INDUCTION OF LYMPHOCYTE TRANSFORMATION BY PERIODATE

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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 (NaIO₄). Some of the characteristic features of the NaIO₄ induced blastogenesis are described in the present communication.

2. Materials and methods
2.1. Materials
5-³H-Uridine (17.25 Ci/mmole) and Me-³H-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 X 10⁶ 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 X 100 mm), loosely capped and incubated at 37° in an atmosphere of air: CO₂ (95:5).

2.4. Incorporation and measurement of radioactivity
In experiments in which the effect of NaIO₄ treatment on RNA synthesis was investigated, the NaIO₄-treated lymphocytes were incubated for 20 hr, 5-³H-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 5-³H-uridine into RNA was determined according to the procedure previously described [5]. The effect of NaIO₄ treatment on DNA synthesis was studied in a similar fashion. Here, the NaIO₄-treated lymphocytes were incubated for 44 hr, Me-³H-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-³H-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 NaIO₄
The effect on RNA and DNA synthesis of incubation of rat lymph node lymphocytes for 10 min at room temperature with NaIO₄, 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 NaIO₄. Lymphocytes were suspended (13.3 X 10⁶/ml) in PBS, containing NaIO₄ 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 X 10⁶/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.

condition used, with NaIO₄ at a concentration of 4 X 10⁻⁴ M. Treatment of the cells with NaIO₄ at higher concentrations (2 X 10⁻³ – 8 X 10⁻³ M) is less stimulating, whereas treatment of the cells at a concentration of 1.6 X 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 NaIO₄

Morphological analysis of lymphocytes treated with NaIO₄ (4 X 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 NaIO₄-treated lymphocytes by horse serum

RNA synthesis in NaIO₄-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 NaIO₄-treated cells is stimulated on incubation of the cells in the absence of serum, the stimulatory effect of NaIO₄ 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 NaIO₄-treated lymphocytes

Table 1 shows that sera from different sources vary in their ability to stimulate RNA synthesis in NaIO₄-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 NaIO₄-treated lymphocytes.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Exp. A</th>
<th>Exp. B</th>
</tr>
</thead>
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<tr>
<td></td>
<td>³H-Uridine incorporation (cpm)</td>
<td>agglutination</td>
</tr>
<tr>
<td>Horse</td>
<td>92211</td>
<td>++++</td>
</tr>
<tr>
<td>Human (blood group B)</td>
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<td>++++</td>
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<td>71630</td>
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</tr>
<tr>
<td>Rat</td>
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<td>0</td>
</tr>
</tbody>
</table>

Exp. A. Lymphocytes were treated with NaIO₄ (4 × 10⁻⁴ M) under the conditions specified in the legend for fig. 1. The cells were suspended (5 × 10⁶/ml) in Dulbecco’s modified Eagle’s medium supplemented with 10% of the appropriate serum (all sera tested were preincubated at 56°C 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 NaIO₄ (4 × 10⁻⁴ M) under the conditions specified in the legend for fig. 1. The cells were suspended (10 × 10⁶/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).

4. Discussion

The mechanism by which treatment of lymphocytes with NaIO₄ induces blastogenesis is not known. It is attractive to assume, however, that the primary action of NaIO₄ in the induction of lymphocyte transformation is the chemical modification of the lymphocyte membrane. This assumption is supported by the observation that NaIO₄-treated lymphocytes are agglutinated by different sera. A similar effect of NaIO₄ on red blood cells was described previously [7]. NaIO₄ 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 NaIO₄, 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 NaIO₄ can be removed readily at any instant from the cell suspension.

Acknowledgements

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References