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Interleukin-2 Production and Lymphocyte Proliferation in Aged and Young Humans

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ABSTRACT

A study was made on the effect of aging on the production of Interleukin-2 (IL-2) and lymphocyte proliferation of human peripheral blood lymphocytes.

1) In comparison with young individuals, IL-2 production tended to decrease in aged individuals, while lymphocyte proliferation showed a significant decrease.

2) A significant correlation was observed between IL-2 production and lymphocyte proliferation in both the aged and young human populations.

3) IL-2 production showed a negative correlation with Leu-2a positive rate and a positive correlation with Leu-3a/Leu-2a ratio in aged individuals.

Since the report of Gillis et al[7] in 1977 on the establishment of antigen-specific IL-2 dependent mouse cytotoxic T cell line (CTLL-2), many IL-2 dependent cell lines have been established[6,18] and with the use of these cell lines the determination of IL-2 activity has been facilitated. Thereafter, IL-2 assay[5] has been primarily employed for the analysis of IL-2 properties, and at the same time it has also been utilized to analyze the kinetics of IL-2 in individual animals and humans with the aim of elucidating the true nature of various diseases.

Systemic lupus erythematosus (SLE), a representative autoimmune disease, can be given as a disease in which the analysis of IL-2 kinetics has been advanced to the greatest extent. As disease models in SLE, MRL/1 mouse, NZB/WF1 female mouse, and BXSB male mouse can be given, and many reports have been published on the kinetics of IL-2 of these mice[2,5,20]. In SLE model mouse, there is a depression in the productivity of IL-2 and responsiveness to IL-2 and in this depression of responsiveness the dysfunction of appearance of IL-2 receptor is involved. This phenomenon is well correlated to the severity of the disease. Many reports including that of Alcocer-Varela[1,12,14] have been published on human SLE, and a phenomenon almost identical to that observed in mouse has been seen in man.

It is well known that there is a depression in various immune functions even in healthy individuals with aging. It has been reported from the past that delayed type hypersensitivity (DTH)[15] and responsiveness of lymphocytes to mitogen and antigen[11,16,19] decrease with aging. In an attempt to analyze the mechanism of depression of immune function due to aging, studies have also been made on the changes in the kinetics of IL-2 due to aging and many reports have been published in the literature. Most of these reports are with regard to mouse or rat[4,10,13,17], but the report of Gillis et al[9] pertains to studies on man. In the present study, IL-2 production and lymphocyte proliferation were determined in aged and young humans and

the correlation between the two was examined with reference made to the relationship between IL-2 production and lymphocyte subsets.

SUBJECTS

Peripheral blood was drawn from 44 aged donors not having any disease composed of 28 males and 16 females with a mean age of 68.9 ± 8.0 years and from 32 young donors composed of 16 males and 16 females with a mean age of 22.6 ± 2.9 years.

METHODS

Preparation of peripheral blood lymphocytes (PBL)

PBL were separated from heparinized peripheral blood by Ficoll-Hypaque gradient separation.

IL-2 production

To RPMI 1640 medium (Gibco) to which was added 2.5% heat-inactivated fresh human AB serum and 1% phytohemagglutinin-M (PHA-M, Difco) PBL were suspended to a level of 1×10^6 cells/ml and after incubation for 24 hr at 37°C in 5% CO_2 culture supernatant was collected and then stored at −80°C.

IL-2 assay

Click's medium to which was added 2% heat-inactivated fetal bovine serum (Flow Laboratories) was used in making 8 stage serial $\log_2$ dilution of IL-2 samples. This was added to 96 well microplate each at 100 μl/well. To each well CTLL-2 cells were added to make 5×10^3 cells/well, followed by incubation for 24 hr at 37°C in 5% CO_2. Four hr prior to completion of incubation, 0.4 μCi ^{3}H-thymidine (^{3}H-TdR) was added and the radioactivity incorporated in CTLL-2 cells was determined.

Determination of units of IL-2 activity

As standard IL-2 (1 unit), 50% titer of the IL-2 sample obtained from one individual in the same manner was employed and by probit analysis[8] IL-2 activity of each sample was obtained as unit.

Lymphocyte proliferation assay

PBL were added to 96 well microplate at 1.25×10^5 cells/well, followed by incubation for 96 hr with 1% PHA (Welcome). 0.4 μ Ci ^{3}H-TdR was added 24 hr prior to completion of incubation and the radioactivity incorporated in PBL was determined as count per minute (cpm).

Detection of lymphocyte subsets by monoclonal antibodies

Fluorescence positive rate was obtained by indirect immunofluorescence method using anti-Leu-1, anti-Leu-2a, and anti-Leu-3a (Becton-Dickinson) as first antibody.

RESULTS

IL-2 production in aged and young subjects (Fig. 1)

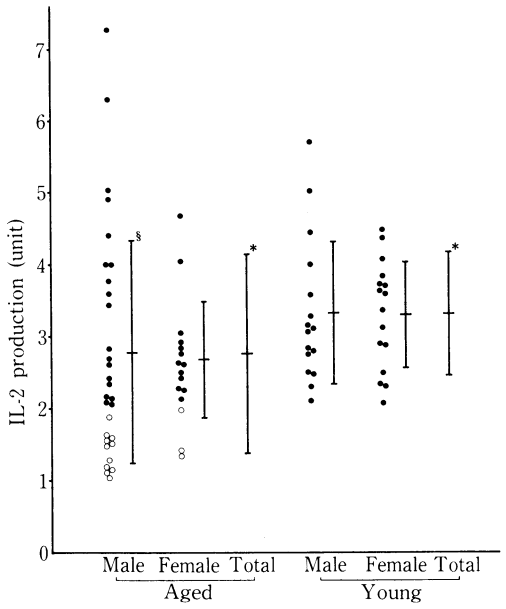

Fig. 1. IL-2 production in aged and young subjects. 44 aged donors (28 males, 16 females) are compared to 32 young donors (16 males, 16 females). Values more than 2 units are expressed as closed circles (●) and values less than 2 units are expressed as open circles (○).
§ Results are expressed as mean ± 1 S.D.
* $p<0.1$

IL-2 production was 2.76 ± 1.38 units in aged donors (male: 2.81 ± 1.61 units, female: 2.67 ± 0.81 units) and 3.31 ± 0.86 units in young donors (male: 3.32 ± 0.99 units, female: 3.30 ± 0.73 units), showing a tendency for aged donors to present a lower value than young donors ($p<0.1$), but the difference was not significant. However, as shown in the Fig.1, those with low production of IL-2 of less than 2 units were not observed in young donors, in aged donors they were observed in 14 out of 44

donors (31.8%) [11 out of 28 males (39.3%) and 3 out of 16 females (18.8%)], indicating that those with low production of IL-2 were frequent among aged donors. No sex difference was observed in IL-2 production.

Mitogenic response to PHA in aged and young subjects (Fig. 2)

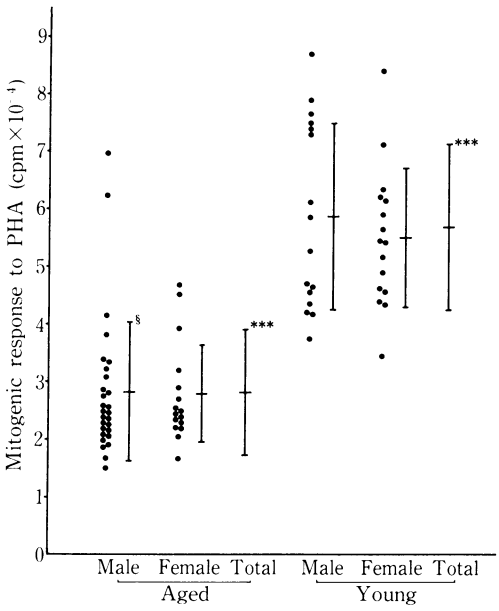

Fig. 2. Mitogenic response to PHA in aged and young subjects. 44 aged donors (28 males, 16 females) are compared to 32 young donors (16 males, 16 females).
§ Results are expressed as mean ± 1 S.D.
*** $p<0.001$

Mitogenic response to PHA was 2.81 ± 1.09 cpm × 10^{-4} (male: 2.83 ± 1.21 cpm × 10^{-4}, female: 2.79 ± 0.84 cpm × 10^{-4}) in aged donors and 5.69 ± 1.44 cpm × 10^{-4} (male: 5.87 ± 1.62 cpm × 10^{-4}, female: 5.50 ± 1.21 cpm × 10^{-4}) in young donors, indicating that the aged donors have a significantly low value ($p<0.001$) when compared with young donors. No sex difference could be observed in mitogenic response to PHA.

Correlation between IL-2 production and mitogenic response to PHA in aged and young subjects (Fig. 3)

A significant correlation was demonstrated between IL-2 production and mitogenic response to PHA in both the aged and young donors ($p<0.001$). In view of the distance between the respective regression lines and of their positional relationship, a depression is suggested in not only IL-2 production but also responsiveness to IL-2 in the aged donors when compared to the young donors.

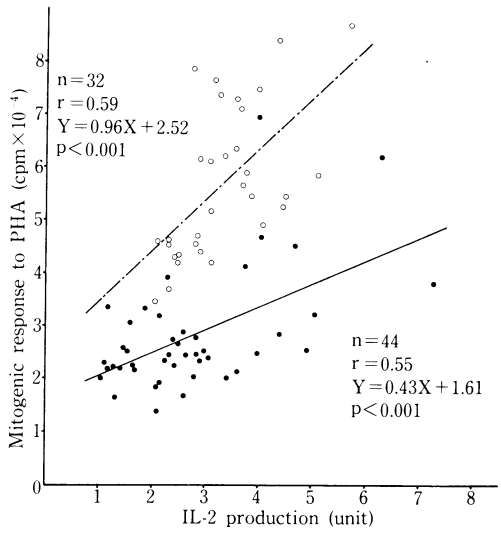

Fig. 3. Correlation between IL-2 production (X) and mitogenic response to PHA (Y). 44 aged donors (●, ——) are compared to 32 young donors (○, —•—).

Correlation between IL-2 production and Leu^+ cells in aged subjects (Fig. 4)

IL-2 production presented a significant negative correlation ($p<0.05$) to Leu-2a positive rate and a significant positive correlation ($p<0.01$) to Leu-3a/Leu-2a ratio in aged donors, but Leu-1 positive rate and leu-3a positive rate did not show a significant correlation. No correlation between IL-2 production and Leu^+ cells were observed in young subjects (data not shown).

DISCUSSION

A good number of reports have been published on the effect of aging on IL-2 productivity of lymphocytes and/or their responsiveness to IL-2 with the use of animals, and of these a brief summary will be made on the reports of four research groups. As experimental animals, Thoman et al[17] have employed C57BL/6J mice, Chang et al[4] C57BL/6, BALB/c and $BC3F_1$ mice, Joncourt et al[14] NMRI mice and Gilman

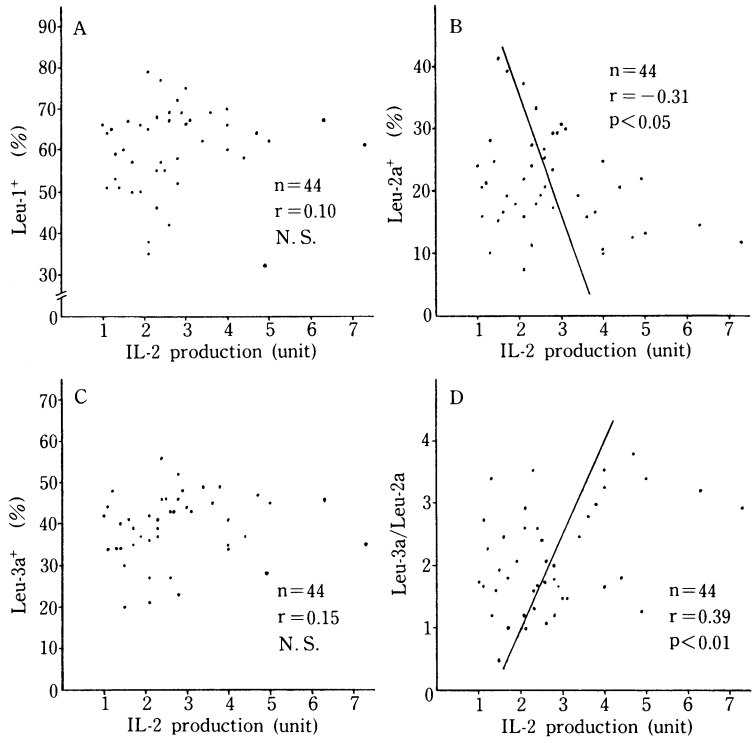

Fig. 4. Correlation between IL-2 production and Leu$^+$ cells in aged subjects. A: Leu-1$^+$ cells B: Leu-2a$^+$ cells C: Leu-3a$^+$ cells D: Leu-3a/Leu-2a

et al[10] Lewis rats. As for IL-2 production, when spleen cells were stimulated with lectin, Thoman et al, Chang et al, and Joncourt et al have observed a remarkable depression in aged animals, but Gilman et al could not demonstrate any significant difference. In general, in aged animals the proliferation of spleen cells by lectin stimulation is depressed. In experiments to determine whether the proliferation of spleen cells can be recovered by adding purified IL-2 together with lectin, Joncourt et al and Gilman et al have been unable to observe such recovery and have thus reported that in aged animals the responsiveness of spleen cells to IL-2 is depressed. To support this, Joncourt et al have observed in spleen cells of aged mice stimulated with lectin that high RNA-content G_1 cells (G_1b cells) which appear as a result of responding to IL-2 are selectively reduced and that this reduction does not recover by the addition of IL-2. Chang et al, however, have observed that by adding IL-2 simultaneously with lectin to spleen cells of aged mice, proliferation can be restored to the same level as that of young mice. Furthermore, with regard to IL-2 absorption by spleen cells stimulated by lectin, Chang et al and Gilman et al have reported the depression of IL-2 absorption in aged animals, indicating the dysfunction in the appearance on IL-2 receptors.

According to the report of Gillis et al[9] on their study of human PBL, there is with aging a remarkable depression in IL-2 production and also a depression in responsiveness to IL-2, and that disturbance in the appearance of IL-2 receptors is involved in this depression in responsiveness to IL-2. This result is almost identical to the data of the aforementioned experiments on animals and also similar to the data of SLE.

Many reports have been published that the mitogenic response to PHA of human PBL is depressed with aging[11,16,19], and that the present authors have observed the same results in their studies. Furthermore, the authors' data on IL-2 production have shown a decreasing tendency with aging, but did not show remarkable depression as observed in the report of Gillis et

al. However, it was observed that individuals with low IL-2 production increase with aging. In examining the correlation between IL-2 production and mitogenic response to PHA, a significant correlation was observed in both the aged donors and the young donors, but the respective regression lines are separated from each other. The depression in mitogenic response to PHA in aged individuals is strongly influenced by the depression of IL-2 production, but at the same time it is suggested that depression in mitogenic response is involved in the depression of responsiveness to IL-2. In examining the correlation between IL-2 production and lymphocyte subsets, IL-2 production has a negative correlation with Leu-2a positive rate and a positive correlation with Leu-3a/Leu-2a ratio, suggesting the involvement of suppressor T cells in IL-2 production. However, inasmuch as IL-2 production is not suppressed even when PBL of aged donors are added to PBL of young donors, Gillis et al[9] have indirectly negated the involvement of suppressor T cells.

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