Enhanced Production of Interferon from Human Lymphoblastoid (Namalwa) Cells Pre-treated with Sodium Butyrate

(Accepted 12 March 1980)

SUMMARY

Pre-treatment of Namalwa cells with 1 mM-sodium butyrate enhanced interferon production induced by Sendai virus. This enhancement was reversed if the cells were incubated in butyrate-free medium. Butyrate treatment increased the rate of interferon production but not its duration. Yields of interferon from Namalwa cells were also enhanced in response to other inducers including the synthetic dsRNA, poly(rI).poly(rC). Butyrate pre-treatment also increased interferon yields from cells of the vervet monkey kidney cell line, V3, but cells of the human diploid fibroblast line, MRC-5, did not respond.

The human lymphoblastoid cell line, Namalwa, is being used increasingly as a source of human interferon (Strander et al. 1975; Bridgen et al. 1977; Johnston et al. 1978). One difficulty found with this cell is that the yields of interferon obtained on induction with Sendai virus can be variable (Johnston et al. 1978), even though culture and induction conditions are apparently held constant. We therefore sought a method of treating Namalwa cells so that interferon titres would be reproducibly high. Johnston (1979) showed that treatment of the cells with 1 mM-sodium butyrate for 48 h enhances interferon yields. This result has been independently confirmed by Adolf & Swetly (1979) who suggested the following alternatives for the mechanism involved: (i) there is an increase in the proportion of cells that are able to make interferon; and (ii) an unaltered proportion of the cells synthesize more interferon, either because the production period is longer, or because of an increase in the efficiency of induction. The results reported here show that the enhanced interferon production is due neither to an extended period of synthesis nor to a direct effect upon the inducer.

For sodium butyrate treatment, Namalwa cells were diluted to a concentration of $1 \times 10^6$ cells/ml in RPMI 1640 medium containing 7% (v/v) calf serum and sodium butyrate was added to 1 mM. After incubation at 37 °C for 48 h, the cells were centrifuged at 800 g and resuspended in RPMI 1640 medium containing 2% (v/v) calf serum, to a concentration of $3 \times 10^6$ cells/ml. To induce formation of interferon 10 ml of cells in a 25 cm² tissue culture flask received 20 haemagglutinating (HA) units/ml of egg-grown Sendai virus and were placed at 37 °C for 24 h.

To investigate the kinetics of interferon production, a stirred culture of cells was treated with 1 mM-sodium butyrate. A control culture was similarly incubated without addition of butyrate. After 48 h, the cells of each culture were distributed in fresh medium into a number of tissue culture flasks and all were induced with Sendai virus. At the subsequent times indicated in Fig. 1 the interferon was harvested from one flask of treated cells and one of control cells. Interferon was first detected at about 2 to 3 h post-induction and production continued until approx. 16 h post-induction. The time period over which interferon was synthesized was approximately the same in both treated and untreated cells. These results show that the enhancement of interferon production by butyrate-treated Namalwa cells is due not to an extended period of production, but rather to an...
increased rate of production. Thus, treatment of Namalwa cells with butyrate increases yields of interferon by a mechanism which is quite distinct from that operating in the superinduction of interferon from fibroblasts (Vilcek & Ng, 1971).

In common with many of the effects described for the action of sodium butyrate on cell lines such as HeLa (Ginsburg et al. 1973; Simmons et al. 1975) and Chinese hamster ovary (Wright, 1973), we found that the enhancement of interferon production from Namalwa cells was reversible. Replicate cultures of Namalwa cells were prepared. Sodium butyrate was added to four of these and after 48 h incubation a butyrate-free culture and one of the butyrate-treated cultures were induced with Sendai virus. These cultures gave interferon titres of 4.05 and 4.57 log_{10} units/ml, respectively. The cells in the remaining butyrate-treated cultures were sedimented and resuspended in fresh growth medium containing no butyrate. One of these cultures was induced on each of the next 3 days and titres of 4.30, 3.95 and 3.92 log_{10} units/ml were obtained at 1, 2 and 3 days, respectively, after butyrate reversal. Butyrate-treated cells therefore produced slightly less interferon when incubated further in butyrate-free medium for 24 h than when induced immediately after butyrate treatment. When incubated for 48 h or more in butyrate-free medium, cells produced only about as much interferon as cells which had never been treated with butyrate.

The enhancement of interferon production by pre-treatment with butyrate has been demonstrated in cells other than lymphoblastoid cells. Results in Table 1 show that when confluent monolayers of V3, a vervet monkey kidney cell line (Christofinis, 1970), were treated with 2 mm-sodium butyrate for 48 h and then induced with Sendai virus, interferon yields were increased almost fivefold. However, when MRC-5 human diploid cells were similarly treated and then induced with Newcastle disease virus (NDV) strain B1, no enhancement of interferon titres was observed. The data in Table 1 also show that the increase in interferon yields from Namalwa cells pre-treated with butyrate is independent of the nature of the inducer. Thus, induction with Sendai virus or NDV strain B1 each gave enhanced interferon yields, although with another virus, Semliki forest virus (SFV), there was no detectable formation of interferon whether or not the cells were butyrate-treated. The synthetic dsRNA, poly(rI).poly(rC), complexed with an equal weight of DEAE-dextran, gave rise to only small amounts of interferon from Namalwa cells; there was nevertheless an enhancement of interferon yields when the cells were pre-treated with
Short communications

Table I. Interferon production by sodium butyrate-treated cells induced with virus and non-virus inducers

<table>
<thead>
<tr>
<th>Cell*</th>
<th>Inducer</th>
<th>Interferon titre, log_{10} units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With butyrate</td>
</tr>
<tr>
<td>MRC-5</td>
<td>NDV BI</td>
<td>&lt;2.20</td>
</tr>
<tr>
<td>V3</td>
<td>Sendai virus</td>
<td>1.32</td>
</tr>
<tr>
<td>Namalwa</td>
<td>Sendai virus</td>
<td>4.56</td>
</tr>
<tr>
<td>Namalwa</td>
<td>NDV BI</td>
<td>3.19</td>
</tr>
<tr>
<td>Namalwa</td>
<td>SFV</td>
<td>&lt;1.22</td>
</tr>
<tr>
<td>Namalwa</td>
<td>poly(rI), poly(rC) + DEAE-dextran</td>
<td>1.66</td>
</tr>
</tbody>
</table>

* MRC-5 and V3 cells were treated with 2 mM-sodium butyrate for 48 h and induced with 10 HA units of NDV BI or 50 HA units of Sendai virus. Namalwa cells were treated with 1 mM-sodium butyrate for 48 h and were induced with Sendai virus at 50 HA units/ml, NDV BI at 10 HA units/ml, SFV at 5 p.f.u./cell, or 50 μg/ml of poly(rI), poly(rC) plus 50 μg/ml DEAE-dextran.

butyrate. Thus, enhancement of interferon yields from treated cells was observed after induction with two different viruses and with a non-virus inducer. We also found that butyrate need not be present during the induction stage (data not shown). Taken together with other observations such as the reversibility of the butyrate effect and the need for a 48 h pre-treatment of the cells, these data strongly suggest that butyrate does not act directly on the inducer but exerts its effect on the host cell.

Like butyrate, pre-treatment of Namalwa cells with bromodeoxyuridine increases yields of interferon from virus-induced Namalwa cells (Tovey et al. 1977). It is of interest that both sodium butyrate and bromodeoxyuridine have been reported to induce the production of Epstein-Barr virus early antigen by those lymphoblastoid cells which carry latently the necessary genetic information (Tovey et al. 1977; Luka et al. 1979). There is no such effect with Namalwa cells which do not contain this information (Klein & Vileck, 1980).

In repeated studies which we have carried out over several years with cells derived from a single mass culture of Namalwa cells, the yields of interferon have been found to vary from time to time by a factor of more than 10. The reason for this is not yet known. The effect of butyrate treatment is to increase yields differentially: batches of cells which in the absence of treatment produce low levels of interferon on induction, then produce far more; batches of cells which give high yields in the absence of butyrate treatment produce only slightly more in its presence. Yields as high as 50,000 units per 10^6 cells have been obtained from butyrate-treated cells. The use of butyrate is therefore of considerable practical importance for the routine production of large amounts of lymphoblastoid interferon.

I would like to thank Mrs E. M. Furze and Mr S. Luker for their excellent interferon assays.

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(Received 11 December 1979)