IMMUNOLOGIC SPECIFICITY OF LOCAL INHIBITORY INTERCELLULAR INTERACTIONS

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The authors described previously local intercellular interactions inhibiting the growth of the number of antibody-forming cells (AFC) in a suspension of spleen cells from unimmunized mice and increasing sharply with an increase in the density of cultured suspensions. The present investigation shows that preliminary immunization of mice with an antigen abolishes or greatly weakens the inhibition of the increase in the number of AFC relative to that particular antigen, but does not affect the inhibition of the increase in the number of cells forming antibodies against another antigen or inhibition of proliferation of the main mass of dividing cells in culture.

KEY WORDS: antibody formation; cell density; antigenic specificity; regulation of proliferation.

The authors described previously local intercellular interactions inhibiting the increase in number of antibody-forming cells (AFC) during the development of antibody formation. These interactions increase sharply with an increase in the density of the cell suspension in culture [1-4]. Inhibitory intercellular interactions are much stronger in suspensions of cells from unimmunized animals than in suspensions of spleen cells taken some time after immunization [1].

The object of this investigation was to study the mechanism of abolition of local inhibitory intercellular interactions after immunization and to determine whether this abolition is antigenically specific.

EXPERIMENTAL METHOD

C57BL/6 mice were used. The antigens for immunization were sheep's (SRBC) or hen's (HRBC) red blood cells. Either the red cells themselves or the water-soluble antigen isolated from SRBC [7] was added to the cultures. Spleen cells taken from immunized or unimmunized mice were cultured by the method described previously in enriched Eagle's nutrient medium in siliconized penicillin flasks [1, 4]. The number of AFC was determined by a modified Jerne's method [6]. [^{3}H]Thymidine (from the Radiochemical Centre, Amersham, England) was added in a dose of 1 μCi/ml to some samples 24 h before the end of incubation and the quantity of ^{3}H incorporated into the cells was measured on a liquid scintillation counter (Intertechnique SL-40).

The results were subjected to statistical analysis, when the geometric or arithmetic mean values and standard error were determined for each group.

EXPERIMENTAL RESULTS

In the experiments of series I the course of the increase in number of AFC in spleen cell cultures of different densities from unimmunized mice and from mice immunized intravenously with different doses of antigen (from $0.5 \cdot 10^6$ to $500 \cdot 10^6$ SRBC per mouse) was compared. In these experiments the spleens were removed 3 days after immunization of the animals and suspended. The cells were then cultured for 4 days as "optimal" ($5 \cdot 10^6$ cells/ml) and "condensed" ($20 \cdot 10^6$ cells/ml) suspensions. It will be clear from Fig. 1 that the number of AFC formed in the "condensed" suspension from unimmunized animals was 20 times smaller than in the optimal suspension. A different picture was observed in cell suspensions from immunized animals. In this case the inhibitory effect of increased density was much weaker, and the larger the dose of the antigen

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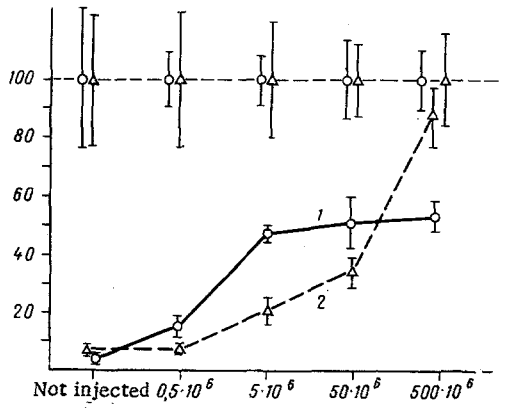

Fig. 1

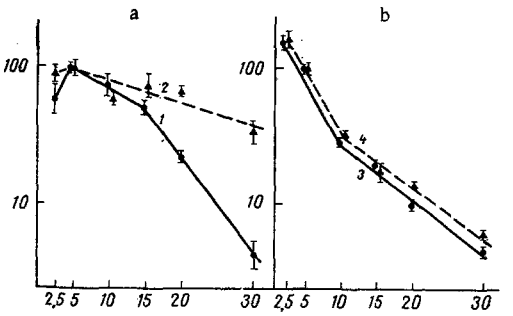

Fig. 2

Fig. 1. Effect of preliminary injection of different doses of antigen into mice on inhibition of antibody formation in "condensed" culture. 1) Antigen in SRBC culture; 2) antigen in culture with water-soluble antigen of SRBC. Abscissa, dose of SRBC injected into mice; ordinate, AFC formation (in percent of "optimal" cultures).

Fig. 2. Comparison of antibody formation (a) and [^{3}H]thymidine uptake (b) in cultures containing different numbers of spleen cells of unimmunized and immunized animals. 1, 3) Unimmunized mice; 2, 4) mice immunized with SRBC. Abscissa, number of cells cultured ($\times 10^6$); ordinate: a) AFC formation (in % of "optimal" cultures), b) incorporation of [^{3}H]thymidine (in percent of "optimal" cultures).

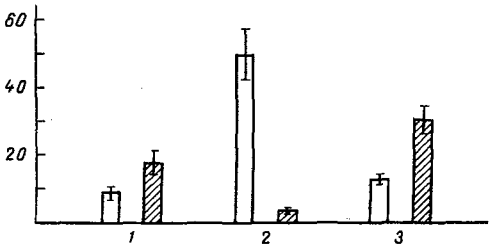

Fig. 3. Effect of "condensation" on increase in number of cells forming antibodies against antigen previously injected into animals, and number of cells forming antibodies against heterologous antigen. Unshaded columns denote cultures to which SRBC were added; shaded columns cultures to which HRBC were added. 1) Unimmunized mice; 2) mice immunized with SRBC; 3) mice immunized with HRBC. Ordinate, formation of AFC in "condensed" cultures (in percent of "optimal" cultures).

with which the animals were immunized, the weaker the effect. In the condensed cell suspension from mice immunized with 500×10^6 SRBC, AFC formation was inhibited by only 10-50%.

In the present experiments, just as previously, an increase in the density of the suspension of spleen cells from unimmunized animals was accompanied by inhibition of the increase in the number of AFC and incorporation of [^{3}H]thymidine into these cells (Fig. 2, curves 1 and 3). However, this parallel was no longer found in suspensions of spleen cells from the immunized animals: with an increase in the density of the suspension the incorporation of [^{3}H]thymidine declined rapidly (curve 4) whereas the number of AFC formed decreased only a very little (curve 2). Preliminary immunization of the animals thus weakened the inhibition of the increase in the number of AFC but did not affect inhibition of DNA synthesis in the main mass of cells of the "condensed" culture.

TABLE 1. Increase in Number of AFC and Incorporation of [^{3}H]Thymidine after Simultaneous Addition of Two Antigens (SRBC and HRBC) to "Condensed" and "Optimal" Cultures of Spleen Cells of Unimmunized Animals and Animals Immunized with SRBC

Dose of antigen injected into mice	Antigen added to culture	Number of cells in culture	Number of cells (per 10^6 living cells) forming anbibodies				Incorporation of ^{3}H thymidine, cpm/10^6 cells	
			against SRBC		against HRBC			
			absolute figures	% of inhibition	absolute figures	% of inhibition	absolute figures	% of inhibition
—	SRBC + HRBC, $5 \cdot 10^6$ of each	$5 \cdot 10^6$	673±134	—	683±144	—	24 797±2 499	—
	$5 \cdot 10^6$	$20 \cdot 10^6$	183±25	73±3,4	276±56	60±8,3	4 127±517	83±2,1
$50 \cdot 10^6$ SRBC per mouse	SRBC + HRBC, $5 \cdot 10^6$ of each	$5 \cdot 10^6$	7 377±664	—	802±83	—	38 997±737	—
	$5 \cdot 10^6$	$20 \cdot 10^6$	6 837±562	7,3±7,6	163±41	80±5,1	4 960±775	87±2,0

The problem of whether immunization of an animal with a given antigen leads to abolition of the inhibitory effect of condensation only with respect to an increase in the number of cells forming antibodies against that particular antigen, or whether it also does so in relation to the increase in the number of AFC against all other antigens, was studied in the experiments of series II. In this series of experiments two antigens (SRBC and HRBC), not giving crossed reactions either at the antibody level or at the T-cell level [5], were used. The mean results of four such experiments are illustrated in Fig. 3. In a suspension of spleen cells taken 3 days after immunization of mice with SRBC, an increase in density led to a decrease in the number of AFC formed by only half (compared with the 90% decrease in the cell suspension from unimmunized animals). This effect was antigen-specific: cells from mice into which the other antigen (HRBC) had previously been injected reacted to an increase in density in the same way as the cells of the unimmunized animal (Fig. 3).

A similar although less marked picture was observed also when the other antigen (HRBC) was used. Preliminary immunization with HRBC weakened the density effect, and preliminary immunization with the other antigen (SRBC) not only did not weaken the interaction effect, but actually potentiated it (Fig. 3).

The relationships described above were seen particularly clearly in the experiments in which two antigens (SRBC and HRBC) were added simultaneously to cell suspensions cultured in vitro, and the number of cells forming antibodies against each of these antigens was determined separately at the end of culture (Table 1). These experiments showed that when both antigens were present simultaneously in the medium condensation inhibited the increase in the number of AFC much less strongly against the antigen which had previously been injected into the animal compared with the other antigen.

There are several possible causes of the weaker inhibition of the increase in the number of AFC following an increase in the density of the cell suspension from the immunized animals: 1) weakening of the effect of the surrounding cells on proliferation of AFC (this suggestion is contradicted by inhibition of the increase in the number of cells forming antibodies against the other antigens observed in the same suspension, and inhibition of the proliferation of the main mass of dividing cells in the culture); 2) after immunization numerous specific T-helper cells, facilitating proliferation of AFC, appear in the animal's spleen; 3) after immunization a population of precursor cells appears in the animal and, in "condensed" cultures these cells may be converted without division into antibody-forming cells; 4) the clone of AFC formed in the immunized animal ceases to be susceptible to inhibitory influences of surrounding cells, in the same way that malignant cells cease to be susceptible to "contact inhibition."

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