EFFECT OF MITOGENS ON INHIBITION OF ANTIBODY FORMATION AND PROLIFERATION IN DENSE CULTURES

A. A. Korukova, A. B. Kim, and A. E. Gurvich

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The study of the regulation of antibody formation during induction of this process in a suspension of mouse spleen cells *in vitro* showed that proliferation of both antibody-forming cells (AFC) and of the other cells is strongly inhibited in dense cultures [1, 7].

The object of the present investigation was to discover whether the inhibition of proliferation observed after an increase in density is effected through the same receptors on lymphoid cells as those through which various mitogens stimulate the proliferation of these cells. This possibility has been discussed by various workers who have studied contact inhibition [2, 4, 5]. In order to examine this problem the effect of mitogens on the number of AFC and on total proliferation in cultures of mouse spleen cells of differing density was studied.

EXPERIMENTAL METHOD

A suspension of spleen cells from female C57BL/6 mice was obtained under sterile conditions. To induce an immune response *in vitro* the suspensions were cultured in the presence of water-soluble antigens isolated from sheep's red blood cells (SRBC-WSA) on enriched Eagle's medium for 4 days [3, 6]. The number of AFC was determined by the method of local hemolysis in gel [8].

In experiments in which only total proliferation was studied, suspensions containing different numbers of cells were cultured for 2 days on RPMI-1640 medium ("Flow-Lab.") containing 4 mM L-glutamine, 2 mg/ml sodium bicarbonate, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and 5% embryonic calf serum (ECS). Two batches (U351401 and U782201) of ECS from "Gibco" and several batches of ECS obtained in the N. F. Gamaleya Institute of Epidemiology and Microbiology* were used. In all cases ^{3}H-thymidine (from "Izotop," specific activity 1 Ci/mmole) was added in a dose of 1 μCi per culture 24 h before the end of incubation. Incorporation of label was measured on an "Intertechnique SL-40" liquid scintillation counter.

The following mitogens were used: 1) sodium salts of dextran sulfate (DS) from "Pharmacia," mol. wt. 500,000; 2) concanavalin A (Con A) from "Calbiochem," mark A; 3) lipopolysaccharide (LPS) from "Sigma," and *E. coli* serotype 0127:B8, phenolic extract.

In the statistical analysis of the results the geometric mean and limiting error (mt) were calculated for each group of values and the confidence intervals $M_g \pm mt$ determined at the $P < 0.05$ level. All calculations were done on 10^6 living cells in culture.

EXPERIMENTAL RESULTS

The calf embryonic sera possessed mitogenic properties to a varied degree. In these experiments incorporation of ^{3}H-thymidine into spleen cells differed by a factor of 10 in the

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Laboratory of Chemistry and Biosynthesis of Antibodies, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Zoophytotoxins, Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 2, pp. 188-190, February, 1981. Original article submitted May 19, 1980.

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TABLE 1. Inhibition of Total Cell Proliferation and of Antibody Formation in Cultures with Increased Density of Suspension Depending on Mitogenicity of ESC Present in Medium

Batch of ESC	Incorporation of thymidine-^{3}H, cpm/10^6 living cells in culture			Number of AFC per 10^6 living cells in culture		
	with optimal density ($5 \cdot 10^6$ cells/ml)	with increased density ($20 \cdot 10^6$ cells/ml)	percent of inhibition	with optimal density ($5 \cdot 10^6$ cells/ml)	with increased density ($20 \cdot 10^6$ cells/ml	percent of inhibition
A3*	9 663 (7 499—12 451)	3 095 (2 396—3 996)	68	1880 (1 613—2 191)	206 (134—316)	89
782201†	12 338 (11 061—13 763)	5 259 (4 208—6 573)	57	2041 (1 662—2 505)	391 (271—565)	81
Museum	21 422 (17 989—25 510)	7 173 (5 147—9 997)	66	2133 (1 472—3 090)	182 (122—70)	91
351401†	76 388 (65 410—89 210)	28 474 (22 224—36 481)	63	1329 (1 098—1 609)	81 (49—132)	94
A_4^*	82 340 (64 581—104 982)	15 166 (11 593—19 840)	82	2016 (1 234—3 295)	207 (92—465)	90

*Obtained from A. V. Izvekova (N. F. Gamaleya Institute of Epidemiology and Microbiology).
†Manufactured by "Gibco."

TABLE 2. Effect of DS on Cell Proliferation and Antibody Formation in Cultures with Increased Density of Suspension

DS concentration in culture, μg/ml	Incorporation of thymidine-^{3}H, cpm/10^6 living cells in culture			Increase in number of AFC during 2nd-4th day per 10^6 living cells in culture		
	with optimal density	with increased density	percent of inhibition	with optimal density	with increased density	percent of inhibition
—	34 890 (29 390—41 419)	12 954 (11 894—14 108)	63	1 127 (774—1 640)	366 (262—511)	67
5	32 035 (28 052—36 585)	12 297 (10 536—14 352)	62	1 278 (810—2 015)	452 (352—581)	65
50	33 130 (28 059—39 116)	7 767 (6 566—9 187)	76	848 (465—1 546)	329 (303—357)	61

Legend. Cells cultured in optimal density of suspension ($5 \cdot 10^6$/ml) for 2 days with SRBC-WSA. Pool of suspensions then prepared. Some suspensions poured into flasks (with no change of density), others centrifuged and the sedimenting cells were resuspended in the same medium in one-quarter of its original volume (a fourfold increase in density), after which this suspension was poured into flasks. DS was then added and culture continued for a further 2 days; ^{3}H-thymidine was added to all cultures on the 3rd day. Incorporation and number of AFC determined on the 4th day. Number of AFC on the 2nd day was 41 per 10^6 living cells.

presence of ECS of different batches; no correlation was found between the mitogenic activity of the ECS and the degree of inhibition of proliferation in cultures with increased density (Table 1).

The action of different mitogens (DS, Con A, and LPS) on proliferative activity of cells cultured in the presence of ESC with differing ability to stimulate DNA synthesis in a lymphoid cell culture was next studied. It was found that for all mitogens the lower the mitogenic activity of the ECS present in the culture medium, the stronger its action (Fig. 1). It is interesting to note that the SRBC-WSA used to induce the primary immune response in the culture possessed the same well-marked ability to stimulate proliferation of spleen cells in culture (Fig. 1).

In the next stage of the work an attempt was made to determine the effects of different mitogens on inhibition of proliferation and the increase in number of AFC in dense cultures. Two series of experiments were undertaken.

The action of the B-cell mitogen DS was first studied. It will be clear from Table 2 that DS had no stimulating action under these conditions either on antibody formation or on incorporation of ^{3}H-thymidine, whether in optimal or dense cultures. DS thus did not abolish

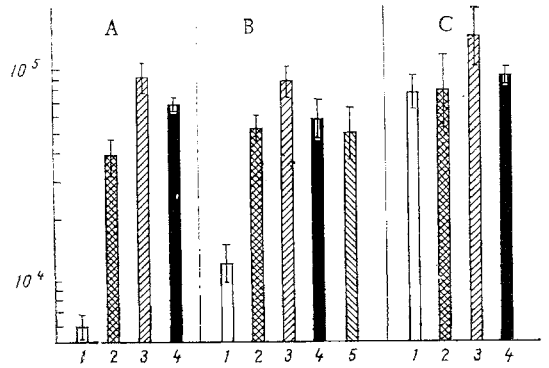

Fig. 1

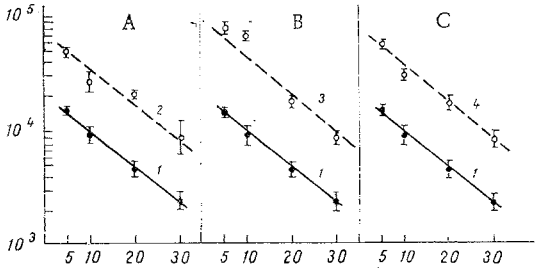

Fig. 2

Fig. 1. Stimulation by mitogens on total proliferation of cells cultured in optimal density of suspensions in the presence of ECS with different mitogenic activity. Culture with ECS: A) batch A3; B) batch U782201; C) batch U351401. 1) Without mitogens; 2) DS (100 μg/ml); 3) Con A (2.5 μg/ml); 4) LPS of *E. coli* (50 μg/ml); 5) SRBC-WSA (50 μg/ml). Ordinate, incorporation of ^{3}H-thymidine (in cpm) per 10^6 living cells.

Fig. 2. Stimulation of total proliferation of cells cultured in different densities of suspension by DS (A), concanavalin A (B), and lipopolysaccharide (C). 1) Control (without mitogen); 2) DS (100 μg/ml); 3) Con A (2.5 μg/ml); 4) LPS of *E. coli* (50 μg/ml). Ordinate, incorporation of ^{3}H-thymidine (cpm) per 10^6 living cells. Abscissa, original number of cells (10^6/ml) in culture.

inhibition of the immune response and of total cell proliferation in cultures with increased density of suspension.

Finally, an attempt was made with mitogens to abolish the inhibition of total cell proliferation caused by an increase in density of the suspension in culture. The results given in Fig. 2 show that addition of any of these mitogens did not affect the character of the fall in proliferative activity of the cells depending on the increase in density of the suspension: The slope of the straight lines representing this relationship was the same in the control (curve 1) and experimental (curves 2, 3, and 4) groups of cultures. The stimulating action of mitogens on cell proliferation in cultures with any density of suspension whatever also can be clearly seen in Fig. 2.

It can be concluded from these results that the two very important mechanisms regulating proliferation of lymphoid cells are independent: regulation through the actions of polyclonal mitogens and regulation through mutual local inhibition of proliferation.

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