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Enzymatic Induction of Interferon Production by Galactose Oxidase Treatment of Human Lymphoid Cells

FERDINANDO DIANZANI,[1]* THOMAS M. MONAHAN,[2] ANNE SCUPHAM,[1] AND MARIO ZUCCA[1]

Department of Microbiology[1] and Department of Human Biological Chemistry and Genetics,[2] University of Texas Medical Branch, Galveston, Texas 77550

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Human lymphocyte cultures produced large amounts of interferon after treatment with the enzyme galactose oxidase. Interferon production was detectable as early as 3 h after enzymatic treatment and reached a level of about 10^4 reference units 20 to 24 h later. Galactose oxidase-induced interferon appeared to be immune interferon on the basis of acid lability, lack of neutralization by antibody to leukocyte interferon, and slow kinetics of activation of the cellular antiviral state. Interferon production was inhibited to the same extent (99%) by pretreatment of the cells with β-galactosidase or with neuraminidase followed by β-galactosidase, suggesting that the critical event for activation of interferon production is the oxidation of exposed galactose residues on lymphocyte membrane.

Human lymphoid cell cultures stimulated by viruses, mitogens, or specific antigens produce two distinct types of interferon (IF) molecules. One type, called leukocyte IF, is induced by viruses (3), by B-lymphocyte mitogens (7), and by foreign or tumor cells (2, 16). This IF is thought to be produced by B or "null" lymphocytes (2, 15). The other type, called immune IF, appears to be produced by T lymphocytes stimulated by specific antigens or by T-cell mitogens (8, 17-19). However, it has not been established whether the production of one type of IF rather than the other is determined by the type of producing cell or by the kind of stimulus it receives (10). Rather, it has been shown that: (i) immune IF can be induced by B-cell mitogens (7), as well as by T-cell mitogens; (ii) leukocyte IF can be induced by specific antigens (14); and (iii) T-cell mitogens can induce immune IF production in purified B lymphocytes (6, 7). Another open question is whether IF production, which always precedes deoxyribonucleic acid (DNA) synthesis, plays a role in lymphocyte activation or whether it is a result of mitogenesis. Finally, only limited information is available to delineate the relationship existing between the events that trigger IF production and the production of the other lymphokines released during lymphocyte activation. Investigation of these questions will require identification of the specific steps of lymphocyte activation. Since all of the substances capable of IF induction in lymphocytes have a complex macromolecular structure and since the active site(s) responsible for the induction has not been identified, the molecular definition of the mechanisms of lymphocyte activation and IF induction has not been possible. Because lymphocyte activation has been obtained after oxidation of membrane-bound galactose residues by galactose oxidase (1, 5, 11, 12), we initiated studies to investigate whether this enzyme, whose mechanism of action is known, is also capable of IF induction.

MATERIALS AND METHODS

Lymphocytes were obtained as previously described (11) from healthy adult donors and, after Ficoll-Hypaque gradient sedimentation, were incubated overnight at 37°C in McCoy 5A medium containing 10% autologous heat-inactivated serum at a concentration of 2×10^6 cells per ml.

Activation by galactose oxidase was performed as previously described (11). Briefly, lymphocyte suspensions (10^7 cells per ml in 0.15 M phosphate-buffered saline) were treated with various concentrations of galactose oxidase (Worthington Biochemicals Corp.) for 30 min at 24°C. The cells were then washed extensively with Eagle minimal essential medium, resuspended in McCoy medium at a concentration of 2×10^6 cells per ml, and incubated at 37°C for various times. Some cultures were pretreated with actinomycin D (10 μg/ml for 1 h at 37°C), β-galactosidase (PL Biochemicals; 800 U/ml for 30 min at 24°C), or *Vibrio cholerae* neuraminidase (Schwarz/Mann; 50 U/ml for 1 h at 37°C) before treatment with galactose oxidase.

DNA synthesis was determined as previously described (11) by a 2-h pulse-labeling with [^{3}H]thymidine (New England Nuclear Corp.; 0.5 Ci/2 × 10^6 cells; specific activity, 2.0 Ci/mmol).

IF was incubated overnight in human WISH cell cultures, and the activity was measured as previously described (4) by inhibition of Sindbis virus hemagglutinin yield after a single growth cycle. The antiviral activity found in the samples was shown to be IF mediated by current criteria (activity against different

viruses, inactivity on heterologous cells, and blocking by actinomycin D).

RESULTS

Effect of treatment with different doses of galactose oxidase on IF induction and DNA synthesis. The results of a representative dose-response experiment are shown in Fig. 1, in which IF production (Fig. 1A) and DNA synthesis (Fig. 1B) have been reported for each dose of galactose oxidase treatment. It may be seen that every dose of the enzyme applied induced both IF production and DNA synthesis. These two parameters show remarkable correlation, both reaching the maximum response at the level of 5 U of galactose oxidase. However, the IF response, which appeared exceptionally high in cultures treated with 5 to 20 U of galactose oxidase, was already maximal at 24 h whereas DNA synthesis developed later, between 24 and 48 h, and reached the maximum level at 72 h. Both IF synthesis and [^{3}H]thymidine incorporation were completely prevented in cultures pretreated with actinomycin D. Pretreatment with β-galactosidase or with neuraminidase followed by β-galactosidase strongly reduced the response (up to 99%). Additionally, IF production did not occur in cells treated with heat-inactivated galactose oxidase (60°C for 30 min).

Kinetics of IF production. To determine the kinetics of IF production more accurately, we treated lymphocyte cultures with galactose oxidase (5 and 20 U/ml) and harvested samples for IF titration after 3, 6, 11, 24, and 48 h. The results of a representative experiment are shown in Fig. 2. It may be seen that very low levels of IF were already detectable at 3 h posttreatment; the levels increased substantially between 3 and 11 h and reached the maximum titer between 11 and 24 h. Lymphocyte cultures from six different donors showed slightly different responses (not exceeding 30% of the response shown) only during the first 6 h, whereas the later samples showed nearly identical responses. No significant differences were observed between cultures induced with 5 U of galactose oxidase and those induced with 20 U.

Characterization of galactose oxidase-induced IF. The properties of representative samples of galactose oxidase-induced IF were studied in comparison with samples of previously identified human leukocyte and immune IF. The results of these experiments are summarized in Table 1.

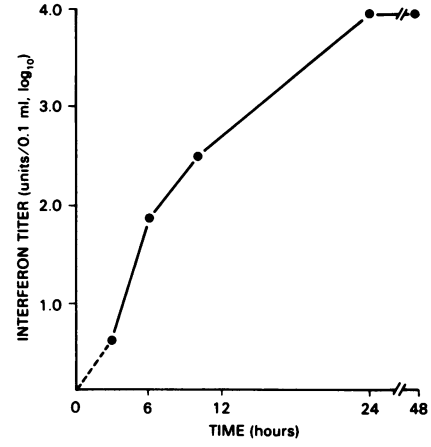

FIG. 2. *Kinetics of IF production in lymphocyte cultures treated with galactose oxidase (20 U/ml).*

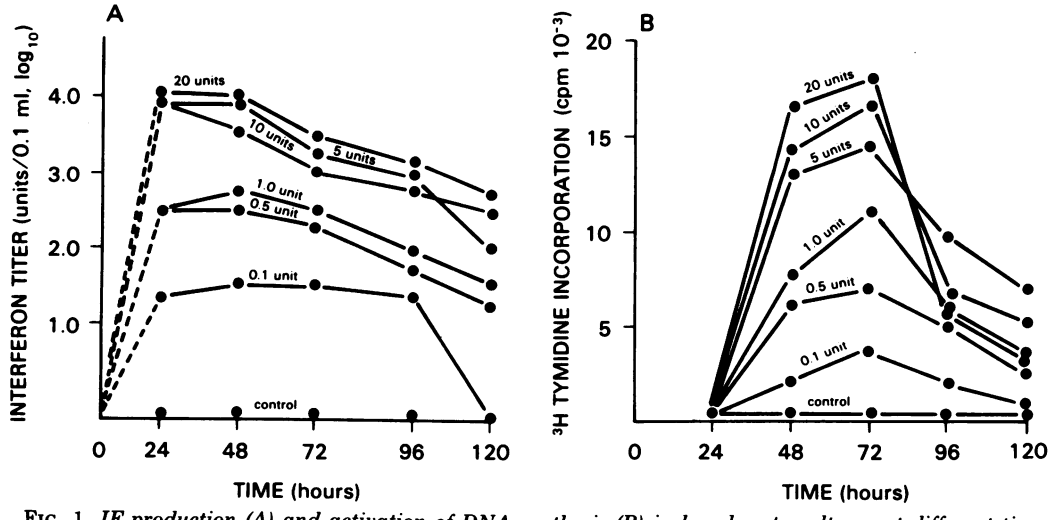

FIG. 1. *IF production (A) and activation of DNA synthesis (B) in lymphocyte cultures at different times after induction with various concentrations of galactose oxidase.*

TABLE 1. *Comparative properties of galactose oxidase-induced IF, leukocyte IF, and immune IF*

Type of IF	% of antiviral activity expressed after:		
	1-h treatment at pH 3.0	Preincubation with antibody to human leukocyte IF	10-min incubation with human WISH cell cultures
Leukocyte	100	0	33
Immune	0	100	0
Galactose oxidase induced	0	100	0

It may be seen that galactose oxidase-induced IF shares with immune IF all the properties tested, and it may therefore be temporarily identifed as immune IF. Final identification will be achieved by determination of other properties, such as molecular weight and sensitivity to mercaptoethanol (13).

DISCUSSION

For the first time, IF production has been obtained after enzymatic treatment of the producing cells, which implies that in this system, oxidation of the membrane galactose residues may be the triggering event for IF induction. The present findings show that galactose oxidase is an extremely potent inducer of immune IF: comparative studies carried out in this and other laboratories, using different mitogens as inducers, have produced substantially lower IF yields (4, 6, 9). Further, with galactose oxidase induction the genetic derepression of the immune IF locus occurs very early, as compared with other inducers, as indicated by the appearance of IF only a few hours after induction. That this IF comes from de novo synthesis and not from the release of preformed material is shown by the inhibition of IF production in cells pretreated with actinomycin D. Induction by the enzyme specifically is shown by the findings that IF induction is prevented by inactivation of the inducer at 60°C or by the cleavage of galactose residues of the cell membrane by pretreatment with β-galactosidase or with neuraminidase followed by β-galactosidase. The hypothesis that IF production could have been stimulated by foreign ribonucleic acid present as a contaminant in the enzyme preparation is invalid because treatment of galactose oxidase with ribonuclease under conditions which would hydrolyze both single- and double-stranded ribonucleic acids did not affect its IF-inducing activity.

This extremely prompt and potent IF production is interesting, not only in view of practical applications, such as large-scale immune IF production, but also because it raises important questions concerning the correlations between IF production and cellular DNA synthesis, which in this system do not appear significantly different from those observed in lymphocytes stimulated with other mitogens. Another interesting question being studied is whether galactose oxidase stimulates IF preferentially over other lymphokines, since our previous studies showed poor production of migration inhibition factor and lymphotoxin in this system (11).

Therefore, we believe, that the identification of at least one specific event which is responsible for activating IF induction may represent an important starting point for a clearer definition of several crucial problems, including: (i) the type(s) of receptors involved in mitogenic, antigenic, or viral induction of IF; (ii) the relationship between IF induction and other parameters of cell activation (lymphokines, DNA synthesis, etc.); and (iii) the availability of the receptors as modulators of cell responses, which will require studies involving treatment with other enzymes (neuraminidase, trypsin, etc.) and aldehyde blocking agents. These problems are now under study.

LITERATURE CITED

1. **Biniaminov, M., B. Ramot, E. Rosenthal, and A. Novogrodski.** 1975. Galactose oxidase induced blastogenesis of human lymphocytes and the effect of macrophages on the reaction. Clin. Exp. Immunol. **19**:93-97.
2. **Blalock, J. E., M. R. Langford, J. Georgiades, and G. J. Stanton.** 1979. Nonsensitized lymphocytes produce leukocyte interferon when cultured with foreign cells. Cell. Immunol. **43**:197-201.
3. **Cantell, K., H. Strander, G. Hadhazi, and H. R. Nevanlinna.** 1968. How much interferon can be prepared in human leukocyte suspensions, p. 223-232. *In* G. Rita (ed.), The interferons. Academic Press Inc., New York.
4. **Dianzani, F., L. Salter, W. R. Fleischmann, and M. Zucca.** 1978. Immune interferon activates cells more slowly than does virus-induced interferon. Proc. Soc. Exp. Biol. Med. **159**:94-97.
5. **Dixon, J. F., J. W. Parker, and R. L. O'Brien.** 1976. Transformation of human peripheral lymphocytes by galactose oxidase. J. Immunol. **116**:575-578.
6. **Epstein, L. B.** 1977. Mitogen and antigen induction of interferon in vitro and in vivo. Tex. Rep. Biol. Med. **35**: 42-56.
7. **Epstein, L. B., H. W. Kreth, and L. A. Herzemberg.** 1974. Fluorescence activated cell sorting of human T and B lymphocytes. Identification of the cell type responsible for interferon production and proliferation in response to mitogens. Cell. Immunol. **12**:407-421.
8. **Green, J. A., S. R. Cooperband, and S. Kibrick.** 1969. Immune specific induction of interferon production in cultures of human lymphocytes. Science **164**:1415-1417.
9. **Johnson, H. M., G. J. Stanton, and S. Baron.** 1977. Relative ability of mitogens to stimulate production of interferon by lymphoid cells and to induce suppression of the in vitro immune response. Proc. Soc. Exp. Biol. Med. **154**:138-142.
10. **Maehara, N., M. Ho, and J. A. Armstrong.** 1977. Differences in mouse interferons according to cell source

and mode of induction. Infect. Immun. **17**:572–579.
11. **Monahan, T. M., and C. W. Abell.** 1978. The requirement for cell surface galactosyl residues during oxidative activation of normal and chronic lymphocytic leukemia lymphocytes. Exp. Cell Res. **116**:47–53.
12. **Novogrodsky, A., and E. Katchalski.** 1973. Induction of lymphocyte transformation by sequential treatment with neuraminidase and galactose oxidase. Proc. Natl. Acad. Sci. U.S.A. **70**:1824–1827.
13. **Osborne, L. C., J. A. Georgiades, and H. M. Johnson.** 1979. Large-scale production and partial purification of mouse immune interferon. Infect. Immun. **23**:80–86.
14. **Rasmunssen, L., and T. Merigan.** 1978. Role of T lymphocytes in cellular immune response during herpes simplex virus infection. Proc. Natl. Acad. Sci. U.S.A. **75**:3957–3961.
15. **Santoli, D., G. Trinchieri, and F. S. Lief.** 1978. Cell mediated cytotoxicity against virus infected target cells in humans. Characterization of the effector lymphocyte. J. Immunol. **121**:526–531.
16. **Trinchieri, G., D. Santoli, and B. B. Knowles.** 1977. Tumor cell lines induce interferon in human lymphocytes. Nature (London) **270**:611–613.
17. **Weelock, E. F.** 1965. Interferon-like virus inhibitor induced in human leukocytes by phytohemagglutinin. Science **149**:310–311.
18. **Wietzerbin, J., S. Stephanos, R. Falcoff, and E. Falcoff.** 1977. Some properties of interferons induced by stimulants of T and B lymphocytes. Tex. Rep. Biol. Med. **35**:205–209.
19. **Youngner, J. S., and S. B. Salvin.** 1973. Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed sensitivity. J. Immunol. **111**:1914–1922.