EFFECT OF FIBROBLASTS FROM MONOLAYER CULTURES OF HEMATOPOIETIC AND LYMPHOID TISSUES ON THE IMMUNE RESPONSE *in vitro*

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Fibroblasts from monolayer cultures of human bone marrow and from guinea pig bone marrow, spleen, thymus, and peripheral blood suppressed the accumulation of antibody-forming cells in suspension cultures of mouse spleens in response to treatment with sheep's red cells *in vitro*. Practically complete inhibition of the immune response was produced during combined cultivation of $20 \cdot 10^6$ mouse spleen cells and $0.2 \cdot 10^6$ fibroblasts. Inhibition was much weaker if the fibroblasts acted on mouse spleen cells immunized *in vivo* 3 days before explantation and it was absent if animals immunized 9 days before the cells were taken for culture were used as donors of spleen cells.

KEY WORDS: immune response *in vitro*; cells of the microenvironment; stromal cells; adherent spleen cells.

Besides hematopoietic cells and cells of the lymphoid series, organs of hematopoiesis and immunopoiesis also contain stromal mechanocytes, for which there is evidence that they actively participate in physiological processes taking place in these organs [4, 7]. Properties of stromal cells such as high radioresistance [2] and a marked ability to adhere to glass [3] suggest that the stromal fibroblasts form part of the population of A- (adherence) cells necessary for reproduction of the immune response in cultures *in vitro* [15]. The method of cloning stromal fibroblasts developed in recent years enables a sufficient number of these cells to be obtained in monolayer cultures, although the concentration of precursor cells for mechanocytes in hematopoietic and lymphoid organs of mammals is low [6, 8].

This paper describes a study of the effect of fibroblasts from monolayer cultures of hematopoietic and lymphoid tissues on the immune response in a suspension of spleen cells incubated *in vitro*.

EXPERIMENTAL METHOD

Stromal cells from human and guinea pig hematopoietic and lymphoid organs were grown in monolayer cultures by the methods described previously [6, 8]. Guinea pig peripheral blood leukocytes were explanted by the method of Panasyuk and Luria [5]. Cultures were used in the experiments after not less than two or three passages, so that it was possible to collect a sufficiently large number of fibroblasts without contamination by histiocytes. Flasks with a complete monolayer of cells were treated with 0.25% trypsin solution and the cells separating from the glass were washed off into fresh medium used for mouse spleen cell cultures.

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TABLE 1. Effect of Fibroblasts from Monolayer Bone Marrow Cultures on the Primary Immune Response

Cells in culture				Antigen (sheep's red cells)	Number of AFCs per 10^6 cells
mouse spleen cells ($\times 10^6$)	human bone marrow fibroblasts $\times 10^6$)	guinea pig bone marrow fibroblasts ($\times 10^6$)	cells from primary suspension of guinea pig bone marrow cells		
20	—	—	—	—	118±23,9
20	0,5	—	—	—	5,7±2,3
20	—	—	—	+	1952±196,3
20	0,5	—	—	+	104±12,4
20	—	—	—	—	100±26
20	—	0,37	—	—	5±0,6
20	—	—	—	+	359±42,6
20	—	0,37	—	+	4±1,4
20	—	—	—	+	1082±86,8
20	—	—	0,3	+	1009±67,5

The immune response *in vitro* was studied on a suspension of C57BL/6 mouse spleen cells. A modification of Mishell and Dutton's method [1, 11, 14] was used. The suspension of spleen cells was poured into silicone-treated [1] penicillin flasks to which the antigen was added (sheep's red cells or the water-soluble antigen isolated from them) and, if necessary, the fibroblasts to be studied. The flasks were filled with 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. The number of antibody-forming cells (AFCs) was determined by the method of Jerne and Nordin [13].

EXPERIMENTAL RESULTS

The presence of fibroblasts obtained during cultivation of human and guinea pig bone marrow suppressed the increase in number of AFCs in suspension cultures of mouse spleens on the addition of antigen to them (Table 1). Human and guinea pig fibroblasts had a similar action. The effect depended on the dose of stromal cells added (Fig. 1). Virtually complete inhibition of the immune response took place by fibroblasts in a concentration of between $0.1 \cdot 10^6$ and $0.2 \cdot 10^6$ cells/ml culture fluid. In some experiments slight stimulation (an increase of 10-20% in the number of AFCs) of the immune response was observed on the addition of small numbers of fibroblasts. However, this increase in the number of AFCs was not statistically significant ($t_d < t_{st}$; $t = 0.95$). The presence of antigens cross reacting with antigens of sheep's red cells in embryonic calf serum leads to the accumulation of large numbers of AFCs capable of synthesizing hemolysins against sheep's red cells even when no antigen is added to the cultures [14]. Addition of fibroblasts to the culture inhibited accumulation also of these "background" AFCs (Table 1). The phenomenon of inhibition of the immune response was due in all probability not to interspecies, but to intercellular (intertissue) differences between the suspensions grown together in culture. This conclusion is confirmed by the fact that the primary suspension of guinea pig bone marrow cells, unlike fibroblasts of bone marrow origin, had no inhibitory action of AFC formation (Table 1). One of the most likely mechanisms of the depressant effect of fibroblasts added to the culture could be a decrease in the number of surviving mouse spleen cells. This possibility was tested by counting the number of cells "surviving" in the culture, as revealed with the aid of Trypan Blue. The results indicate that the "survivability" of the cells after 4 days in culture together with fibroblasts did not differ significantly from that of spleen cells kept in flasks without fibroblasts.

The study of the action of fibroblasts on the increase in numbers of AFCs in suspensions of spleen cells from an immunized animal was of great interest. Spleens of mice immunized by intravenous injection of sheep's red cells 3-9 days before explantation of the spleen cells into cultures were used. The results of these experiments are given schematically in Fig. 2. Depression of the immune response on the addition of fibroblasts to spleen cells from animals immunized 3 days before explantation was less than twice that observed in intact animals, although it was statistically significant ($t_\alpha > t_{st}$; $t = 0.95$). In cultures of spleen cells from mice immunized 9 days before explantation, very slight inhibition of the immune response, on average by 15-20% and not statistically significant ($t_d < t_{st}$; $t = 0.95$) was observed on the addition of fibroblasts.

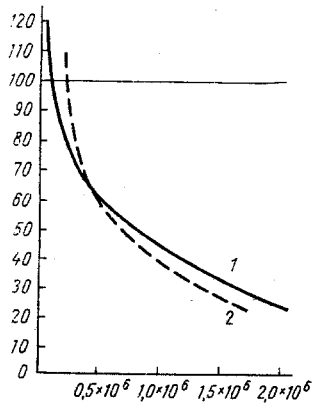

Fig. 1

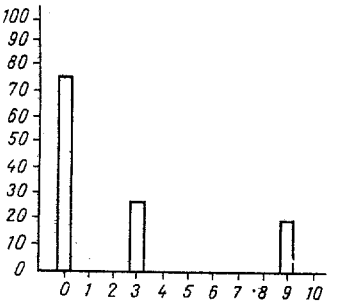

Fig. 2

Fig. 1. Level of immune response as a function of number of fibroblasts in culture: 1) guinea pig bone marrow fibroblasts; 2) splenic fibroblasts. Abscissa, dose of fibroblasts added to culture; ordinate, level of immune response (in %). Number of AFCs in control culture without fibroblasts taken as 100%.

Fig. 2. Inhibitory activity of fibroblasts in cultures of spleen cells of animals immunized *in vivo*. Abscissa, time of explantation of spleen cells into cultures after preliminary immunization *in vivo* (in days); ordinate, degree of inhibition of immune response (in %).

In a series of experiments the effect of fibroblasts obtained from different hematopoietic and lymphoid organs of guinea pigs on the immune response *in vitro* were compared. The results showed that, besides stromal cells from bone marrow, fibroblasts from spleen, thymus, and peripheral blood also had an inhibitory action on development of the immune response; the relationship between the number of inhibitory cells and the degree of inhibition of the immune response was similar for stromal cells of different origin.

The experimental results described in this paper indicate that stromal cells of human bone marrow and of various hematopoietic and lymphoid organs of guinea pigs inhibit the formation of the immune response to heterologous red cells in suspension cultures of mouse spleen cells. It is interesting to compare these results with the evidence of inhibition of the immune response *in vitro* by high concentrations of A-cells [12]. The medium used for the mouse spleen cell cultures contained 2-mercaptoethanol. This substance can completely prevent the restorative action of A-cells in the immune response *in vitro* when added to nonadherent cells [9]. Meanwhile the inhibitory effect of high concentrations of A-cells on antibody production is exhibited to the full in cultures even in the presence of 2-mercaptoethanol [10]. The system used in the present experiments to reproduce the immune response was thus perhaps capable of recording only the effect of high concentrations of stromal cells. Inhibition of the immune response observed in the present experiments was in all probability due to intercellular and not interspecific differences in the suspensions mixed together in the culture. However, a further study of the phenomenon described above must call for the creation of more adequate experimental systems excluding the possible influence of xenogeneic differences between cells grown together in culture as a relevant factor.

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