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GALACTOSE OXIDASE-INDUCED BLASTOGENESIS OF HUMAN LYMPHOCYTES AND THE EFFECT OF MACROPHAGES ON THE REACTION

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SUMMARY

Treatment of human lymphocytes with neuraminidase and galactose oxidase induced extensive blastogenesis. A less pronounced effect was observed after treatment of the cells with galactose oxidase alone.

Macrophage-depleted human lymphocytes had a markedly reduced blastogenic response after treatment with neuraminidase and galactose oxidase. Incubation of the purified lymphocytes on macrophage monolayers markedly enhanced their response to neuraminidase and galactose oxidase. Furthermore, a stimulation of thymidine incorporation into purified untreated lymphocytes was noted after incubation of the cells on neuraminidase and galactose oxidase-treated macrophage monolayers.

INTRODUCTION

Treatment of mouse spleen cells with galactose oxidase after their incubation with neuraminidase (NAGO treatment) was demonstrated to induce extensive blastogenesis. The cells were not affected by either one of the enzymes alone (Novogrodsky & Katchalski, 1973). It was suggested that the galactosyl residues exposed by the action of neuaminidase on the cell surface are oxidized by galactose oxidase, and the aldehyde moiety thus formed is involved in the triggering process (Novogrodsky & Katchalski, 1973).

In the present study we investigated the blastogenic effect of galactose oxidase on normal human peripheral blood lymphocytes. The effect of glass-adherent cells (macrophages) on this process was also studied.

MATERIALS AND METHODS

Materials

Materials consisted of phytohaemagglutinin (Wellcome, England), neuraminidase

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(Behringwerke AG, Germany), galactose oxidase and catalase (Sigma Chemical Co., U.S.A.), and methyl [^{3}H]thymidine (5 Ci/mmol) (Nuclear Research Centre, Israel).

Cell preparation

Blood samples were obtained from normal individuals. The mononuclear cells were separated on a Ficoll–metrizoate (Biniaminov, Ramot & Novogrodsky, 1974), and these cells (unfractionated lymphocytes) consisted of 70–90% lymphocytes, 10–30% monocytes and a few granulocytes.

The Ficoll-separated mononuclear cells were further purified on nylon fibre columns (Leukopak, Fenwall Laboratories, Illinois). The non-adherent cells which were eluted from the column with phosphate-buffered saline (PBS) consisted of 95–99% lymphocytes and a few monocytes (purified lymphocytes).

Macrophage monolayers were obtained by incubation of the Ficoll-separated cells suspended in fresh plasma in tissue culture tubes (Falcon No. 3033). Each aliquot contained $10–20 \times 10^5$ monocytes. After incubation for 2 hr at 37°C, the non-adherent cells were removed by extensive washing with PBS.

Treatment with neuraminidase and galactose oxidase

Unfractionated or purified lymphocytes ($2–10 \times 10^6$/ml PBS) were incubated with neuraminidase (50 units/ml) and galactose oxidase (GO) 0·25 units/ml (NAGO treatment) or with GO alone, for 30 min at 37°C. In some experiments the cells were treated sequentially with neuraminidase and GO. After incubation the cells were washed twice with PBS and suspended in culture medium.

The adherent macrophages were treated with NAGO under the same conditions.

Cell cultures

The cells were suspended at a final concentration of 10^6/ml in Dulbecco's modified Eagle's medium, containing 10% human heat-inactivated AB plasma supplemented with penicillin 100 units/ml and streptomycin 100 μg/ml. One-millilitre aliquots were incubated in loosely capped polystyrene tubes (Falcon 3033) at 37°C in an atmosphere of 95% air and 5% CO_2 for 69 hr. At the end of the incubation, [^{3}H]thymidine 1·25 μCi was added to each culture tube. After additional incubation for 2 hr with shaking, the incorporation of [^{3}H]thymidine into DNA was determined (Novogrodsky & Katchalski, 1970). The results are expressed as the mean value of two determinations.

RESULTS

Stimulation of human blood lymphocytes by galactose oxidase

Treatment of human blood lymphocytes with GO alone resulted in a mild blastogenic response, varying in degree among the subjects (Table 1). In order to prove the specificity of this reaction the cells were incubated with GO in the presence of an excess of D-galactose and catalase. The latter was added to prevent damage by hydrogen peroxidase formed during the enzymatic reaction. The transformation of lymphocytes incubated with GO under these conditions in the presence of catalase and D-galactose was markedly reduced, while the response to PHA was unaffected (Table 2).

TABLE 1. Response of human blood lymphocytes to treatment with neuraminidase and galactose oxidase: [^{3}H]thymidine incorporated (ct/min)

	Treatment				
Subject	None	GO	Neuraminidase	NAGO	PHA
1	1700	27550	640	63170	168790
2	770	49800	3180	132090	—
3	1190	3700	1360	40950	60450
4	2940	—	—	111200	183860
5	340	5200	4750	35180	37300
6	1130	48250	3970	138260	—
7	1530	—	—	44860	68160
8	1990	7557	10920	124700	181500
9	1700	16482	7810	64240	—

Cells were treated and incubated under conditions specified in Materials and Methods section.

TABLE 2. Inhibition of the action of galactose oxidase by D-galactose

	[^{3}H]Thymidine incorporated (ct/min)	
Treatment	Exp. 1	Exp. 2
None	2920	5828
GO	19720	28270
GO + D-galactose + catalase	5420	8370
PHA	48740	50550
GO + D-galactose + catalase + PHA	66870	32190

Unfractionated cells suspended in PBS were treated where indicated with galactose oxidase. For inhibition reaction D-galactose (20 mg/ml) and catalase (450 units/ml) were added during the incubation. PHA was added where indicated. [^{3}H]thymidine incorporation was determined after incubation for 69 hr.

Stimulation of human blood lymphocytes by neuraminidase and galactose oxidase

Incubation of peripheral blood lymphocytes with neuraminidase followed by GO induced more extensive blastogenesis than that obtained by GO alone, comparable to that obtained after stimulation with PHA (Table 1). There was no difference in the transformation of lymphocytes between simultaneous and sequential treatment with neuraminidase and GO. Morphological examination revealed over 90% of the cells transformed to blasts.

Neuraminidase treatment alone usually resulted in a small blastogenic response, although there was a notable response in some cases in contradiction to reports in the literature (Han, 1972; Lundgren & Simons, 1971). This phenomenon is currently under investigation and these cases were not included in Table 1.

TABLE 3. Effects of macrophages on NAGO-induced [^{3}H]thymidine incorporation in normal human lymphocytes

Unfractionated lymphocytes treated with:			Purified lymphocytes treated with:			Untreated macrophages incubated with:	NAGO-treated macrophages incubated with:		
None	NAGO	PHA	None	NAGO	PHA	NAGO-treated purified lymphocytes	None	Untreated purified lymphocytes	NAGO-treated purified lymphocytes
670	10,6468	12,9978	790	13,117	79,850	—	—	—	—
8236	13,9823	74,901	725	14,389	120,417	15,9747	12,286	229,778	94,171
5660	11,3107	71,444	2748	37,500	63,504	17,6320	7242	200,550	124,422
3029	76,120	74,172	591	54,272	40,140	—	5908	164,064	208,218
1130	77,904	50,812	690	4906	41,880	—	—	—	—
—	—	—	247	1183	19,544	—	3204	55,884	44,543
920	41,748	38,509	1033	11,340	32,575	—	—	—	—

The results are expressed as ct/min/1×10^6 cells. Unfractionated lymphocytes, purified lymphocytes and macrophages were treated with neuraminidase (50 units/ml) and galactose oxidase (0·25 units/ml). PHA was added in a concentration of 5 μg/ml. [^{3}H]-Thymidine incorporation was determined after 69 hr.

The effect of macrophages on lymphocytes treated with neuraminidase and galactose oxidase (NAGO)

The response of human purified lymphocytes to NAGO was quite low in comparison with unfractionated lymphocytes (Table 3). In a few cases the response of the purified lymphocytes to PHA was also somewhat reduced. When NAGO-treated purified lymphocytes were cultured on macrophage monolayers a markedly increased blastogenic response was observed. Furthermore, untreated purified lymphocytes cultured on NAGO-treated macrophages were also significantly stimulated (Table 3). It should be noted that thymidine incorporation into these cells cultured as described exceeded even that of enzymatically stimulated unfractionated lymphocytes incubated alone.

DISCUSSION

Our results show that NAGO treatment of human lymphocytes induced an extensive blastogenesis similar to mouse lymphocytes. However, contrary to mouse spleen cells, some human blood lymphocytes were stimulated somewhat by GO alone, the response varying between individuals. The response to GO was proven to be specific, as evidenced by the inhibition of the reaction with an excess of D-galactose.

The fact that GO alone induces blastogenesis in some normal human lymphocytes might indicate the presence of a lymphocyte sub-population with exposed galactosyl sites which are susceptible to the mitogenic action of GO. The size of such a lymphocytic sub-population may vary according to physiological or pathological conditions accounting for the observed variation in lymphocyte response to GO.

Macrophage-depleted human lymphocytes have a markedly reduced blastogenic response after treatment with NAGO, as compared to unfractionated lymphocyte preparations. The varied responses observed with different lymphocyte preparations is probably related to unequal degrees of macrophage contamination among them.

Since the response of purified lymphocytes treated with NAGO and cultured on a monolayer of untreated macrophages exceeded that of the enzymically treated unfractionated lymphocytes cultured alone, it appears that the macrophages might be the rate-limiting factor in this process. Similar observations on the optimal macrophage–lymphocyte ratio were made by Hersh & Harris (1968) in antigen-induced blastogenesis.

It is also important to note that untreated purified lymphocytes undergo transformation upon incubation on macrophage monolayers treated with NAGO.

The mechanism by which macrophages enhance the response of human lymphocytes to NAGO is not known. It is well known that macrophages are essential in antigen-induced blastogenesis (Hersh & Harris, 1968; Oppenheim, Leventhal & Hersh, 1968), and were reported essential also in PHA-induced lymphocyte transformation (Levis & Robbins, 1970). There are several possible explanations of the role of macrophages in the antigen initiation of blastogenesis (Unanue, 1972). It has been suggested that macrophages are essential for the presentation of the antigen to the lymphocytes (Waldron, Horn & Rosenthal, 1973).

In our study the blastogenic response of NAGO-treated lymphocytes was markedly enhanced by macrophages. It seems, therefore, that the role of macrophages in this system is not confined to the triggering signal *per se*, but is required for the blastogenic transformation to occur.

Similar to the effect of macrophages on NAGO-induced blastogenesis described here, we recently reported the enhancing action of macrophages on the transformation of periodate-treated lymphocytes as well.

We further showed that, similar to NAGO, periodate-treated macrophages enhance the response of purified lymphocytes to periodate more than do untreated macrophages (Biniaminov *et al.*, 1974).

Stimulation of untreated lymphocytes by NAGO-treated macrophages might result from a direct interaction between the enzymically modified macrophages and lymphocytes. The possible role of a Schiff base in NAGO-induced blastogenesis has been previously discussed (Novogrodsky & Katchalsky, 1973). On the other hand, it is possible that the mitogenic enzymes are pinocytosed by the macrophages and subsequently released during incubation with the lymphocytes. Finally, a soluble factor might mediate the effect of macrophages, although we have yet to demonstrate its presence.

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