MECHANISM OF DEPRESSION OF ANTIBODY FORMATION IN HIGH-DENSITY SUSPENSION CULTURES

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The cause of the decrease in number of antibody-producing cells formed on induction of the primary immune response *in vitro*, observed when the surface density (the number of cells per square centimeter floor of the incubation vessel) was increased was studied. Depression of antibody formation in high-density cultures was found to be due, not to disturbance of induction of the clone of antibody-forming cells, but to inhibition of the subsequent proliferation of the cells of that clone. The disturbance of proliferation is reversible: A decrease in the density of the cultures 1 or 3 days after the beginning of incubation led to resumption of the increase in number of antibody producers. The depressant effect cannot be transferred humorally from high-density to low-density cultures. It cannot be abolished by changing the incubation medium of high-density cultures daily for fresh nutrient medium.

KEY WORDS: *antibody formation in vitro; proliferation.*

In previous experiments the writers showed that during induction of the primary immune response *in vitro* [4] an increase in the surface density (the number of cells per square centimeter floor of the incubation vessel) of cells taken for cultivation leads to sharp depression of antibody formation [2]. This depression is not connected with a deficiency of antigen, serum, or nutrients [2].

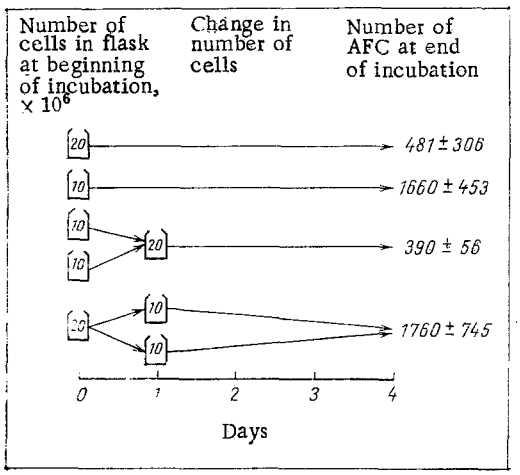

Fig. 1. Effect of change in density of cultures 1 day after beginning of incubation on AFC formation.

To study the mechanism of depression of antibody formation experiments were carried out to discover whether it is due to a disturbance of induction or of subsequent proliferation of the antibody-forming clone; the possibility of humoral transfer of the depression described also was investigated.

EXPERIMENTAL METHOD

C57BL/6 mice were used. Induction of antibody formation was studied *in vitro* by a modified method of Mishell and Dutton [2, 3, 6]. The spleens were removed with sterile precautions, suspended, and the cell suspension in enriched Eagle's medium [3]; this was poured into silicone-treated penicillin flasks (diameter 20-22 mm), which were filled with

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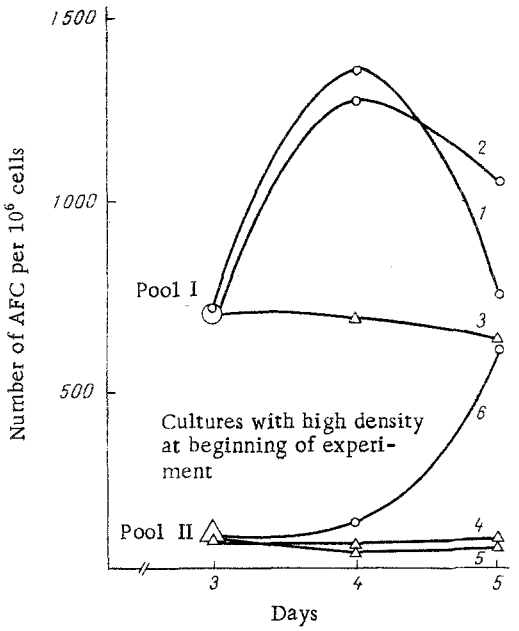

Fig. 2

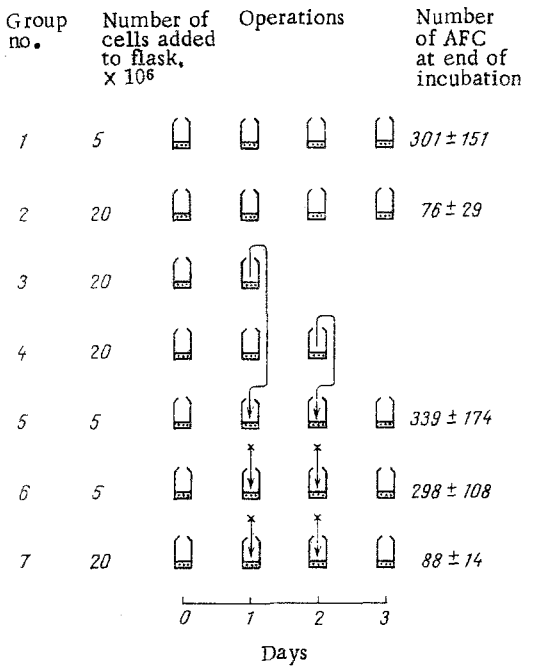

Fig. 3

Fig. 2. Effect of change in density of cultures 3 days after beginning of incubation on AFC formation: 1) samples containing $3.9 \cdot 10^6$ cells from beginning of incubation and not subjected to additional treatment; 4) samples containing $15.6 \cdot 10^6$ cells from beginning of incubation and not subjected to additional treatment; pool I obtained on 3rd day after beginning of incubation by combining cultures with low density ($3.9 \cdot 10^6$); 2) samples prepared from pool I with no change in density; 3) samples prepared from pool I after fourfold concentration of suspension; pool II obtained on 3rd day after beginning of incubation by combining cultures with high density ($15.6 \cdot 10^6$); 5) samples prepared from pool II with no change in density; 6) samples prepared from pool II after fourfold dilution of suspension.

Fig. 3. Absence of effect of transfer of culture medium from high-density suspensions and also of change of medium on AFC formation. Arrows indicate transfer of medium; asterisks denote change of half of medium for fresh.

a gas mixture (5% CO_2, 10% O_2, 85% N_2), closed with rubber stoppers, and incubated for 1-4 days at 37°C with antigen. Sterile sheep's red cells (RBC) or water-soluble antigen (WSA) extracted from them [7], were used as the antigen. After the end of incubation the total number of living cells (by the test with eosin and trypan blue) and the number of IgM antibody-forming cells (AFC) in the samples were determined [5].

EXPERIMENTAL RESULTS

To discover whether the phenomenon observed is connected with a disturbance of induction of antibody formation in high-density cultures or with delay of subsequent proliferation of the AFC clone formed, the density of the cells in culture was changed 24 h after the beginning of incubation.

It was assumed that induction of antibody formation is largely complete at the end of the first day of incubation with antigen *in vitro*, and that this is then followed by proliferation of the clone thus formed [4].

In one of these experiments RBC was used as the antigen. In the flasks to which $20 \cdot 10^6$ cells were added, 481 AFC were formed, compared with 1660 AFC (per 10^6 living cells) in the flasks with $10 \cdot 10^6$ cells.

Some of the samples to which $10 \cdot 10^6$ cells (in 1 ml) were added were pooled after incubation with antigen for 24 h. The volume of the samples was made up to 1 ml by drawing off the excess of nutrient medium, and the samples thus obtained were incubated for another 3 days. The number of AFC formed in these samples corresponded approximately to the number of AFC in samples to which $20 \cdot 10^6$ cells were added at the beginning of incubation (Fig. 1).

In the same experiment, samples containing $20 \cdot 10^6$ cells (in 1 ml) were incubated for 24 h with antigen and then divided into two equal halves. The volume of the samples was made up to 1 ml and they were incubated for another 3 days (to avoid the addition of fresh medium, the volumes were equalized with medium in which $10 \cdot 10^6$ cells had been incubated for the previous 24 h). The results showed that the number of AFC formed in samples containing initially $20 \cdot 10^6$ cells, and divided into two halves after 24 h, was the same as the number of AFC in samples containing $10 \cdot 10^6$ cells from the very beginning (Fig. 1).

Special experiments were carried out to discover how a change in cell density affects the change in the number of AFC during the period of fastest exponential growth between the 3rd and 4th days of incubation *in vitro*. In one such experiment, in the samples with a low cell density ($3.9 \cdot 10^6$) relatively many AFC were formed in 3 days and their number continued to rise (from 736 per 10^6 living cells on the 3rd day to 1362 per 10^6 cells on the 4th day; Fig. 2).

On the 3rd day after the beginning of incubation the cells of several such samples were pooled. Some suspensions of this pool were poured into flasks with no change of density (treatment control), and other samples containing 4 times more cells were prepared. The increase in number of AFC in the samples prepared from the general pool, and containing only a few cells, was about the same as in the samples containing the same number of cells from the beginning of the experiment (Fig. 2). A different picture was observed in samples prepared from the same cell pool but with a fourfold increase in density. An increase in density completely prevented the increase in number of AFC (Fig. 2).

The effect of a decrease in the surface density 3 days after the beginning of incubation of samples containing many cells ($15.6 \cdot 10^6$ cells in the experiments described), and forming only a few AFC, also was studied (Fig. 2). These samples were pooled on the 3rd day. From this pool samples with the original number of cells and also samples containing 4 times fewer cells were prepared. These experiments showed that a decrease in density led to a sharp increase in the number of AFC. The treatment itself, but with no change in density of the samples, had no appreciable effect (Fig. 2).

It was of great interest to discover whether the depressive effect of the high-density cultures could be transferred humorally to suspension with low density. The results of one experiment carried out for this purpose are illustrated in Fig. 3. Four times more AFC were formed in the flasks to which $5 \cdot 10^6$ cells were added than in flasks to which $20 \cdot 10^6$ cells were added. A daily change of half of the incubation medium in samples with low cell density by medium from samples in which many cells were incubated did not lead to depression of AFC formation. Daily replacement of half of the incubation medium by fresh nutrient medium likewise had no effect on AFC formation in samples with low or high density (Fig. 3).

The experiments described above show that depression of antibody formation in high-density cultures is not due to disturbance of formation of the cell clone synthesizing the particular antibody, but is due to a sharp and reversible depression of the proliferation of the cells of that clone, and also evidently of the proliferation of other cells present in the suspension. Depression of AFC proliferation described in this paper during an increase in the surface density of the incubated cells bears a close resemblance to contact inhibition [1, 8], although it differs from it by the absence of a monolayer (for in the silicone-treated flasks used in the present experiments the cells did not adhere to the bottom of the flask) and by its independence of the quantity of serum in the incubation medium [2].

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