INDUCTION OF LYMPHOCYTE TRANSFORMATION BY PERIODATE

A. NOVOGRODSKY and E. KATCHLASKI
Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

The lectins, phytohemagglutinin (PHA) and concanavalin A (Con A) induce transformation of lymphocytes by the binding to specific, saccharide containing sites of the cell membrane [1, 2]. While studying the nature of the Con A binding sites of rat lymph-node lymphocytes, we found that lymphocytes undergo extensive transformation after mild treatment with sodium periodate ($NaIO_4$). Some of the characteristic features of the $NaIO_4$ induced blastogenesis are described in the present communication.

2. Materials and methods

2.1. *Materials*

5-^{3}H-Uridine (17.25 Ci/mmole) and Me-^{3}H-thymidine (5 Ci/mmole) were obtained from the Radiochemical Centre, Amersham. Sodium periodate, analytical reagent, was obtained from BDH Chemicals Ltd., England.

2.2. *Preparation of lymphocytes*

Male Wistar rats weighing 200–240 g were used, after being killed by ether. Peripheral and mesenteric lymph nodes were removed aseptically and minced in phosphate buffered saline (PBS) [3]. The large pieces of connective tissue were allowed to settle and the supernatant suspension was harvested.

2.3. *Lymphocyte cultures*

Lymphocytes, at a final concentration of 5×10^6 cells/ml, were suspended in Dulbecco's modified Eagle's medium [4], supplemented with serum (source and concentration specified for each experiment), penicillin (100 units/ml) and streptomycin (100 μg/ml). Cultures of 1 ml were prepared in triplicate in sterile polystyrene tubes (17 mm × 100 mm), loosely capped and incubated at 37° in an atmosphere of air: CO_2 (95 : 5).

2.4. *Incorporation and measurement of radioactivity*

In experiments in which the effect of $NaIO_4$ treatment on RNA synthesis was investigated, the $NaIO_4$-treated lymphocytes were incubated for 20 hr, 5-^{3}H-uridine 2.5 μCi (specific activity 17.25 /mmole) per ml lymphocyte culture was then added and the culture incubated for an additional 2 hr at 37° with agitation. The incorporation of 5-^{3}H-uridine into RNA was determined according to the procedure previously described [5]. The effect of $NaIO_4$ treatment on DNA synthesis was studied in a similar fashion. Here, the $NaIO_4$-treated lymphocytes were incubated for 44 hr, Me-^{3}H-thymidine 2.5 μCi (specific activity 5 Ci/mmole) per ml of lymphocyte culture was then added and incubation continued for an additional 2 hr at 37° with agitation. The incorporation of Me-^{3}H-thymidine into DNA was determined according to the procedure previously described [5].

3. Results

3.1. *Stimulation of RNA and DNA synthesis in rat lymph node lymphocytes treated with $NaIO_4$*

The effect on RNA and DNA synthesis of incubation of rat lymph node lymphocytes for 10 min at room temperature with $NaIO_4$, at different concentrations, is shown in fig. 1. The synthesis of both types of nucleic acids is maximally stimulated upon treatment of the cells, under the experimental con-

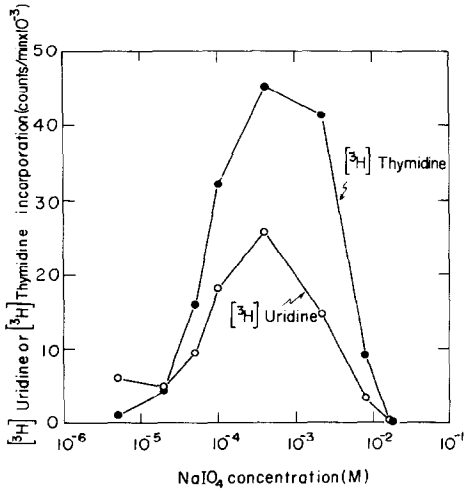

Fig. 1. Stimulation of RNA and DNA synthesis in rat lymph node lymphocytes treated with $NaIO_4$. Lymphocytes were suspended (13.3×10^6/ml) in PBS, containing $NaIO_4$ at concentrations specified on the abscissa, and incubated at room temperature for 10 min. The cells were then centrifuged, washed once with PBS (in amount equal to the original volume of the cell suspension) and suspended (5×10^6/ml) in Dulbecco's modified Eagle's medium, supplemented with 20% horse serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). One ml aliquots were incubated for 20 hr and RNA synthesis was measured as described in Materials and methods. DNA synthesis was measured after an incubation period of 44 hr.

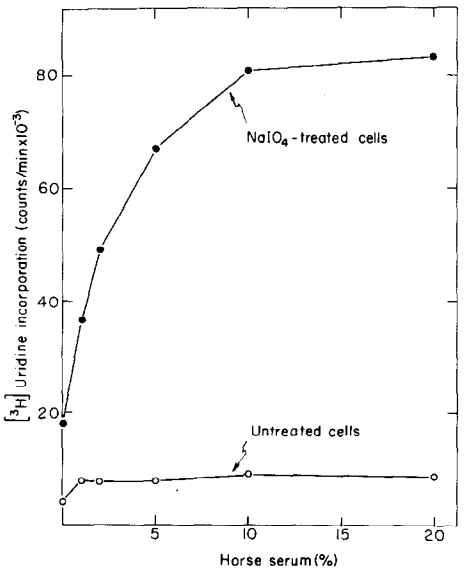

Fig. 2. Effect of horse serum, at different concentrations on RNA synthesis, in $NaIO_4$-treated lymphocytes. Lymphocytes were treated with $NaIO_4$ (4×10^{-4} M) under the conditions specified in the legend for fig. 1. The cells were suspended (5×10^6/ml) in Dulbecco's modified Eagle's medium, supplemented with horse serum at the concentrations specified on the abscissa, penicillin (100 units/ml), and streptomycin (100 μg/ml). One ml aliquots were incubated for 20 hr and RNA synthesis was determined as described in Materials and methods.

dition used, with $NaIO_4$ at a concentration of 4×10^{-4} M. Treatment of the cells with $NaIO_4$ at higher concentrations ($2 \times 10^{-3} - 8 \times 10^{-3}$ M) is less stimulating, whereas treatment of the cells at a concentration of 1.6×10^{-2} M is inhibitory. A similar dose response curve was recorded for PHA and Con A stimulated RNA and DNA synthesis of lymphocytes [2,6].

3.2. *Morphology of transformed lymphocytes induced by $NaIO_4$*

Morphological analysis of lymphocytes treated with $NaIO_4$ (4×10^{-4} M, under the experimental conditions outlined in the legend for fig. 1), after 44 hr in culture, revealed that 76 percent of the cells underwent blast formation. The large transformed cells (12–22 μm) contained large nuclei with fine chromatin structure and basophilic, often vacuolated cytoplasm. Many mitoses were also observed.

3.3. *Enhancement of RNA synthesis in $NaIO_4$-treated lymphocytes by horse serum*

RNA synthesis in $NaIO_4$-treated lymphocytes is markedly enhanced on incubation of the cells in the presence of horse serum. The effect of horse serum, at different concentrations, on RNA synthesis is shown in fig. 2. Although RNA synthesis in $NaIO_4$-treated cells is stimulated on incubation of the cells in the absence of serum, the stimulatory effect of $NaIO_4$ treatment on RNA synthesis is much more pronounced if the cells are incubated in the presence of serum.

3.4. *Effect of different sera on RNA synthesis in $NaIO_4$-treated lymphocytes*

Table 1 shows that sera from different sources vary in their ability to stimulate RNA synthesis in $NaIO_4$-treated lymphocytes (exp. A). It is of interest that sera which are most potent in enhancing RNA syn-

Table 1
Effect of different sera on RNA synthesis in and agglutination of $NaIO_4$-treated lymphocytes.

Serum	Exp. A ^{3}H-Uridine incorporation (cpm)	Exp. B agglutination
–	9907	0
Horse	92211	++++
Human (blood group B)	106920	++++
Human (blood group A)	106533	++++
Human (blood group O)	101183	++++
Human cord blood	85536	0
Goat	93889	+++
Calf	71630	++
Calf fetus	66788	0
Rabbit	68049	0
Rat	27953	0

Exp. A. Lymphocytes were treated with $NaIO_4$ (4×10^{-4} M) under the conditions specified in the legend for fig. 1. The cells were suspended (5×10^6/ml) in Dulbecco's modified Eagle's medium supplemented with 10% of the appropriate serum (all sera tested were preincubated at 56° for 30 min), penicillin (100 units/ml), and streptomycin (100 μg/ml). One ml aliquots were incubated for 20 hr, and RNA synthesis was determined as described in Materials and methods (second column).
Exp. B. Lymphocytes were treated with $NaIO_4$ (4×10^{-4} M) under the conditions specified in the legend for fig. 1. The cells were suspended (10×10^6/ml) in PBS, supplemented with 20% of the appropriate serum and 0.5 ml aliquots were incubated at room temperature. Agglutination of the cells was determined microscopically after incubation for 4 hr. A serological scale of 0 to ++++ was used to estimate the degree of clumping (third column).

thesis in $NaIO_4$-treated lymphocytes, such as the human sera, are also most potent in agglutinating the chemically modified cells (see exp. B of table 1). However, the agglutinating activity of serum does not seem to be essential for the enhancement of RNA synthesis in $NaIO_4$-treated cells. Thus sera from human cord blood, calf fetus, and rabbit which lack the agglutinin for $NaIO_4$-treated lymphocytes, are active in stimulating RNA synthesis in these cells. It should be noted that the sera used in exps. A and B (table 1) were preincubated at 56° for 30 min, since it was observed that $NaIO_4$-treated lymphocytes undergo lysis on incubation with some of the fresh sera tested.

4. Discussion

The mechanism by which treatment of lymphocytes with $NaIO_4$ induces blastogenesis is not known. It is attractive to assume, however, that the primary action of $NaIO_4$ in the induction of lymphocyte transformation is the chemical modification of the lymphocyte membrane. This assumption is supported by the observation that $NaIO_4$-treated lymphocytes are agglutinated by different sera. A similar effect of $NaIO_4$ on red blood cells was described previously [7]. $NaIO_4$ was also reported to inactivate blood group receptors on human erythrocyte surface [8] and to modify the antigenic properties of human red cells [9].

Transformation of rat lymph node lymphocytes induced by $NaIO_4$, provides a useful system for the study of blastogenesis. The transformation in this system is extensive and is comparable to that obtained by treatment of rat lymph node lymphocytes with PHA or Con A under optimal conditions [2]. Furthermore, excess of $NaIO_4$ can be removed readily at any instant from the cell suspension.

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References

[1] H. Borberg, I. Yesner, B. Gesner and R. Silber, Blood 31 (1968) 747.
[2] A. Novogrodsky and E. Katchalski, unpublished results.
[3] R. Dulbecco and M. Vogt, J. Expl. Med. 99 (1954) 167.
[4] J.D. Smith, G. Freeman, M. Vogt and R. Dulbecco, Virology 12 (1960) 185.
[5] A. Novogrodsky and E. Katchalski, Biochim. Biophys. Acta 215 (1970) 291.
[6] S.D. Handmaker, H.L. Cooper, B.G. Leventhal, in: Proc. of the Third Annual Leucocyte Culture Conference, ed. W.O. Rieke (Appleton-Century-Crofts, New York, 1969) p. 53.
[7] M. Moskowitz and H.P. Treffers, Science 111 (1950) 717.
[8] W.T.J. Morgan and W.M. Watkins, Brit. J. Expl. Pathol. 32 (1951) 34.
[9] F.S. Stewart, J. Pathol. Bacteriol. 61 (1949) 456.