Isolation of Mutants of Namalwa Cells Differing in their Ability to Produce Interferon

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SUMMARY

We have generated several variants of the human lymphoid cell line Namalwa which were characterized as high producers, non-producers and spontaneous producers of interferon (IFN). All of them were stable for more than 1 year, suggesting chromosomal inheritance of their mutations. We show the kinetics and quantity of IFN production following treatment with several inducers, including polyriboinosinic–polyribocytidylic acid (poly(rI).poly(rC)).

Human lymphoblastoid cell lines, such as the Namalwa line, are excellent tools for studying the induction of interferon (IFN). The cells can be grown to large numbers for biochemical studies and a variety of virus agents will induce them to synthesize IFN in large amounts (Bridgen et al., 1977). However, no mutants have been generated and characterized which would facilitate studies of the mechanisms involved in IFN synthesis. Furthermore, Namalwa cells produce only minute amounts (Tovey et al., 1977) of IFN when induced with poly(rI).poly(rC) despite the fact that this synthetic double-stranded RNA is an excellent inducer in human fibroblasts (De Clercq, 1974).

We describe here the isolation of variants differing in their ability to produce IFN upon induction with various inducers. These variants may facilitate the elucidation of the events leading up to the control of IFN synthesis.

Mutagenesis and cloning were performed as follows. $10^7$ Namalwa cells were treated with 3 mM-ethyl methanesulphonate (EMS) for 2 h at 37 °C at $10^5$ cells/ml in serum-free RPMI 1640 medium (Boehringer, Mannheim). After extensive washing, the cells were incubated in fresh medium supplemented with 8% foetal calf serum (Gibco). After 4 days, about 0.1% of the cells were still alive and started growing. When the cell number approached $10^7$, the cells were mutagenized a second time as described above. As soon as the surviving cells started to grow again, they were distributed into 100 microtitre plates (10 to 30 cells/well). Since about 10 clones arose in one microtitre well, as judged by microscopical observation, we screened of the order of $10^5$ clones. The cells in the microtitre wells were grown to stationary phase before testing for IFN production.

In a parallel experiment $10^7$ Namalwa cells were mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) at a concentration of 1 μg/ml (Thompson et al., 1970). Since there is no positive selection procedure for IFN overproducers or spontaneous producers, we developed a simple procedure for screening large numbers of cells. We set up the experiment so that only overproducers gave positive results above the limit of detection of the assay. In each assay series, a standard (B69/19, National Institute of Biological Standards and Control, Holly Hill, Hampstead, London, U.K.) of 25 international units (IU) α-IFN/ml was included. Under the conditions used, non-induced Namalwa cells gave 10 to 30 IU IFN/ml. The culture supernatants were diluted eightfold into the IFN assay. In this way most of the wells which were protected from lysis after vesicular stomatitis virus (VSV) infection contained supernatant fluids from overproducers or spontaneous producers of IFN.

In a first experiment, supernatant fluids of uninduced, mutagenized cells were tested for spontaneously produced IFN. Two protected wells were found: 49E5 and 69A2. On further
Table 1. Comparison of the IFN induction of different mutants of Namalwa cells by different inducers

<table>
<thead>
<tr>
<th>Variant</th>
<th>Interferon induced (IU/ml) in the presence of</th>
<th>NDV*</th>
<th>Sendai virus*</th>
<th>Poly(rI).poly(rC)†</th>
<th>No inducer‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 HA</td>
<td>200 HA</td>
<td>100 HA</td>
<td>25 µg/ml</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>6E2-6</td>
<td>7000§</td>
<td>24000</td>
<td>60</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td>72H7-26</td>
<td>8000</td>
<td>32000</td>
<td>250</td>
<td>150</td>
<td>ND</td>
</tr>
<tr>
<td>12C5-13</td>
<td>10000</td>
<td>27000</td>
<td>470</td>
<td>75</td>
<td>ND</td>
</tr>
<tr>
<td>24F3-18</td>
<td>9600</td>
<td>25600</td>
<td>3100</td>
<td>150</td>
<td>23000</td>
</tr>
<tr>
<td>35F6-14</td>
<td>0</td>
<td>1600</td>
<td>200</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>49E5-36</td>
<td>400</td>
<td>1600</td>
<td>200</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>69A2-3</td>
<td>ND</td>
<td>ND</td>
<td>600</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Namalwa</td>
<td>2000</td>
<td>4500</td>
<td>2200</td>
<td>10</td>
<td>4400</td>
</tr>
</tbody>
</table>

* Haemagglutination units (HA) of NDV or Sendai virus per 10⁶ cells; cells were pretreated for 2 days with 1 mM-sodium butyrate, and then for 2 h with virus and finally incubated in fresh medium for 22 h.
† Cells were treated with 1 mM-butyrate for 48 h, followed by poly(rI).poly(rC) plus 200 µg/ml DEAE-dextran.
‡ IU/ml/10⁶ cells in 24 h.
§ IU/10⁶ cells; average of three to seven measurements.
|| ND, Not done.

analysis both of them spontaneously produced IFN. In a second series of tests the cells of each well were induced with 25 µg/ml poly(rI).poly(rC) (Boehringer, Mannheim) in serum-free medium. At 8 h after induction, 25 µl of a pancreatic RNase solution (10 µg/ml in phosphate-buffered saline), was added to each well and the incubation was continued for a total of 20 h at which time the supernatants were assayed for IFN according to the semi-micro dye-binding assay of Armstrong (1971). Briefly, 50 µl of RNase-treated supernatant of the induced cells were added to microtitre plates containing IFN-sensitive L-132 human lung epithelial cells. The plates were incubated overnight at 37 °C. VSV was then added at a concentration of 500 p.f.u./well and incubation was continued for an additional 24 h at 37 °C. The assay was terminated by staining the unlysed cells with crystal violet in 50% methanol containing 5% formaldehyde. All titres are expressed in IU of human IFN-α using the B69/19 standard.

Four positive wells were found: 12C5, 24F3, 6E2 and 72H7. The cells from these wells were cloned in semi-solid medium containing 1% methyl cellulose (Iscoe & Schreier, 1979). The cloning efficiency in RPMI 1640 medium containing 1% methyl cellulose was close to 30%. In a parallel experiment, it was not possible to clone the same Namalwa cells either on agar plates or by limit dilution in microtitre plates.

Individual colonies were picked from the plates and grown in microtitre plates. Induction and screening were performed as described above. Table 1 shows a selected group of clones and the properties of their IFN production following induction by different inducers. Compared to Namalwa cells, clones 6E2-6, 12C5-13, 24F3-18 and 72H7-26 produced 3.5 to 6 times more IFN upon Newcastle disease virus (NDV) induction at both concentrations tested. On the other hand, when induced with Sendai virus, clones 6E2-6, 12C5-13 and 72H7-26 produced less IFN than Namalwa cells while production in 24F3-18 clone was about the same.

The two clones spontaneously producing several hundred IU IFN (49E5-36 and 69A2-3) did not respond to poly(rI).poly(rC) or to poly(rI).poly(rC) in combination with DEAE-dextran and butyrate pretreatment. While screening for overproducers, we found a variant, 35F6-14, lacking the ability to produce IFN. This mutant is resistant to
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Fig. 1. Kinetics of IFN production by different inducers. Fifty ml cultures of Namalwa cells (10⁶ cells/ml) were pretreated for 44 h with 10⁻³ M-sodium butyrate. IFN was induced by 25 HA/ml Sendai virus (●), 100 HA/ml NDV (▲), or 100 μg/ml poly(rI).poly(rC) in combination with 250 μg/ml DEAE-dextran (○). Samples were taken at the times indicated and stored at pH 2 at 4 °C for 2 days before assaying.

concentrations of poly(rI).poly(rC) which are toxic for normal Namalwa cells (≥250 μg/ml). The resistance to double-stranded RNA was used as a positive selection for non-producers. Mutagenized cells were incubated in RPMI 1640 medium containing 250 μg/ml poly(rI).poly(rC). After several days all but a few cells died. The remaining ones started to grow after 6 days in the presence of the high concentration of poly(rI).poly(rC). One of the surviving cells, designated 11E6, lacked the ability to produce IFN upon induction as did clone 35F6-14.

An interesting observation was made with respect to the kinetics of α-IFN production after poly(rI).poly(rC) induction. Human fibroblasts produce β-IFN within hours after poly(rI).poly(rC) induction (Kohase & Vilček, 1977), but much later after virus induction (Havell & Vilček, 1972). In contrast to fibroblasts, Namalwa cells as well as mouse L-929 cells (Trapman, 1979) show the same kinetics of IFN production upon both poly(rI).poly(rC) and NDV induction, while Sendai virus-induced IFN appeared very rapidly (Fig. 1). The kinetics of IFN production in response to the different inducers were compared between Namalwa cells and the mutants listed in Table 1. No differences were found between the parent and mutant cells (data not shown), although the amount of IFN produced varied between different mutants as shown in Table 1.

Using rabbit antibodies directed against human IFN-α (kindly provided by K. Paucker) we analysed the IFN produced by the variants listed in Table 1. IFN was precipitated with the antibodies, pelleted and both the supernatant fluid as well as the pellet (dissolved in glycine-HCl pH 3) were analysed. IFN from all variants was quantitatively precipitated by the antibodies (90 to 100%) and the supernatants were devoid of any measurable IFN, suggesting that the variants produced IFN-α irrespective of the type of inducer. It remains to be shown by monoclonal antibodies whether spontaneously produced IFN-α and IFN produced upon Sendai virus induction belong to the same subspecies as the IFN-α produced upon NDV or poly(rI).poly(rC) induction.

The variants listed in Table 1 fell into three categories, namely high producers, non-producers and spontaneous producers of IFN. The four high producers showed comparable levels of IFN production after NDV induction, but reacted differently when induced with Sendai virus. Neither higher nor lower amounts of Sendai virus yielded higher IFN titres than those shown in Table 1. Only clone 24F3-18 produced IFN in comparable
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amounts to Namalwa cells when induced with Sendai virus. The other three clones produced rather small amounts and 6E2-6 produced almost no IFN. The data shown in Table 1 for poly(rI).poly(rC) induction were in the absence of butyrate, but for some clones also in the presence of DEAE-dextran and butyrate pretreatment. These results were comparable to those obtained by NDV induction.

The non-producer mutant 35F6-14 did not respond to any of the inducers tested. These cells clearly lost the ability to produce IFN, but so far we do not know the location of the mutation along the path of IFN induction and production.

The group of spontaneous IFN producers, represented by clones 49E5-36 and 69A2-3 in Table 1, are inducible by viruses but not by poly(rI).poly(rC). Another lymphoblastoid cell line, LukII, isolated by Pickering et al. (1980) and producing IFN spontaneously, can also be induced by viruses, but poly(rI).poly(rC) induction has not been described. We do not know at present if there is any direct correlation between spontaneous IFN production and the failure of poly(rI).poly(rC) to induce IFN.

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