REACTION OF PERIPHERAL BLOOD LYMPHOCYTES TO MITOGENS FROM THE AGE ASPECT

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The reaction of peripheral blood lymphocytes from healthy blood donors aged from 19 to 49 years to the mitogens phytohemagglutinin, concanavalin A (con A), and rabbit serum against human thymocytes (ATS) was investigated. A significant decrease in the proliferative response of the lymphocytes to con A was found in subjects over 30 years old. Significant negative correlation was found between the indices of the proliferative response and the age of the donors during stimulation by con A for the whole group of subjects, but for stimulation by ATS only for subjects aged 30-49 years. Analysis of the intensity of incorporation of [^{3}H]-thymidine shows a decrease with age in the proportion of cells with intensively labeled nuclei and an increase in the number of cells with weak labeling of the nucleus both with a decrease in the indices of the proliferative response with age and also when no significant differences were found in these indices.

KEY WORDS: lymphocyte; mitogens; age; blast transformation.

The blast transformation reaction of peripheral blood lymphocytes (PBL) in culture in vitro on addition of mitogens is widely used for evaluating the state of the T-cell system of immunity. Among the factors influencing this reaction, the age of the subjects is nowadays being mentioned increasingly often [1, 11].

The object of this investigation was to compare some features of the proliferative response of PBL of healthy subjects of different ages to mitogens: phytohemagglutinin (PHA), concanavalin A (con A), and rabbit serum against human thymocytes (ATS). Three stimulators were used because they have different binding sites on the cell surface and also some differences in their mechanism of action [8-10].

EXPERIMENTAL METHOD

PBL from 42 healthy blood donors (23 men and 19 women) aged from 19 to 49 years were tested. The lympocytes were obtained by allowing heparinized venous blood to stand for 80 min at 37°C. The plasma with the cells (top layer) was transferred to test tubes and centrifuged for 10 min at 1000 rpm. The supernatant was drawn off with a Pasteur pipet. The residue was resuspended in Eagle's medium containing 5% heated embryonic calf serum (ECS) to a final concentration of 1 million cells/ml. The mitogens were used in doses of: PHA 50 μg/ml, con A 25 μg/ml, and ATS 20% of the total volume of culture medium. The concentration of ECS in the flasks containing the phytomitogen (PHA and con A) was adjusted to 20%. Preparations of PBL cultured in medium containing 20% ECS without addition of the mitogens or containing 20% normal rabbit serum (NRS) were used as the controls. NRS and ATS were obtained by the method described earlier [5]. Each culture was duplicated. Cultivation took place in flasks at 37°C for 70-72 h. [^{3}H]Thymidine was added to the flasks 1 h before the end of the incubation time in a concentration of 2 μCi/ml. The cultures were fixed and the specimens treated by the method described earlier [3].

The number of labeled cells in 1000 cells counted in the films was determined and the index expressed in percent. The proliferative response of the PBL to each mitogen was expressed as the difference between the percentage of labeled cells in cultures stimulated by the mitogen and the percentage of labeled cells in the corresponding controls. The result was determined as the arithmetic mean of two identical cultures.

Depending on the intensity of label in the nucleus the following classes of cells were distinguished: 1) cells with weakly labeled nuclei (with an intensity of labeling of between 4 and 30 grains of silver per nucleus),

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2) cells with an average intensity of labeling of the nucleus (from 30 to 100 grains per nucleus), and 3) cells with intensively labeled nuclei (over 100 grains per nucleus).

EXPERIMENTAL RESULTS

The number of labeled cells in the control films varied from 0 to 2%, with a mean value of 0.38% for specimens with ECS and 0.57% for those with NRS ($P > 0.05$). Films of PBL treated with mitogens had the typical appearance of stimulated cultures, in which the proliferative response was much greater than that in the control cultures ($P < 0.001$).

Analysis of the results obtained by stimulation of the lymphocytes with mitogens was carried out both for the group of subjects as a whole and for the age subgroups: 19-29, 30-39, and 40-49 years (Table 1).

The response of the PBL of the whole group of subjects to con A under these experimental conditions was significantly lower on average than that to PHA or ATS ($P < 0.001$). The index of the proliferative response to con A varied from 8.4 to 37.2%, mean 23.33%. Negative correlation was found between the number of labeled cells in the films and the subjects' age ($r = -0.48$, $n = 40$, $P < 0.01$). Analysis of the mean values of the proliferative response for the age subgroups (Table 1) showed a significant decrease in the response to con A in subjects over 30 years of age ($P < 0.05$).

The number of labeled cells in films of PBL from the same donors when stimulated by ATS varied from 22.9 to 53.1% (mean 36.73%). The mean values of the proliferative response for the various age subgroups did not differ significantly (Table 1). No correlation was found with age for the whole group of subjects. However, in subjects over 30 years of age negative correlation was found between the number of labeled cells in the films with ATS and the subjects' age ($r = -0.59$, $n = 14$, $P < 0.05$).

The percentage of labeled cells in preparations treated with PHA varied from 22.9 to 49.0, with a mean value of 34.74, and on the whole it did not correlate with the subjects' age. Analysis of the results for the age subgroups revealed a decrease, which was not significant, in the proliferative response in subjects aged 40-49 years (Table 1); negative correlation was found in this same age subgroup between the index of the proliferative response and the subjects' age ($r = -064$, $n = 7$, $P > 0.05$).

The ability of PBL to react to con A thus exhibits age changes much sooner than their reaction to the other two mitogens. This can be explained by a whole series of causes, including a decrease with age in the number of younger peripheral T cells reacting mainly to con A [4] or the appearance of a functional defect in these cells. Thymosine, a hormone from the thymus, which is considered to play the role of one of the factors controlling proliferation and differentiation of T cells [7], may play a not unimportant role in this case, for thymosine production is known to diminish appreciably starting from the age of 30 years [7]. Probably if the disturbance affects the less mature subpopulation of T lymphocytes to a greater degree, it will be reflected at the level of the proliferative response to con A (mostly) and to ATS (less so), for ATS, as a stimulator of the whole thymus-dependent population of PBL, stimulates both the younger (reacting mainly to con A) and the mature (reacting mainly to PHA [4]) T lymphocytes.

The results of these experiments, and also those cited in the literature [1, 11], thus indicate that changes with age in the subpopulation of PBL reacting to PHA takes place gradually and reach a significant level in persons over 49 years of age.

TABLE 1. Percentage of Labeled Cells in Cultures of Peripheral Blood Lymphocytes Stimulated by Mitogens, for Age Subgroups ($M \pm m$)

Age of subjects, years	No. tested	Mitogens		
		con A	ATS	PHA
19—29 (1)	26	26,22±1,21	37,50±1,50	35,46±1,25
		$P_{1-3}<0,05$	$P_{1-2}>0,05$	$P_{1-2}>0,05$
30—39 (2)	7	18,68±3,24	37,01±3,38	35,33±2,67
		$P_{2-3}>0,05$	$P_{2-3}>0,05$	$P_{2-3}>0,05$
40—49 (3)	7	16,96±2,36	33,61±3,31	31,31±1,82
		$P_{1-3}<0,05$	$P_{1-3}>0,05$	$P_{1-3}>0,05$

The results of the study of the intensity of nuclear labeling showed that the proportion of intensively labeled nuclei falls significantly with age ($P < 0.05$ for all mitogens) whereas the number of weakly labeled nuclei rises correspondingly (for lymphocytes stimulated by PHA and con A $P < 0.01$, for lymphocytes stimulated by ATS $P < 0.05$), whether the indices of the proliferative response decrease with age or no significant differences in these indices are found. Since the incorporation of [^{3}H]thymidine is known to reflect DNA synthesis by the cell in the S phase of the cell cycle [2], the results described above can be interpreted as a decrease with age in the proportion of cells intensively synthesizing DNA under the influence of the mitogens on the third day of culture. Considering these findings, and also those of Barbaruk [1], who observed an earlier decrease with age in the number of mitoses than in the number of blast cells in cultures stimulated by PHA, and the reported shift of the maximum of the response to phytomitogens in cultures of PBL from old people compared with young from the third to the fifth day [6], it can be postulated that with age there is a reduction in the proportion of cells among the PBL that "react most quickly" to stimulation by mitogens. At the same time, the relative proportion of cells "reacting slowly" to mitogens increases. Under these circumstances qualitative changes in reactivity of the PBL to mitogens evidently precede the decrease in magnitude of the proliferative response.

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BIORHYTHMS OF HISTOPHYSIOLOGICAL INDICES OF THE THYROID GLAND AT DIFFERENT AGES

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Rhythmic changes in several histophysiological indices of the thyroid gland were studied in 144 A/He mice of three age groups: young (sexually immature), sexually mature, and aging. The rhythm of each index was found to be multicomponent in character, including a circadian component and also infradian and ultradian fluctuations. The leading role of the circadian component in the formation of biorhythms of the organ was established, whereas their adjustment during ontogeny is due mainly to a reduction in the power of the ultradian components.

KEY WORDS: histophysiological indices; biological rhythms; thyroid gland; age changes.

Numerous biochemical, radioisotopic and morphological investigation have demonstrated the cyclic character of thyroid gland activity. At the same time, there is indirect evidence of the multicomponent composition of biorhythms, as is shown in particular by the irregular shape of the curves and their several maxima [6, 9, 11], although the authors just cited did not mention this. No spectral analysis of the rhythms has been carried out. The writers have studied the spectral composition of rhythms of the structural elements of the thyroid

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