RECORDING OPTICAL INTERACTION BETWEEN LYMPHOCYTES DURING ACTIVATION BY PHYTOHEMAGGLUTININ WITH A YEAST DETECTOR

G. P. Altynkov

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The writer showed previously [1] that lymphocytes activated by phytohemagglutinin (PHA) induce blast transformation in nonactivated lymphocytes by means of optical contact effected through UV radiation. Recognizing that this fact is itself debatable, it was decided to study whether the presence of emission from antigenically activated lymphocytes can be demonstrated by other methods. The photoelectronic equipment available, with a sensitivity of about 10^3 photons/sec, was unable to record reliable emission from PHA-activated lymphocytes. This could be done only by means of "biological detection" of very weak ultraviolet radiation. Because of the satisfactory sensitivity and reproducibility of this method, it was possible to record emission both from directly activated lymphocytes and from immunocompetent lymphocytes which had been only in optical contact with the former.

The object of the present investigation was to study the dynamics of the emission accompanying these activation processes in the earliest stage, during the first hour after addition of PHA.

EXPERIMENTAL METHOD

Lymphocytes were obtained by gradient centrifugation of isotonically diluted blood (2:1) in Ficoll–Urografin solution (specific gravity 1.076). Blood was obtained from healthy volunteers aged 29–44 years.

To standardize the experimental conditions, the test lymphocyte cultures were incubated simultaneously at 37°C in special chambers (Fig. 1) in darkness. Three chambers with a diameter of 15 mm were sealed underneath with thin Teflon film, as also was an additional small chamber (Teflon transmits light starting from a wavelength of 168 nm). The chambers contained 0.7 ml of different mixtures: chamber I) a culture of lymphocytes (10^6 cells in 1 ml) activated with 0.001 solution of PHA (PHA-P, from Difco, USA); chamber II) control, pure nutrient medium (Difco medium 199) with the addition of 0.001 PHA; chamber III) an inactivated culture of lymphocytes (10^6 cells in 1 ml) irradiated from the culture activated in the small chamber.

The cells incubated in chamber III were exposed to optical contact with activated lymphocytes from the small additional chamber. This consisted of a short cylinder sealed underneath with Teflon film. A culture of lymphocytes activated with PHA was poured into the small chamber, just as into chamber I. The nonactivated lymphocytes were stimulated through the Teflon bottom of the small chamber with radiation from the activated lymphocytes.

Agar cylinders (detectors) were cut out with the aid of a metal stencil, taking care not to damage the yeast layer. The detectors were exposed for 15 sec to the Teflon bottoms of chambers I, II, and III so that the distance between the layer of yeast and the bottom was 6 mm. After exposure, the detectors were incubated at 26°C for 8 min. During the 15-sec exposure the small chamber was taken away. Before exposure the chambers with lymphocytes were kept in an incubator at 37°C in darkness.

After exposure and incubation for 8 min the yeast was fixed directly to the agar with 10% formalin. Films were prepared from the yeast cultures in the usual way and stained with 5% methylene blue solution. The budding index was determined in 1000 yeast cells by means of an immersion objective. The preparations were counted in a coded form.

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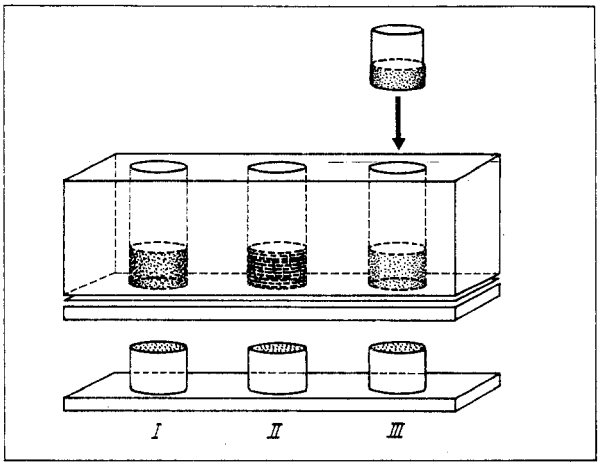

Fig. 1

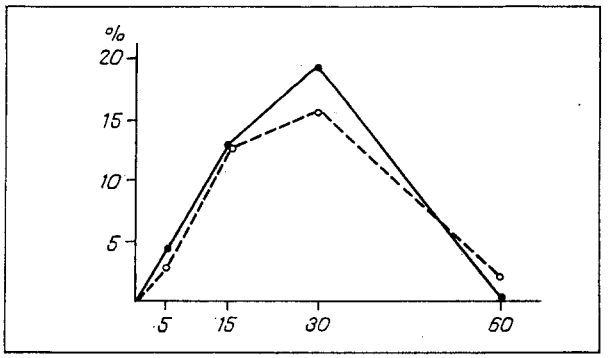

Fig. 2

Fig. 1. Scheme of experiment to detect emission from lymphocytes. Top row: I) chamber with lymphocytes activated by PHA; II) control; III) chamber with inactivated lymphocytes irradiated above by activated lymphocytes. Bottom row: yeast radiation detectors.

Fig. 2. Time course of emission from lymphocyte cultures from chamber I (continuous line) and III (broken line).

The number of "young buds" (budding index) in 1000 cells formed a variance series which obeyed the law of the normal distribution. The confidence interval of the mean budding index for a given preparation was accordingly calculated by means of Strelkov's tables [2] for a level of significance of 0.95.

EXPERIMENTAL RESULTS

Altogether 73 series of lymphocyte cultures from four healthy donors were tested. Each series contained the results of counting 3000 yeast cells (Table 1) under three chambers.

The presence of radiation from activated lymphocytes (chamber 1) was proved by the fact that the budding index on the yeast cells was significantly increased at the 15th and 30th minutes after exposure for 15 sec compared with the budding index in a yeast culture after being in optical contact with the control. These two indices did not differ significantly after 5 and 60 min.

The budding index also was significantly increased after optical contact between yeast and lymphocytes from chamber III, which were not treated with PHA, but which "saw" PHA-activated lymphocytes in the small chamber. In this case also differences from the budding index in the control were significant at the 15th and 30 minutes, but not significant during the first 5 min and toward the end of the first hour after exposure.

The results obtained with the yeast detector cannot be interpreted in photometric units. Accordingly it was impossible to judge either the intensity or the spectral composition of the observed radiation. However, the fact that radiation was present and its time course

TABLE 1. Budding Indices of Lymphocytes under Different Conditions of Stimulation (arithmetic means with confidence intervals at p = 0.95)

Time, min	No. of series	Chamber I	Chamber II	Chamber III
5	9	22,7±3,0	21,7±2,4	21,3±2,2
15	28	23,6±1,5	20,9±1,1	23,6±1,2
30	28	24,3±1,8	20,4±1,4	23,6±1,4
60	8	22,0±4,0	22,0±3,1	22,4±2,5

during the first hour can be regarded as reliably established. It shows that electron-excited states which are deactivated by emission are present in the activated lymphocytes. In the writer's opinion another no less important fact is that the activated lymphocyte culture induces radiation in a culture not activated by optical contact, and, moreover, it does so very rapidly. As will be clear from Fig. 2, the two curves have a practically identical time course.

Taking all these circumstances into account and also having regard to opinions of other workers that electron-excited states play an important role in the course of vital processes in the cell [5, 6], the results described above can be interpreted as reflecting optical interaction between lymphocytes. The view expressed previously [1] that optical contact is a possible mechanism of nonspecific activation of lymphocytes during realization of the immunologic response at the cellular level is confirmed.

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ROLE OF CELL SURFACE H-2 ANTIGENS IN REGULATING PROLIFERATION OF HEMATOPOIETIC STEM CELLS

I. L. Chertkov and O. A. Gurevich

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Transplantation antigens of a nonsyngeneic recipient can appear as "histocompatibility molecules" on the surface of a donor's hematopoietic stem cells (HSC), which facilitate their subsequent growth in the same recipient [2]. This suggests that transplantation antigens take part in intercellular interactions of HSC with their microenvironment that are essential for regulating HSC proliferation.

In the investigation described below proliferation of antigenically modulated HSC, transplanted into a syngeneic irradiated recipient, was studied.

EXPERIMENTAL METHOD

C57BL/6 (subsequently abbreviated to B6) and (CBA × C57BL)F_1 mice (abbreviated to CBF_1), of both sexes, were used. HSC were determined by cloning in the spleen of irradiated mice [4]. To study induction of proliferation in resting HSC the "suicide" method with hydroxyurea (HU) was used. Normal or antigenically modulated HSC were injected in a dose of 6×10^6 to 25×10^6 bone marrow cells into irradiated (11-12 Gy) intermediate recipients. The spleen was removed from the latter 1-3 h later and the suspension of splenocytes was divided into two equal parts, one of which was incubated in medium 199 enriched with 2 mM L-glutamine and 5% embryonic calf serum, with 1 mg/ml (16 mM) HU at 37°C for 1 h; the other half was incu-

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