DEPENDENCE OF ANTIBODY FORMATION IN LYMPHOCYTE CULTURES ON INCUBATION TEMPERATURE

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The increase in the number of antibody-forming cells (AFC) and in the incorporation of [^{3}H]thymidine with a rise in the incubation temperature from 2 to 37°C was studied in cultures of spleen cells from immunized and nonimmunized C57BL/6 mice. An exponential increase in the number of AFC was found with an increase in temperature, and the existence of a "critical" temperature was demonstrated, above which the rate of increase in the number of AFC rises sharply. The curves showing increased incorporation of [^{3}H]thymidine with an increase in temperature in some cases diverged from the curves of the increase in the number of AFC. Culture of immune cells at low temperatures leads to the accumulation of factors stimulating AFC formation in the medium.

KEY WORDS: antibody formation in culture; antibody-forming cells; temperature

The role of temperature in the development of antibody formation has received very little study [3-5]. As the temperature falls, not only the rate of synthesis of these proteins, but also the rate of their "assembly" and secretion has been shown to decrease.

The object of this investigation was to study the effect of temperature on induction of antibody formation and subsequent proliferation of antibody-forming cells (AFC).

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6 mice weighing 18-20 g (from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR). The animals were immunized intravenously with sheep's red blood cells (SRBC) in a dose of 500×10^6 cells per mouse. The method of Mishell and Dutton [9], as modified by Click et al. [6] and by Gurvich et al. [2], was used for the in vitro experiments. For immunization of the culture of mouse spleen cells SRBC were used in a dose of 5×10^6 cells per culture or a water-soluble SRBC antigen (WSA-SRBC) [10] in a dose of 50 mg per culture.

[^{3}H]Thymidine with a specific activity of 1 Ci/mmole (from CEA-France Service, France) or 11 Ci/mmole (from "Izotop," USSR) was used. The label was added for 24 and 96 h in concentrations of 1 and 0.1 μCi/ml respectively. Incorporation of [^{3}H]thymidine was determined by the Mark II (Nuclear Chicago, USA) counter.

In all cases the content of IgM of AFC was determined by Jerne's method [8] and the number of living and dead cells was counted by staining with eosin and trypan blue.

EXPERIMENTAL RESULTS

The object of the experiments of series I was to determine how induction and the development of antibody formation take place in vitro at different temperatures. On incubation of a suspension of spleen cells of an unimmunized mouse, no appreciable increase in the number of AFC was observed in the course of 4 days at 2-17°C. A further increase in the incubation temperature led to an increasingly rapid increase in the number of AFC (Fig. 1). This process was well marked at temperatures of over 20°C and, in particular, of over 33°C. Between 28 and 33°C the number of AFC doubled with a rise in temperature of 2.7°C*, whereas between 33 and 37°C it doubled with a rise in temperature of only 1.6°C.

*Later the rise in temperature which led to doubling of the number of AFC and of incorporation of [^{3}H]thymidine will be designated as ΔT_2.

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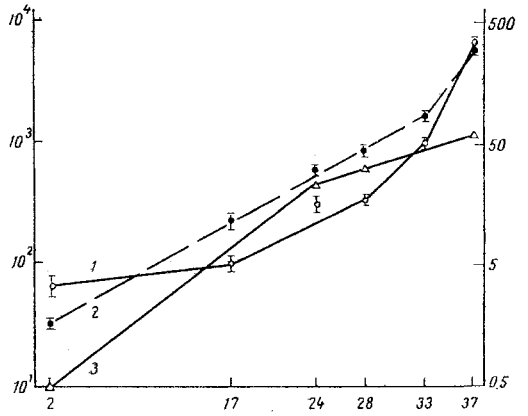

Fig. 1. Effect of temperature on number of AFC and rate of incorporation of [^{3}H]-thymidine during primary immune response in vitro. Number of AFC (1) and incorporation of [^{3}H]thymidine (2) during incubation of culture for 96 h. Increase in number of AFC (3) during 24 h after change in temperature of culture from 37°C to 2, 24, and 28°C. Abscissa, incubation temperature (in °C); ordinate: on left—incorporation of [^{3}H]thymidine per 10^6 living cells (in cpm), on right—number of AFC per 10^6 living cells.

A rather different pattern was found when the incorporation of [^{3}H]thymidine into these cells was studied. Up to 33°C incorporation increased exponentially with a rise of temperature, and was doubled by an increase in temperature of 5.7°C. After 33°C, incorporation began to intensify twice as quickly.

In these experiments the cells were incubated at different temperatures both in the period of induction of antibody formation and in the period of proliferation of the AFC clone formed. To analyze the effect of temperature on each of these phases separately, the cells were first cultured at one temperature and their culture was then continued at another temperature. The results of one such experiment in which the samples, after incubation for 3 days at 37°C, were incubated for a further 24 h at 2, 24, 28, and 37°C, are given in Fig. 1. With a rise in temperature to 24°C the number of AFC increased exponentially and ΔT_2 was 4°C, whereas above 24°C the rate of their increase changed and ΔT_2 became 10°C.

In the experiments of series II the effect of temperature was studied on culture of cells taken from a previously immunized animal. This system appeared to be useful because in it the number of AFC rises rapidly [1, 2, 7]. During incubation for 4 days, elevation of the temperature from 2 to 33°C was accompanied by an almost exponential increase in the number of AFC formed, with ΔT_2 of 12-9°C, whereas at the higher temperature the number of AFC began to rise more rapidly: ΔT_2 became 3.5°C (Fig. 2). The rate of incorporation of [^{3}H]thymidine increased almost uniformly with a rise in temperature over the whole range studied ($\Delta T_2 = 4$-5°C).

In a series of experiments the temperature was changed a short time after culture. For example, the cells were incubated for 3 days at 37°C, and then for a further 24 h at 2, 29, 33, and 37°C. It was found that the number of AFC in the system described above remained almost unchanged during incubation between 2 and 29°C. With a further increase in temperature the number of AFC began to increase more and more rapidly: $\Delta T_2 =$ 2.2°C (Fig. 2). Incorporation of [^{3}H]thymidine followed a rather different pattern after a change in temperature of the culture from 37°C to the other values. Whereas in the interval between 2 and 29°C the number of AFC was unchanged, incorporation of the label was increased more than sixfold. With a further increase in temperature the rate of incorporation of [^{3}H]thymidine began to rise, as also did the rate of increase in the number of AFC. The increase in the number of AFC followed a different course in samples incubated the whole time at a certain temperature and in samples previously incubated for 3 days at 37°C (Fig. 2). In the first case the number of AFC rose steadily with an increase in temperature from 2 to 29°C, but in the second case it remained at a constant level. It was accordingly postulated that a certain factor stimulating the increase in number of AFC accumulates in the culture fluid of cells grown at temperatures below 29°C. To test this hypothesis, a suspension of immune cells was preincubated for 3 days at 2°C, and then incubated at 37°C. Under these conditions, the increase in the number of AFC was found to begin at a faster rate than the increase in their number during incubation at 37°C from the very beginning. As a result of this, the maximal number of AFC was greater than normal (Fig. 3). In three cases the number of AFC formed during the 24 h after the change of temperature was the same as that formed during incubation for 2-3 days at 37°C from the very beginning.

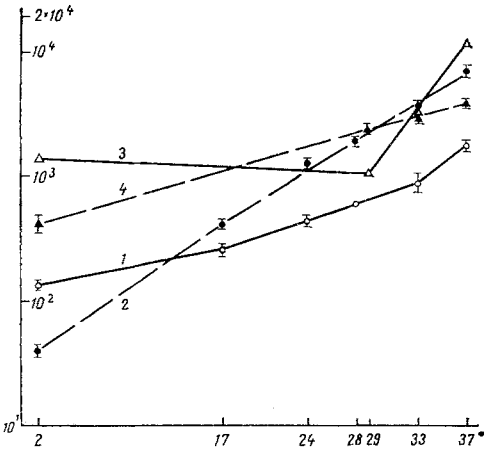

Fig. 2

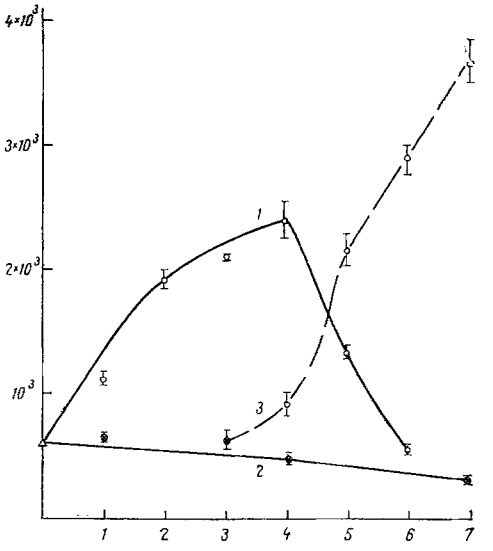

Fig. 3

Fig. 2. Effect of temperature on number of AFC and incorporation of [^{3}H]thymidine during culture of immune lymphocytes taken from spleens 4 days after injection of antigen into animal. Number of AFC (1) and incorporation of [^{3}H]thymidine (2) during incubation for 96 h. Increase in number of AFC (3) and incorporation of [^{3}H]thymidine (4) during 24 h after change in temperature of culture from 37°C to 2, 29, and 33°C. Abscissa, incubation temperature (in °C); ordinate, number of AFC and incorporation of [^{3}H]thymidine per 10^6 living cells.

Fig. 3. Comparison of increase in number of AFC during incubation of culture of immune lymphocytes from very beginning at 37°C (1) and at 2°C (2) and after change in incubation temperature on third day from 2 to 37°C (3). Abscissa, incubation time (in days); ordinate, number of AFC per 10^6 living cells.

Accumulation of the stimulating factor during incubation of cells from the immunized animal also was confirmed in experiments with a change of medium. The medium in which these cells were preincubated at 2°C was found to support the viability and antibody formation better than fresh medium in cultures transferred after 3 days from incubation at 2°C to 37°C.

The stimulating factor evidently did not accumulate in the cell culture from the unimmunized animal. This is shown by the fact that the number of AFC in these cell cultures also increased at low temperatures (29, 24, and 17°C) both when the samples were incubated from the very beginning at different temperatures and after preincubation for 3 days at 37°C, followed by a change to the other temperatures. This was confirmed in experiments with preincubation of a culture of cells from a normal animal at 2°C for 3 days followed by their transfer to 37°C. In that case the number of AFC increased just as in the case of incubation of the cells from the very beginning at 37°C, and after 4 days almost equal numbers of AFC were found in the two suspensions.

The study of the effect of temperature on the increase in number of AFC thus revealed the existence of a "critical" temperature (29-33°C), above which the intensity of this process rises rapidly. In culture of cells from an immunized animal the curves of the change in number of AFC and the curves of the change in incorporation of radioactive label into DNA in some cases diverged. This indirectly confirms that the increase in the number of AFC was due to two independent processes: proliferation of an existing clone of AFC and the involvement of cells which previously had not formed antibodies in the process of antibody formation. Culture of immune cells at low temperatures evidently leads to the accumulation of certain factors which stimulate the formation of AFC.

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ENTEROTOXIC ACTIVITY OF LIVING CULTURES OF *Shigella sonnei* AND ENTEROTOXIN FORMATION *in vivo*

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The overwhelming majority of virulent strains of Shigella sonnei caused the accumulation of fluid in the lumen of an isolated segment of rabbit small intestine; the fluid contained large quantities of mucus and sometimes blood; the mucous membrane of the segment was hyperemic and had petechial hemorrhages. Avirulent strains of Sh. sonnei as a rule did not cause exudation into the loop of intestine. The sterile and concentrated contents of the intestinal loops of rabbits responding to injection of the virulent strain of Sh. sonnei or a toxigenic strain of Shigella shigae invariably gave a positive reaction in other rabbits. The character of the exudate and the changes in the mucous membrane under these circumstances were indistinguishable from those following injection of living cultures.

KEY WORDS: Shigella sonnei; Shigella shigae; enterotoxin

Investigations have shown [7] that most recently isolated strains of Shigella sonnei can induce the accumulation of fluid in the isolated loop of rabbit small intestine. However, Floyd and Arm were unable to reproduce this phenomenon by injecting filtrates of these cultures, and Arm et al. [1] also were unsuccessful when injecting lysates obtained by ultrasonic treatment.

These workers observed correlation between the enterotoxicity of the strains and their virulence.

More recent data [2-7] indicate that Sh. sonnei, unlike Shigella shigae, is unable to produce an enterotoxin.

In the present investigation the isolated loop of rabbit small intestine was used as a model to study the action of recently isolated strains of Sh. sonnei and also of the sterile contents of isolated segments of intestine obtained during tests of virulent strains.

EXPERIMENTAL METHOD

All strains, which were generously provided by the bacteriological laboratory of the Leningrad District Public Health Station in Moscow (Head T. A. Lakshtanova), were kept in the lyophilized state at 4°C. Altogether 14 recently isolated strains and 2 reference strains (Nos. 9090 and 7478) were tested. The strains were grown in dishes on nutrient agar, the round colonies were chosen as far as possible, and a suspension prepared from them in physiological saline with a density of 1 billion bacterial cells/ml according to the optical standard of the State Control Institute, and 1 ml of this suspension was injected into the lumen of an isolated loop of rabbit small intestine.

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