen contained significantly lower activity of TCGF ($P < 0.001$) than did supernatant fluids from young lymphocyte cultures.

In each experiment the proliferation of lymphocytes from elderly subjects incubated with Con A or PHA after 24 or 48 h of culture was also significantly lower ($P < 0.001$) than that of young lymphocytes. Because TCGF is the driving force for T cell proliferation, we chose to show proliferation and TCGF concentration at the time points where each parameter reached its peak respective value (72 h for maximum ^{3}H-Tdr incorporation, 48 h culture supernatant fluids for maximal TCGF activity). Accordingly, although TCGF present in 72-h culture supernatant fluids of elderly PBL was significantly less than factor concentrations present in cultures of identically activated young PBL ($P < 0.001$), their numerical values were lower than those observed 24 h earlier. These findings agree with previous studies indicating that the TCGF activity in supernatant culture medium results from a delicate balance between the amount of factor produced and that consumed by activated T cells (5–7, 11, 12).

Response of lymphocytes from old and young subjects to exogenous TCGF. The close linear correlation between ^{3}H-Tdr incorporation and TCGF production by mitogen-activated PBL from old and young subjects (shown in Fig. 1) was consistent with previous studies which have investigated the relationships between

TABLE I
Summary of TCGF Production and ³H-Tdr Incorporation by PBL from Old and Young Donors

| | Culture stimulant | | | | | |
| | None | | Con A | | PHA | |
	³H-Tdr incorporation	TCGF activity	³H-Tdr incorporation	TCGF activity	³H-Tdr incorporation	TCGF activity
Aged donors (10)‡	0.6±0.3	0.0±0.0	36.2±10.1	2.1±0.9	73.8±21.9	3.4±1.6
Young donors (7)	0.5±0.3	0.0±0.0	81.6±17.2	5.3±0.9	116.2±11.5	8.9±2.2
Significance of difference between old and young PBL response	NS	NS	$P < 0.001$	$P < 0.001$	$P < 0.002$	$P < 0.001$

* Lymphocytes from aged or young donors were cultured in media, media containing 10 μg/ml Con A, or media containing 1% PHA. TCGF and ³H-Tdr incorporation was measured as described in Methods.

‡ Number of individuals tested in parentheses.

TCGF production and resultant activated T cell proliferation. For example, glucocorticoid hormone-induced suppression of mitogen-activated human T cell replication was previously shown to be directly proportional to the inhibition of TCGF production (14, 15). Based on the data detailed in Table I and Fig. 1 it appeared that, in part, the meager lymphoproliferative responses of elderly T cells was due to an impaired production of TCGF.
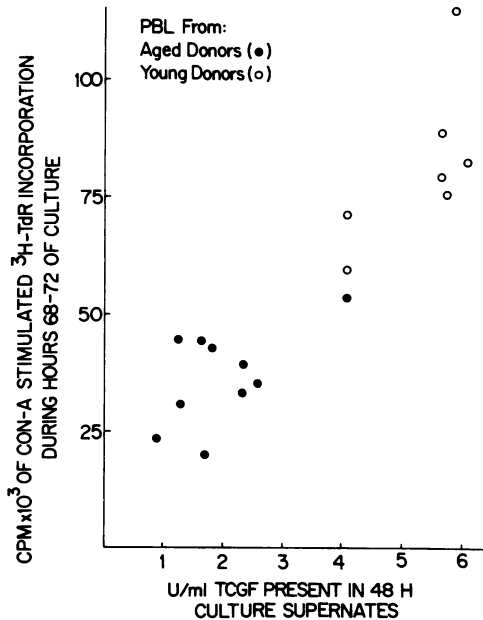
The capacity of exogenous TCGF to correct the proliferative defect of lymphocyte from old donors was tested. Lymphocytes were cultured with mitogenic concentrations of PHA either alone or with purified human TCGF (1 U/ml). After 3 d the cultures were pulsed with ³H-Tdr to assess cellular proliferation. As shown in Fig. 2, PHA-activated PBL from each of the seven young donors incorporated significantly more ³H-Tdr in

FIGURE 1 Lymphocytes from 7 young and 10 old individuals were cultured with Con A. The concentration of TCGF in the supernatant fluid after 48 h and ³H-Tdr incorporation after 72 h was measured. Correlation between concentration of TCGF Activity and ³H-Tdr incorporation is presented. PBL from aged donors (●); young donors (○).
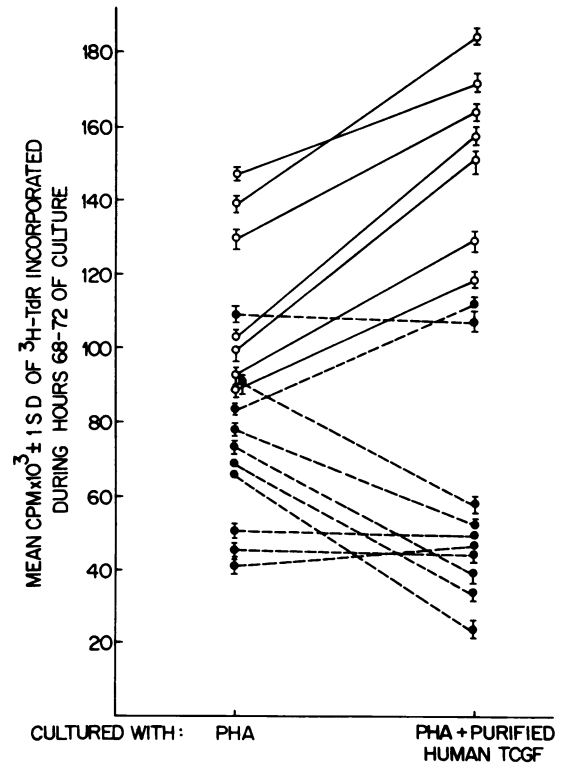

FIGURE 2 Lymphocytes from young and old individuals were cultured with PHA alone or with PHA and 1 U/ml of TCGF. ³H-Tdr incorporation by lymphocytes was measured after 68 h incubation. PBL from aged donors (●); young donors (○).

the presence of the exogenous TCGF as compared with cells stimulated solely with PHA. The mean increase in ^{3}H-Tdr incorporation observed was $36\pm6.8\%$. In contrast, PHA-stimulated PBL from only 2 of 10 elderly donors demonstrated increased ^{3}H-Tdr incorporation in the presence of exogenous TCGF. Lymphocytes from eight elderly donors showed either no change or a decrease in ^{3}H-Tdr incorporation after 72 h in culture in the presence of PHA and TCGF compared to identical cultures stimulated by PHA alone. As lymphocytes from old donors do not interact with TCGF normally (see below), it was possible that larger amounts of TCGF might be necessary to drive lymphocyte proliferations. Incubation with 5- to 20-fold larger amounts of TCGF did not augment but inhibited the proliferation of lymphocytes from old donors (Table II).

The ability of an activated T-cell population to proliferate in response to TCGF has previously been found to be related to the capacity of the activated cells to remove TCGF from culture medium (6, 7, 12). Antigen or mitogen-activated T cell populations can absorb TCGF after short incubations at 4°C or 37°C, whereas lymphocytes not previously activated do not absorb TCGF (6, 7, 12, 13, 16). The capacity of lymphocytes from old and young donors to bind TCGF was investigated. Viable PHA-induced lymphoblasts from elderly or young donors were collected by density sedimentation and adjusted to identical cell concentrations. The ability of these cells to absorb a known concentration of TCGF was tested during a 4-h incubation at either 37° or 4°C. As shown in Fig. 3, at either temperature, PHA blasts prepared from young donors were far more efficient in absorbing TCGF than were activated T cells prepared from elderly individuals. For example, at 37°C, PHA-activated lymphocytes from young donors (7.5×10^6 cells/ml) removed 88% of the TCGF activity present. Identical concentrations of PHA-activated lymphocytes from elderly donors absorbed

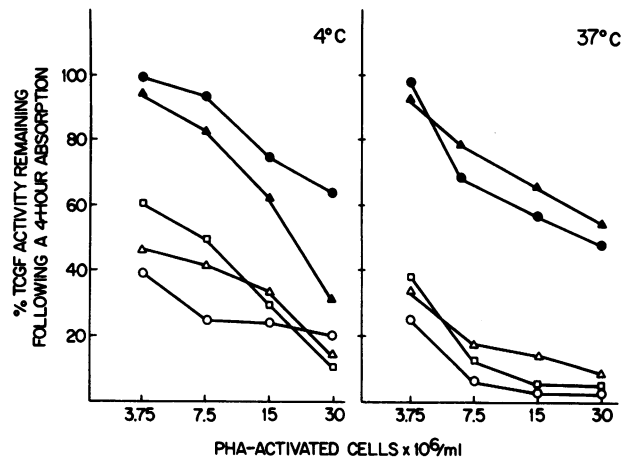
FIGURE 3 Varying numbers of PHA-induced lymphoblasts were incubated with 4 U/ml of TCGF for 4 h at 4° or 37°C. The percentage of TCGF remaining in the supernatant medium was determined after the incubation period. Closed symbols represent cultures with cells from two old subjects. Open symbols represent cultures from three young subjects.

only 20–40% of the TCGF present. These results suggested that lymphocytes from old individuals were impaired in their capacity to bind TCGF receptor sites on activated T cells from elderly donors or changes in the affinity of these receptors must await the development and application of radiolabeled TCGF binding assays.

Lymphocytes from elderly donors do not suppress TCGF production by PBL from young donors. It was possible that the low concentration of TCGF produced by mitogen-stimulated PBL from old as compared with young donors was due to a suppression of TCGF production in the old donor cultures. To investigate this possibility, TCGF production and ^{3}H-Tdr incorporation were measured in the PHA-stimulated lymphocyte cultures from either old or young persons and compared to responses observed in mixed cultures containing equal numbers of PBL from both old and young donors. As shown in Table III TCGF production and ^{3}H-Tdr-incorporation responses in mixed cultures (containing cells from both old and young donors) observed were those predicted from the TCGF activity found or ^{3}H-Tdr incorporated by either cell population cultured alone. For example, a 48-h culture supernatant fluid, conditioned by PHA-stimulated PBL from elderly subject 276, had 1.0 U/ml TCGF activity, whereas medium from identical cultures conditioned by PBL from young subject KAH contained 12.9 U/ml TCGF. Cultures containing equal numbers of cells (same final concentration of cells) from both donors yielded a supernatant medium containing 6.7 U/ml of TCGF activity. Culture of KAH PBL at ½ the normal concentration (5×10^5 cells/ml as opposed to 10^6 cells/ml) generated only half the quantity of TCGF (6.3 U/ml).

TABLE II
Response of PBL from Young or Old Donors to TCGF

Donor	^{3}H-Tdr Incorporation by lymphocytes in presence of PHA and TCGF			
	No TCGF	1 U/ml TCGF	5 U/ml TCGF	20 U/ml TCGF
Y-APB	125.6	165.7	198.0	210.7
Y-JC	108.6	146.2	166.2	171.2
Y-PCK	146.4	181.5	190.0	212.2
A-JH	72.3	61.1	59.0	55.4
A-CH	81.6	71.6	66.5	63.4
A-GTB	53.0	41.4	43.5	41.1

* Lymphocytes from young (Y) or aged (A) donors were incubated with 1% PHA for 72 h in the presence or absence of TCGF (cpm $\times$ 10^{-3}). ^{3}H-Tdr incorporation was measured between 68 and 72 h.

TABLE III
*PBL from Old Donors Do Not Suppress TCGF Production or ^{3}H-Tdr Incorporation Response of PBL from Young Donors**

PBL donors	TCGF activity	^{3}H-Tdr incorporation
	U/ml	cpm $\times$ 10^{-3}
A-276	1.0	67.7±1.9
A-76	3.5	88.1±6.2
Y-KAH	12.9	139.1±5.8
A-276 + Y-KAH	6.7	98.2±1.9
A-76 + Y-KAH	7.5	112.3±1.0
A-276 + A-76	2.6	79.1±0.8
Y-KAH‡	6.5	78.7±1.4

* Lymphocytes from aged (A) or young (Y) donors were incubated alone or with PHA. TCGF concentration in supernatant medium from cultures was measured after 48 h. ^{3}H-Tdr was measured after 68 h incubation. Except for one culture, the final concentration of cells was 10^6/ml medium.
‡ Final concentration of cells 5×10^5 cells/ml.

Thus, the impaired capacity of PBL from elderly donors to produce TCGF did not appear to be the result of suppression of TCGF production.

DISCUSSION

We have presented evidence that lymphocyte cultures from elderly individuals incubated with PHA or Con A do not have as much TCGF in the medium as similar cultures from young persons. Furthermore the augmentation of ^{3}H-Tdr incorporation seen when TCGF was added to lymphocyte cultures from young people was not found when TCGF was added to cultures from old donors. Finally, mitogen-activated lymphocytes from elderly donors did not absorb as much TCGF as did lymphocytes from young donors. The concentration of TCGF in culture medium depends upon both the production and the consumption of TCGF by activated T lymphocytes (5–7). Because stimulated elderly lymphocytes did not respond well to TCGF in culture and did not bind more TCGF than young stimulated T cells, we concluded that the concentration of TCGF present in culture medium conditioned by PHA or Con-A-activated elderly PBL reflects a true decrease in production of TCGF. The impaired production of TCGF by PBL from elderly donors did not reflect active "suppression" of TCGF production. TCGF activity in medium from mixed cultures (containing PHA-activated lymphocytes from both old and young donors) was that predicted from the mixture of TCGF containing supernatant fluids produced by lymphocytes from old and young subjects cultured separately.

These results may explain the cellular defects previously observed: (a) decreased numbers of mitogen-responsive lymphocytes and (b) impaired proliferation of such mitogen-responsive cells. The number of lymphocytes that proliferate when cultured with mitogen depends on the number of cells that can be stimulated by mitogen to express a responsiveness for TCGF, the driving force for T cell division. Presumably such responsiveness is mediated by acquisition of a normal number of high affinity cell surface TCGF receptors (5–7, 12, 13). Our results suggest that fewer T cells from elderly people acquire TCGF receptors of sufficient number or affinity to mediate a cellular response to TCGF. This may explain the presence of fewer mitogen-responsive lymphocytes in the blood of elderly people (3). There is no evidence that binding of PHA by lymphocytes from elderly mice is decreased with age (17). Preliminary studies in our laboratory have shown that the number and affinity of PHA receptors on T lymphocytes from young and old human donors are the same.

The impaired capacity of mitogen-responsive lymphocytes from old persons to divide in culture has recently been directly assessed in our laboratory using bromodeoxyuridine quenching of chromatid fluorescence (18). This technique permits the identification of cells dividing for the first, second, or third time in culture. Cultures of lymphocytes from elderly persons incubated for 72 h with PHA contained the same number of lymphocytes dividing from the first time but less than ½ the number of cells dividing a second time and less than ¼ the number of cells dividing for a third time found in cultures of lymphocytes from young persons. This was not because of a delayed response to PHA or prolonged cell cycle in cultures from aged humans. The decreased production of TCGF in cultures from old persons may be an important factor limiting the successive division of T lymphocytes.

At this time we can not identify the cellular or subcellular basis for the impairments in TCGF production. TCGF production depends on the activity of both monocytes and T lymphocytes (19). Because there is little evidence that monocyte function is comprised with age (20), the cellular basis of the impaired production of TCGF must await further study. The impaired production of TCGF may reflect the loss of a cell population or an intracellular defect in the biosynthetic pathway of TCGF.

ACKNOWLEDGMENTS

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