EFFECT OF A RISE OF TEMPERATURE ON MITOGENIC LYMPHOKINE PRODUCTION BY HUMAN LYMPHOCYTES *in vitro*

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A rise of temperature affects the immune response *in vivo* [6] and also potentiates the response of lymphoid cells to stimulation by lectins, antigens, and allogeneic cells *in vitro* [1, 4, 5, 8, 10]. Increased production of leukocyte migration inhibition factor when the temperature of lymphocytes in culture is raised has been described [9]. Since stimulation of lymphocytes by antigens or mitogens takes place with the participation of soluble mediators giving rise to proliferation, it was decided to study the effect of a rise of temperature on production of mitogenic lymphokines (ML) in cultures of lymphocytes stimulated by phytohemagglutinin (PHA).

EXPERIMENTAL METHOD

Lymphocytes were isolated from heparinized blood from healthy blood donors on a Ficoll–Verografin density gradient (1.077 g/ml) [7]. The cells were washed twice with medium 199 containing 10 mM HEPES buffer (from Microbiological Associates), and were incubated in a concentration of 3 million cells/ml with PHA (PHA-P, from Difco, 30 μg/ml) for 40 min at 37°C [2]. The stimulated lymphocytes were washed once to remove PHA and cultured in serum-free MEM medium (Dulbecco MEM, from Flow Laboratories), containing antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin) at 37-42°C for 2-3 days in an atmosphere of 7% CO_2. At the end of culture the cells were sedimented by centrifugation, resuspended in fresh nutrient medium, and their viability was tested (the absolute number of living cells not incorporating trypan blue in 1 mm^3 medium was counted) and DNA synthesis was determined. Supernatants containing ML were sterilized by filtration through millipore filters (0.2 μ, from Millipore Co.) and investigated in test cultures of allogeneic lymphocytes after dilution of the supernatants in a ratio of 1:4 with fresh medium containing 10% heat-inactivated autologous serum. The concentration of cells in the test cultures was 0.6-0.8 million in 1 ml. To inactivate the trace quantities of PHA, the globulin fraction of rabbit antiserum against PHA [2] was added to the test supernatants. One determination of ML activity included the study of four parallel test cultures. DNA synthesis was investigated with respect to incorporation of ^3H-thymidine per culture (2 μCi/ml, 4.1 Ci/mmole, 4 h at 37°C). The labeled cells were washed with 0.85% NaCl, incubated for 24 h in 5% TCA at 5°C, and precipitated on millipore filters. Radioactivity was counted on a Packard 2450 liquid beta-spectrometer. ML activity was expressed as a stimulation index, the quotient obtained by dividing the level of DNA synthesis in the test cultures in the presence of the test supernatant by the level of DNA synthesis in the control test cultures [3].

EXPERIMENTAL RESULTS

The number of surviving cells at the end of culture remained constant when the temperature was raised from 37 to 41°C, but a further rise of temperature to 42°C led to a sharp fall in viability of the cells (Fig. 1). With a rise of temperature to 39-41°C DNA synthesis in cultures of PHA-stimulated lymphocytes increased significantly, but with a rise of temperature to 42°C DNA synthesis fell sharply (Table 1), in agreement with data obtained previously [5, 8].

An increase in the temperature of culture significantly affected ML production. At 39-

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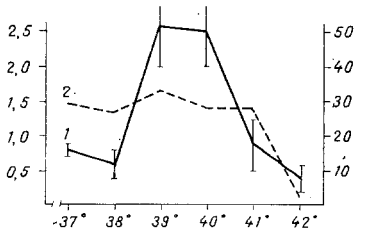

Fig. 1

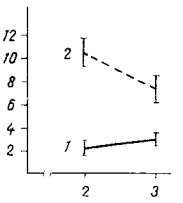

Fig. 2

Fig. 1. Production of mitogenic lymphokines (1) and viability of cells (2) during culture of PHA-stimulated lymphocytes at different temperatures. Abscissa, temperature (in °C); ordinate: on left — number of surviving cells in culture (in millions/ml); on right — index of stimulation.

Fig. 2. Dynamics of mitogenic lymphokine production in cultures of PHA-stimulated lymphocytes at 37 and 40°C. 1) Activity of ML obtained at 37°C; 2) activity of ML obtained at 40°C. Abscissa, time of culture (in days); ordinate, index of stimulation.

TABLE 1. Effect of Temperature of Culture on DNA Synthesis and Production of Mitogenic Lymphokines during Stimulation of Human Lymphocytes with PHA (M ± m)

Expt. No.	Temp. of culture, °C	DNA synthesis, as incorp. of ^{3}H-thymidine, cpm (n = 3)	Activity of mitogenic lymphokines (index of stimulation, n = 4)
1	37	2 072±688	1,2±0,1
	38	4 143±503	3,2±0,1
	39	4 400±444	3,8±0,5
	40	5 785±617	3,6±0,9
	41	5 046±567	1,0±0,3
	42	96±4	1,4±0,3
2	37	16 215±822	2,3±0,4
	40	19 425±286	6,0±0,9
	42	122±10	1,6±0,1
3	37	—	16,0±2,1
	38	—	11,8±4,1
	39	—	51,6±12,4
	40	—	50,4±10,6
	41	—	17,4±7,9
	42	—	8,5±4,7

40°C the greatest increase in ML production was observed (by 2-4 times, see Fig. 1 and Table 1). A further rise in the temperature of culture to 41-42°C sharply depressed the mitogenic activity of the supernatants. Raising the temperature also altered the dynamics of ML secretion. At 37°C the maximum of accumulation of ML in cultures of PHA-stimulated lymphocytes on serum-free nutrient media is observed on the 3rd day [2]. With a rise of temperature to 40°C the maximum of ML accumulation was shifted to the 2nd day, i.e., production of ML was "accelerated" (Fig. 2).

The increase in ML production during a rise of temperature described above may be made use of experimentally in order to obtain highly active ML. It can be tentatively suggested that stimulation of ML secretion is one cause of the altered reactivity of lymphoid cells *in vivo* and *in vitro* during a rise of temperature.

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EFFECT OF IMMUNIZATION ON THYROID FUNCTION AND THYROXINE BINDING BY RAT ORGANS

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Besides causing immunogenesis, an antigen which is an extraordinary factor for the living organism [3, 8], gives rise to a marked nonspecific neuroendocrine reaction. The concurrent development of these two processes in the course of evolution has become closely connected, but neurohumoral regulation of immune processes has been studied extremely inadequately [7,8]. The contradictory data obtained by investigation of the effect of administration of hormones in different periods of the immune response on the dynamics of antibody formation and on intercellular cooperation [2, 6, 9] demand a careful study of the hormonal background. A theory of neurohormonal regulation of antibody synthesis, in which the leading role is ascribed to the hypothalamo-hypophyseo-adrenocortical system, has been developed in Zdrodovskii's [6] and Rappoport's [10] laboratories. Relations between the functions of other neuroendocrine systems and, in particular, the thyroid system, and antibody formation have received much less study by present standards. There have been virtually no observations on thyroid function during the inductive phase of immunogenesis [2, 6, 9].

The object of the present investigation was to make a detailed study of thyroid function in the productive and, in particular, in the inductive phase, which determines all the subsequent events of immunogenesis [3, 7].

EXPERIMENTAL METHOD

Experiments were carried out on 220 male albino rats weighing 150-180 g, divided into two groups. The animals of group 1 were immunized simultaneously with formol typhoid vaccine (suspension of 1 billion cells). The animals of group 2 (control) were injected with the same volume (0.2 ml) of 0.2% formalized physiological saline. Assimilation of ^{131}I was determined 1, 2, 3, 4, 7, 10, 15, 20, and 25 days after injection of the antigen, after subcutaneous injection of 0.5 μCi of the isotope, with a DCU-61 instrument. The level of protein-bound ^{131}I (PBI) in the blood serum and the coefficient of conversion of radioactive iodine were determined [12] on a Gamma (Hungary) instrument. On the 1st, 4th, and 20th days after the antigenic stimulus the content of cyclic AMP in the thyroid gland tissue was determined by means of a Cyclic AMP Assay Kit (Radiochemical Centre, Amersham, England) on a Mark III (The Netherlands) system. The total thyroxine (T_4) and triiodothyronine (T_3) in the blood serum were determined by means of Res-O-Mat T_4 and Ria-Mat T_3 kits (Byk Mallinckrodt, West Germany) on a Gamma 400 instrument (West Germany). The content of ^{131}I-thyroxine (Polish Institute for Nuclear Research), injected subcutaneously in a dose of 1 μCi/

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