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# EFFECT OF MACROPHAGES ON PERIODATE-INDUCED TRANSFORMATION OF NORMAL AND CHRONIC LYMPHATIC LEUKAEMIA LYMPHOCYTES

M. BINIAMINOV,* B. RAMOT* AND A. NOVOGRODSKY†

** Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer and Tel Aviv University Medical School, Israel, and † Department of Biophysics, The Weizmann Institute of Science, Rehovot, Israel*

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SUMMARY

Human lymphocytes which have been purified from mononuclear leucocyte suspensions by removal of cells which adhere to nylon fibres have a reduced blastogenic response to periodate treatment as compared to the whole cell suspensions. Incubation of the purified lymphocytes on macrophage monolayers markedly enhanced their response to periodate. Periodate-treated macrophages enhance the response of purified lymphocytes to periodate more than do untreated macrophages. Stimulation of thymidine incorporation into purified lymphocytes untreated with periodate, was also noted after their incubation on periodate-treated macrophage monolayers. Chronic lymphatic leukaemia lymphocytes which have an impaired blastogenic response to periodate can be stimulated by this agent upon incubation on macrophage monolayers. Alternatively untreated chronic lymphatic leukaemia lymphocytes are stimulated on incubation on periodate-treated macrophage monolayers.

## INTRODUCTION

Previous studies have shown that glass adherent mononuclear cells are necessary for human blood lymphocytes to undergo blastogenesis in response to specific antigens (Oppenheim, Leventhal & Hersh, 1968; Hersh & Harris, 1968). Conflicting data have been obtained on the PHA-induced blastogenic response of purified lymphocytes as compared to unseparated leucocyte suspensions (Hersh & Harris, 1968; Oppenheim *et al.*, 1968; Rabinowitz, 1964; Walker & Fowler, 1965; Wilson, 1966). An obligatory role for glass-adherent cells in the response of purified lymphocytes to PHA has recently been reported (Levis & Robbins, 1970).

Periodate treatment of rat lymph node or mouse spleen lymphocytes induces extensive blastogenesis (Novogrodsky & Katchalski, 1971; Novogrodsky & Katchalski, 1972). While normal human blood lymphocytes are also stimulated by periodate, lymphocytes from

Correspondence: Dr M. Biniaminov, Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer, Israel.

chronic lymphatic leukaemia patients (CLL-lymphocytes) do not respond to this treatment (Parker, O'Brien, Lukes & Steiner, 1972). In this paper, we report the effect of macrophages on the periodate-induced transformation of human blood lymphocytes.

Transformation of lymphocytes by periodate provides a useful system for studying the separate effects of the mitogen on lymphocytes or macrophages in the induction of blastogenesis.

## MATERIALS AND METHODS

### *Materials*

Phytohaemagglutinin-M was obtained from Difco Laboratories, Detroit, Michigan. [$^3$H-methyl]thymidine (5 Ci/mmol) was obtained from the Nuclear Research Center, Negev, Israel. Sodium periodate ($NaIO_4$) was obtained from BDH Chemicals Ltd, England.

### *Cell preparation*

*Mononuclear leucocytes.* Heparinized human venous blood was incubated at room temperature for 45 min in test tubes sloped at an angle of 45°C. The supernatant plasma containing the leucocytes was removed and the cells after centrifugation were suspended ($30 \times 10^6$/ml) in a PBS:plasma mixture (1:1). Mononuclear leucocytes obtained by separation from the erythrocytes and granulocytes on a Ficoll–Hypaque gradient (Harris & Ukaejiofo, 1969) contained 70–90% lymphocytes, 10–30% monocytes and a few granulocytes.

*Purified lymphocytes* were obtained by the following procedure: mononuclear leucocytes ($15 \times 10^6$) in 0·5 ml plasma were added to a glass column (0·7 cm × 6 cm) containing nylon wool (Leukopak from Fenwal Laboratories, Morton Grove, Illinois). After incubation for 20 min at 37°C, the nonadhering cells containing 95–99% lymphocytes were collected by elution with 2 ml PBS.

*Macrophage monolayers.* Aliquots (0·5 ml) of a mononuclear leucocyte suspension (1·2–$2{\cdot}8 \times 10^6$ cells/ml plasma) were incubated for 2 hr at 37°C in tissue culture tubes (16 mm × 125 mm, Falcon, number 3033). The non-adhering cells were then removed by repeated washings with PBS.

*Treatment of cells with periodate.* Mononuclear leucocytes, purified lymphocytes or CLL-lymphocytes were treated with periodate under the conditions specified in the appropriate legends to the figure and tables. Macrophage monolayers were treated with PBS containing periodate at a final concentration of $5 \times 10^{-4}$ M and incubated for 10 min at 22°C. The periodate solution was then decanted and the cell monolayers washed twice with PBS.

*Cell cultures.* Mononuclear leucocytes, purified lymphocytes or CLL-lymphocytes were suspended at a final concentration of $10^6$/ml in Dulbecco's modified Eagle's medium, containing autologous human serum (10%, heat-inactivated at 56°C for 30 min), and supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml). One millilitre portions were placed in loosely capped polystyrene tubes (16 mm × 125 mm, Falcon, number 3033), and incubated at 37°C in an atmosphere of 95% air:5% $CO_2$.

### [$^3$*H*]*Thymidine incorporation into DNA*

[$^3$H]Thymidine (1·25 μCi) was added to cell cultures (1 ml) that had been incubated for time intervals as specified in the appropriate legends to the figure and tables. After additional

incubation for 2 hr with shaking, incorporation of [$^3$H]thymidine into DNA was determined (Novogrodsky & Katchalski, 1970). In each experiment, cultures were incubated in duplicate and the results are expressed as the mean value of two determinations.

*Patient material*

The patients with chronic lymphatic leukaemia were clinically and haematologically in a steady state. None of the patients received any therapy in the course of this study.

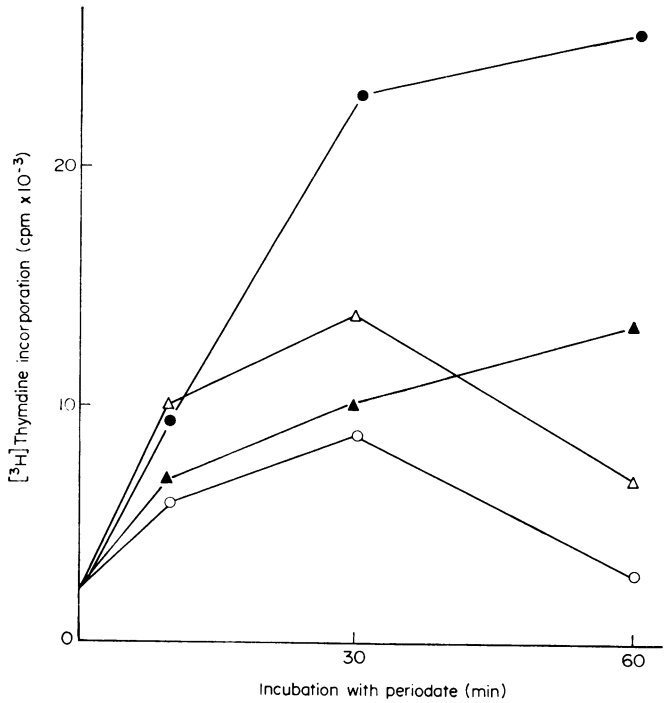


FIG. 1. Stimulation of [$^3$H]thymidine incorporation into human blood lymphocytes treated with periodate. Mononuclear leucocytes ($2 \times 10^6$/ml) in PBS were treated with periodate at the specified concentrations and incubated for different time intervals at 0°C or at 22°C. The cells were then centrifuged, washed once with PBS and suspended in culture medium. After incubation for 72 hr [$^3$H]thymidine incorporation within 2 hr was then determined. (●) Periodate ($5 \times 10^{-4}$ M) at 0°C; (▲) periodate ($10^{-3}$ M) at 0°C; (△) periodate ($2 \times 10^{-3}$ M) at 0°C; (○) periodate ($5 \times 10^{-4}$ M) at 22°C.

## RESULTS

*Stimulation of [$^3$H]thymidine incorporation into human blood lymphocytes treated with periodate*

In studies carried out previously on the induction of lymphocyte transformation by periodate (Novogrodsky & Katchalski, 1971; Novogrodsky & Katchalski, 1972), the cells were treated with the oxidizing agent at room temperature. We found that under the experimental conditions outlined in the legend to Fig. 1, treatment of the cells with periodate

TABLE 1. Effect of macrophages on periodate-induced transformation of normal human lymphocytes *

| Normal subjects | [$^3$H] Thymidine incorporation (cpm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mononuclear leucocytes treated with | | | Purified lymphocytes treated with | | | Untreated macrophages incubated with | | | Periodate-treated macrophages incubated with | | |
| | None | Periodate | PHA | None | Periodate | PHA | Alone | Untreated purified lymphocytes | Periodate-treated purified lymphocytes | Alone | Untreated purified lymphocytes | Periodate-treated purified lymphocytes |
| 1 | 1600 | 18500 | 78300 | 680 | 1450 | 27600 | — | — | — | — | — | — |
| 2 | 730 | 23500 | 10450 | 200 | 800 | 11050 | — | — | — | — | — | — |
| 3 | 1250 | 70100 | 75600 | 1330 | 13250 | 42100 | — | — | — | — | — | — |
| 4 | 910 | 4450 | — | 820 | 1850 | — | 1210 | 1850 | 7800 | — | — | — |
| 5 | 550 | 24700 | 33300 | 1100 | 640 | — | 320 | 2300 | 40500 | 250 | 13300 | 36500 |
| 6 | 910 | 6630 | 32500 | 1300 | 3120 | 98800 | 880 | 1300 | 55900 | 730 | 11700 | 64500 |
| 7 | 350 | 9600 | 92200 | 530 | 2650 | 62400 | — | — | 34900 | — | 20700 | 55600 |
| 8 | 300 | 14700 | — | 830 | 170 | — | — | — | 15800 | 1560 | 16300 | 44200 |
| 9 | — | — | — | 420 | 1860 | — | 490 | — | 25800 | 310 | — | 57100 |
| 10 | — | — | — | 310 | 480 | — | 150 | 860 | 8950 | 280 | 2250 | 9770 |

* Lymphocyte suspensions ($2 \times 10^6$/ml) in PBS were treated, as indicated, with periodate at final concentration of $5 \times 10^{-4}$ M and incubated for 60 min at 0°C. The cells were then washed with PBS suspended in culture medium, and incubated, as indicated, in test tubes containing autologous macrophage monolayers. PHA–M was added, as indicated, to a final concentration of 400 $\mu$g/ml. [$^3$H]Thymidine incorporation, within 2 hr, was determined after incubation for 48 hr (subjects 1–3) and 72 hr (subjects 4–10).

at 0°C is much more effective than at 22°C in the induction of blastogenesis. The periodate-induced blastogenic response of lymphocytes isolated from different subjects was quite variable (Table 1). However, in most of the cases maximal response was attained after treatment of the lymphocytes with periodate at a final concentration of $5 \times 10^{-4}$ M for 60 min at 0°C. [$^3$H]Thymidine incorporation into the periodate-treated normal human lymphocytes reached its maximal value after incubation of the cells for 48 to 72 hr. It should be pointed out that maximal [$^3$H]thymidine incorporation into PHA-treated cells (Table 1) is seen after incubation of the cells for 72–96 hr.

*Effect of macrophages on periodate-induced transformation of normal human lymphocytes*

The response of human blood lymphocytes depleted of macrophages to periodate treatment is markedly decreased. However, upon incubation of the depleted cells with macrophages there is a markedly increased response. Periodate-treated macrophages

TABLE 2. Effect of macrophages on periodate-induced transformation of chronic lymphatic leukaemia lymphocytes *

| Patient | [$^3$H]Thymidine incorporation (cpm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | CLL-lymphocytes treated with | | Untreated macrophages incubated with | | Periodate-treated macrophages incubated with | | |
| | None | Periodate | Alone | Periodate-treated CLL-lymphocytes | Alone | Untreated CLL-lymphocytes | Periodate-treated CLL-lymphocytes |
| 1 | 300 | 490 | 590 | 13600 | 630 | 690 | 6640 |
| 2 | 400 | 250 | 430 | 1260 | 150 | 2560 | 2720 |
| 3 | 2250 | 2590 | 1200 | 25100 | 1850 | 25250 | 43400 |
| 4 | 740 | 670 | 940 | 3100 | 980 | 9750 | 14100 |
| 5 | 1240 | 6500 | — | — | 970 | 4070 | 20210 |
| 6 | 3800 | 3100 | — | — | 480 | 8620 | 23020 |

* CLL-lymphocytes were not purified on nylon fibre columns. Experimental conditions were similar to those outlined in the legend to Table 1 except that homologous macrophages from normal subjects were used. [$^3$H]Thymidine incorporation was determined within 2 hr after an incubation for 5 days.

enhance the response of purified lymphocytes to periodate treatment to an even greater extent. Stimulation of lymphocytes, untreated with periodate, was also noted after their incubation in the presence of periodate-treated macrophages (Table 1). The response of purified lymphocytes to PHA was also lower, in some of the cases tested, than that of the unfractionated cells.

*Effect of macrophages on periodate-induced transformation of chronic lymphatic leukaemia lymphocytes*

In a number of cases tested, lymphocytes from patients with chronic lymphatic leukaemia were found to be impaired in their response to periodate treatment (Table 2); an enhanced response is observed when such periodate-treated CLL-lymphocytes are incubated in the presence of macrophages. Periodate-treated macrophages are even more active cells in

enhancing such a response. CLL-lymphocytes, untreated with periodate are also stimulatec upon their incubation in the presence of periodate-treated macrophages.

## DISCUSSION

Human blood lymphocytes purified on a nylon fibre column have a reduced blastogenic response to periodate treatment as compared to unseparated cells. Culture of these purified lymphocytes on macrophage monolayers markedly enhanced the cell response to periodate. However, [$^3$H]thymidine incorporation into periodate-treated purified lymphocytes cultured on macrophage monolayers is markedly greater than in unseparated cells. It is thus possible that macrophage activity in the whole lymphocyte cultures is a rate-limiting factor which determines the extent of the blastogenic response of the lymphocytes to periodate. It is of interest to note that treatment of macrophages with periodate increases their activity in enhancing the blastogenic response of blood lymphocytes. While it is possible that other mitogens (i.e. lectins) might also affect macrophages in a similar way, it might be very difficult to evaluate their separate effects on the lymphocytes or macrophages alone.

The role of macrophages in enhancing the response of human lymphocytes to periodate is not known. Trials to demonstrate a soluble factor which mediates the above-mentioned effect of the macrophages are as yet unsuccessful. In this connection it is pertinent to note that adherent mouse cells and human peripheral leucocytes produce a soluble factor which potentiate the response of mouse thymocytes to PHA or periodate (Gery, Gershon & Waksman, 1972; Gery & Waksman, 1972; Novogrodsky & Gery, 1972). The production of this factor was enhanced by treatment of the producing cells with lipopolysaccharide, PHA, concanavalin A or periodate. Of special interest are the preliminary finding that the blastogenic response of CLL-lymphocytes to periodate is enhanced upon incubation of the cells on macrophage monolayers. It is possible that the impaired response of CLL-lymphocytes to periodate treatment might be due in part to the reduced number of macrophages in the cell cultures. Further studies are required for identification of the cell classes in normal and CLL-lymphocytes which respond to periodate treatment.

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