Selection and Characterization of an Interferon-responsive Clonal Cell Line of HeLa Cells

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SUMMARY

HeLa cells generally do not respond well to interferon (IFN). We have used is-1, an IFN-sensitive mutant of mengovirus, to select a clone of IFN-responsive HeLa cells (F-H12). At moderate levels of human α/β IFN, is-1 yields were fivefold lower in these cells than in similarly protected control cells. In contrast, wild-type mengovirus, vesicular stomatitis virus and a wild-type and thymidine kinase-negative strains of herpes simplex virus type 1 grew equally well in both cell lines. By a cell survival assay, the F-H12 line was up to 100 times more responsive to IFN than the parental line when challenged by is-1. 2'-5'-Oligo(A)-dependent endonuclease activity was the same in both lines. These observations cannot be accounted for by enhanced induction of IFN following infection.

INTRODUCTION

Interferon (IFN) suppresses a wide variety of viruses, both DNA and RNA, at all levels of growth (i.e. penetration, uncoating, replication and release). However, the antiviral and antiproliferative effects of IFN vary considerably among cell lines (Adams et al., 1975; Baglioni et al., 1982; Sen & Herz, 1983). It is quite possible for a protected cell to be resistant to infection by one virus and not another (Fout & Simon, 1981, 1983; McMahon & Kerr, 1983; Sen & Herz, 1983; Czarniecki & Allen, 1984). Attempts to define a standard and uniform set of events that must occur to attain an IFN-mediated antiviral state have been unsuccessful, and its has become clear that, to a considerable extent, each cell line has a unique response to IFN (Baglioni et al., 1982; Cayley et al., 1982; Daher & Samuel, 1982; Silverman et al., 1982; McMahon & Kerr, 1983; Sen & Herz, 1983; Whitaker-Dowling & Youngner, 1984, 1986).

The best characterized pathways in the IFN-mediated antiviral response are the double-stranded RNA-dependent protein kinase pathway and the double-stranded RNA-dependent endonuclease pathway (e.g. see DeClercq, 1982; McMahon & Kerr, 1983; Revel, 1984; Samuel et al., 1984; Whitaker-Dowling & Youngner, 1986). But there is evidence for other pathways as well (Knight & Korant, 1979; Rubin & Gupta, 1980; Chebath et al., 1983; Fout & Simon, 1983; Dron et al., 1985).

In 1976 Simon et al. isolated an IFN-sensitive mutant of mengovirus which they designated is-1. This mutant was found to have a number of unique properties. In the absence of IFN it grew almost as well as the wild-type (is+), but in the presence of 10 to 20 units/ml IFN only 1% of the cells yielded virus. The rest survived. In contrast, all is+-infected cells died and the yield of virus, although delayed, was similar to that of the untreated controls. In mixed infection, is+ was dominant to is-1, and both were equally effective in inhibiting cellular RNA and protein synthesis, either in the presence or absence of IFN. The is-1 phenotype is expressed only in certain sublines of mouse L cells. Furthermore, addition of actinomycin D to protected cells immediately before infection phenotypically converts is-1 into is+. The growth of is+ is not affected. Based on these observations and others, Fout & Simon (1983) suggested that is-1 was responding to a unique aspect of the IFN-induced antiviral state.
The isolation of cell lines with altered IFN responses has provided an important tool for studying the mode of action of IFN. Many of these cellular mutants have been IFN-resistant lines (Chany & Vignal, 1970; Gresser et al., 1974; Kuwata et al., 1976; Silverman et al., 1982; Affabris et al., 1982; Dron & Tovey, 1983; Dron et al., 1985). HeLa cells generally do not respond well to IFN. We have used \( \text{is}^{-1} \) to select an IFN-responsive line (F-H12) of HeLa cells. Preliminary karyotypic analysis has revealed that the two lines are very similar, although it appears that cells of the F-H12 line have, on average, one more chromosome than a clone of the parental cell line.

**METHODS**

**Cell lines.** H1 line HeLa cells, obtained from Dr R. R. Rueckert (University of Wisconsin, Madison, Wis., U.S.A.), were cultivated in Eagle’s essential growth medium supplemented with 10% newborn calf serum (KC Biological). A subclone of L929 mouse fibroblast cells (designated G3), isolated from an original stock obtained from Dow Chemical and Pharmaceutical Co. (Zionsville, Ind., U.S.A.), was used for all mengovirus plaque assays.

*Interferon.* Human \( \alpha \) or \( \beta \) IFN was obtained either from Dr W. Stewart II (Sloan-Kettering Institute, New York, N.Y., U.S.A.) or the National Institute of Allergy and Infectious Diseases (NIH, Bethesda, Md., U.S.A.). These samples were expressed in terms of human leukocyte reference (Ga 23-902-530) units as established by NIH. Human \( \gamma \) IFN was obtained from Dr W. R. Fleischmann (University of Texas, Galveston, Tx., U.S.A.). All IFN samples were stored at \(-100 \, ^\circ\text{C} \), with the exception of the \( \gamma \) IFN which was stored at \( 4 \, ^\circ\text{C} \).

**Viruses.** The origin of \( \text{is}^{-1} \), an IFN-sensitive mutant of mengovirus, has been described by Simon et al. (1976). The virus was assayed as described by Fleischmann & Simon (1973). All virus stocks were stored at \(-100 \, ^\circ\text{C} \).

**Isolation of the F-H12 clonal line.** An IFN gradient of 1 to 100 units/ml human \( \alpha \) or \( \beta \) IFN was set up in 96-well microtitre trays (Nunc). Approximately \( 5 \times 10^5 \) H1 line HeLa cells were then added to each well. Twenty-four h later the IFN was removed, and the cells were washed with phosphate-buffered saline (PBS; 0.137 M-NaCl, 0.003 M-KCl, 0.008 M-Na2HPO4, 0.0015 M-KH2PO4, 0.001 M-CaCl2, 0.003 M-MgCl2, pH 7.2), and infected with \( \text{is}^{-1} \) or \( \text{is}^{+} \) at a m.o.i. of 10 p.f.u./cell in 0.025 ml, unless noted otherwise. Approximately 0.1 ml of fresh medium was added to each well immediately following infection. The cells were examined for c.p.e. at 24 and 48 h. (C.p.e. values are expressed as a percentage of apparently surviving cells as determined by direct counts of representative microscope fields.) Virus was then removed, cells were washed with PBS, and rescued by adding anti-mengovirus antibody to a final concentration sufficient to reduce the virus titre 103-fold in 1 h at 37 \( ^\circ\text{C} \). Five days later, cells at the lowest IFN pretreatment level that permitted survival (12.5 units/ml IFN) were transferred. Antibody was maintained until the cells were free of mengovirus (three or four passages). Cells from this new subline (AB) were treated with lower IFN levels (ranging from 1 to 50 units/ml), infected and rescued as before along with unselected H1 line controls. Survivors (subline C) were obtained from wells pretreated with 3.1 units/ml IFN. This procedure was repeated three more times, with survivors appearing successively at 0.8, 0.2 and 0.2 units/ml IFN. This procedure yielded the D, E and F HeLa sublines. The F-H12 clone was isolated from the latter line. For comparison, a clone (designated NOR) was also isolated from the H1 parental line.

**Cell survival assay.** Cells (\( 1 \times 10^5 \)) were added to 1 cm Nunc multidish wells and treated with different concentrations of IFN. The wells were infected with \( \text{is}^{-1} \) or \( \text{is}^{+} \) and rescued at 48 h as described previously, except that a viral m.o.i. of 1-0 was used. Percentage cell survival at 48 h was recorded (Table 1). Five days after rescue, the number of clones in each well was determined.

**Mengovirus inactivation and IFN induction assay.** Mengovirus (\( \text{is}^{-1} \) or \( \text{is}^{+} \)) was inactivated at pH 2.0 (E. A. Bakich & E. H. Simon, unpublished method). Supernatants from infected cell monolayers that had been pretreated with zero or 20 units/ml IFN were acidified to pH 2.0 with 1.0 M-HCl and then incubated at 37 \( ^\circ\text{C} \) for 4 h. The pH was adjusted back to 7.0 with 1.0 M-NaOH. As a control, 20 units/ml IFN and virus (m.o.i. 10) were added to medium from uninfected cell monolayers. This was inactivated with the other samples. Supernatants from infected cell monolayers that had been pretreated with \( \text{is}^{-1} \) or \( \text{is}^{+} \) at a m.o.i. of 10 p.f.u./cell in 0.025 ml, unless noted otherwise. Approximately 0.1 ml of fresh medium was added to each well immediately following infection. The cells were examined for c.p.e. at 24 and 48 h. (C.p.e. values are expressed as a percentage of apparently surviving cells as determined by direct counts of representative microscope fields.) Virus was then removed, cells were washed with PBS, and rescued by adding anti-mengovirus antibody to a final concentration sufficient to reduce the virus titre 103-fold in 1 h at 37 \( ^\circ\text{C} \). Five days later, cells at the lowest IFN pretreatment level that permitted survival (12.5 units/ml IFN) were transferred. Antibody was maintained until the cells were free of mengovirus (three or four passages). Cells from this new subline (AB) were treated with lower IFN levels (ranging from 1 to 50 units/ml), infected and rescued as before along with unselected H1 line controls. Survivors (subline C) were obtained from wells pretreated with 3.1 units/ml IFN. This procedure was repeated three more times, with survivors appearing successively at 0.8, 0.2 and 0.2 units/ml IFN. This procedure yielded the D, E and F HeLa sublines. The F-H12 clone was isolated from the latter line. For comparison, a clone (designated NOR) was also isolated from the H1 parental line.

**Binding assay.** Monolayers of 1 x 10⁶ cells were set up in 1 cm Nunc multidish wells in the presence or absence of IFN and incubated for 24 h. Medium was then removed, wells were washed with PBS and infected with \( \text{is}^{-1} \) or \( \text{is}^{+} \) at an m.o.i. of 0-1, 1-0 or 10 p.f.u./cell. Forty-eight h later, supernatants were collected and virus was inactivated at low pH as described.

To assay IFN, twofold dilutions of the supernatants were dispensed into microtitre trays. IFN of known concentration was included as a control. Approximately 2 x 10⁶ F-H12 cells were added to each well, incubated for 24 h, and infected with \( \text{is}^{-1} \) at an m.o.i. of 4 p.f.u./cell. Percentage c.p.e. was recorded 24 h later.

**IFN binding assay.** Monolayers of 3 x 10⁶ cells in 6 cm Nunc Petri plates were incubated with 1000 units IFN in a volume of 1-0 ml for 2 h at 4 \( ^\circ\text{C} \). Medium was removed and cells were washed twice with cold PBS. Cells were scraped into 1-0 ml of cold medium and spun down. The supernatant was decanted, the cells were resuspended in
Selection of interferon-responsive HeLa cells

Table 1. Percentage c.p.e. observed in NOR and F-H12 clonal lines and two intermediate non-clonal lines 48 h after infection with is-1*

<table>
<thead>
<tr>
<th>IFN pretreatment (units/ml)</th>
<th>0 0.005 0.01 0.04 0.08 0.15 0.31 0.62 1.25 2.5 5 10 20 40 80 160</th>
<th>NOR</th>
<th>C line</th>
<th>E line</th>
<th>F-H12</th>
</tr>
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<tbody>
<tr>
<td>45</td>
<td>0 0 0 0 0 0 0</td>
<td>20</td>
<td>10</td>
<td>15</td>
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</tr>
<tr>
<td>5</td>
<td>- - - - - -</td>
<td>10</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>- - - - - -</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>- - - - - -</td>
<td>1</td>
<td>1</td>
<td>5</td>
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<tr>
<td>1</td>
<td>- - - - - -</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>- - - - - -</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* The symbol (-) indicates a cell line was not tested at this IFN level.

0·2 ml cold PBS, and sonicated for 3 min in a Heat Systems-Ultrasound, Inc. sonic bath. Tenfold dilutions were made of each sonicate and IFN was assayed as described above, except that an m.o.i. of 0·1 p.f.u./cell of is-1 was used.

Ribosomal RNA assay for 2'-5'-oligo(A)-dependent endonuclease. The procedure was a modification of that described by Wreschner et al. (1981), as developed by J. G. Calvert in our laboratory. Cells were grown on 35 x 10 mm Falcon tissue culture dishes to a concentration of 10^6 to 2 x 10^6 per plate (to give 10 to 20 μg RNA). The cells were treated with 20 units/ml human α/β IFN and infected with is-1 or is* at an m.o.i. of 20. Pellets were suspended in 0·3 ml saturation buffer [0·1 M-Tris-HCl pH 8·0, 1·0 mM-EDTA, 0·2% (v/v) 2-mercaptoethanol] and 0·044 ml 3 M-sodium acetate pH 5·2 was added to them. After gentle shaking, 0·6 ml of a 1 : 1 mixture of phenol/chloroform saturated with aqueous (saturation) buffer, and containing 0·1% 8-hydroxyquinoline was added. Samples were allowed to stand at room temperature for 10 min with periodic vortex-mixing. They were then centrifuged for 10 min in a microfuge, and the upper aqueous phase was collected. RNA was precipitated, denatured with formamide and subjected to constant voltage electrophoresis in 1·8% agarose gels containing 2·2 M-formaldehyde. The gels were stained (1 μg/ml ethidium bromide) and visualized according to standard procedures.

Chromosome analysis. The NOR and F-H12 HeLa lines were karyotyped using the trypsin-Giemsa banding technique of Seabright (1971). Tightly contained spreads were analysed to determine the average number of chromosomes in each line.

RESULTS

Selection of a HeLa line with increased IFN response

After five cycles of selection, a limit to IFN-responsiveness was reached. Both the E and F sublines were grown in wells from survivors which had been primed with 0·2 units/ml α/β IFN prior to infection with is-1. No cells could be successfully rescued and cured of mengovirus at lower IFN concentrations. The E line appeared to be more IFN-responsive than the E line at the time it was selected. Both lines came from a mixture of cells, and there appeared to be a gradual loss of the phenotype upon passage. The E line may have lost some degree of sensitivity by the time the F line was selected and tested. A second F line was rescued at the 0·05 unit/ml IFN pretreatment level, but could not be maintained. The F-H12 line was subcloned from the F line immediately after selection of the latter, and was found to be equally or more IFN-responsive than two subsequent lines (G and H). Of all HeLa sublines tested, F-H12 was the most responsive to IFN.

Increased cell survival in F-H12

Discrete clones could only be counted in wells that contained relatively few surviving cells upon rescue (i.e. 90% c.p.e. or greater). This was found to be the case particularly for the NOR line, which divided more rapidly than the others. Fig. 1 illustrates some typical clones observed in our rescue experiments. In general, no more than 300 clones/well could be counted 5 days after rescue. IFN responsiveness was selected for quickly since it was seen that 1·25 and 0·6 units/ml IFN pretreatment of the C, E and F-H12 lines resulted in comparable numbers of surviving clones (Fig. 2). However, at the lower IFN pretreatment levels (0·3 to 0·005 units/ml α/β IFN), significantly greater survival was consistently noted in the F-H12 line.

The values plotted reflect the number of surviving clones that grew, minus the background numbers (survivors in 0 units/ml IFN wells). In general, there was no background in the NOR
Fig. 1. Example of some typical clones observed 5 days after rescue. F-H12 clones in a well were pretreated with 0.6 μg/ml α/β IFN and infected with is-1 at an m.o.i. of 1.0 p.f.u./cell. Clones of both lines were discrete and well separated. Bar marker represents 100 μm.

line, 13 clones in the C and E lines, and 10 clones in the F-H12 line. The IFN levels at which 50 clones were obtained were used as a means of comparing the IFN-responsiveness of NOR and F-H12 lines, since this number fell well above background counts. Furthermore, this was deemed to be a more accurate estimate than comparisons of percentage confluence 5 days after rescue, as the NOR line divided in 25 h compared to 31 h for the F-H12 line.

Using the 50 clone endpoint, in four independent experiments the F-H12 line was 30- to 100-fold more IFN-responsive than the NOR line. No clones were observed when either line was infected with is+ (highest IFN pretreatment level 40 units/ml). In similar experiments using γ IFN, the F-H12 line was about 16 times as IFN-responsive as the NOR line (Fig. 3).

Growth of mengovirus in the NOR and F-H12 cell lines

It was evident that the is-1 and is+ yields were decreased by increasing levels of IFN for both lines, and that yields of is-1 were less than those of is+ (Fig. 4). However, the difference between them is much less than the 50- to 200-fold difference seen in the G3 subline of mouse L cells (Simon et al., 1976; Fout & Simon, 1983). Yields of vesicular stomatitis virus were not suppressed to any significant extent in either the NOR or F-H12 line after pretreatment with up to 100 units/ml IFN (data not shown). Likewise, no difference in yields of either wild-type or thymidine kinase-negative strains of herpes simplex virus type 1 was observed in either line following pretreatment with 10 units/ml IFN (R. W. King, personal communication). In the absence of IFN, yields of is-1 in both lines were almost the same. Pretreatment with 1.0 unit/ml IFN or more resulted in an average fivefold decrease of is-1 yields in F-H12 relative to NOR. Yields of is+ were reduced by a factor of less than 2.
Selection of interferon-responsive HeLa cells

Fig. 2. Clone recovery from α IFN-treated NOR line (○), 'C' line (●), 'E' line (□) and F-H12 line (△) HeLa cells infected with is-1 and rescued with mengovirus antibody. Numbers of clones shown at each IFN level represent total counted minus background. No clones were ever observed in wells infected with is+.

Fig. 3. Clone recovery from γ IFN-treated NOR line (○) and F-H12 line (△) HeLa cells infected with is-1 and rescued with anti-mengovirus antibody. Numbers of clones represent total minus background. No clones were ever obtained in wells infected with is+.
A more detailed experiment shows that the kinetics of is⁻¹ and is⁺ growth were virtually identical in both lines in the absence of IFN (Fig. 5). A different pattern was seen in cells pretreated with 10 units/ml IFN. In the NOR line, both is⁻¹ and is⁺ yields peaked at 36 h. In the F-H12 line, yields of neither virus peaked until 60 h; however, is⁻¹ yields were 10-fold lower than is⁺ yields until after 48 h. Fig. 5 presents data from one of six similar experiments, all of which gave comparable results.

**IFN induction by is⁻¹ and is⁺**

The pH inactivation procedure described in Methods effectively destroyed mengovirus without significantly lowering the IFN titre (see IFN and IFN/virus controls in Table 2). Low levels of IFN were induced in NOR and F-H12 cells by is⁻¹, but only if they had first been pretreated with IFN (data from non-pretreated samples not shown). Is⁺ did not induce IFN under any circumstances.

**IFN binding**

In experiments using two different IFN preparations, on NOR cells and two F-H12 sublines, there was no difference in the amount of IFN bound to the cells (data not shown).

**2′-5′-Oligo(A)-dependent nuclease activity**

Evidence of endonuclease activity was found in IFN-treated cells of both the F-H12 and NOR lines. RNA was extracted 8, 10 and 12 h after infection with is⁻¹ or is⁺. Fig. 6 shows that similar amounts of rRNA cleavage products were obtained in both lines 10 and 12 h after infection with either virus.

Two rRNA degradation products were observed. The first migrated slightly ahead of the 18S ribosomal subunit, and the second slightly ahead of the 28S subunit. Treatment of cells with IFN
Selection of interferon-responsive HeLa cells

Fig. 5. Mengovirus yields from NOR HeLa cells infected with is-1 (○) and is+ (●), and from F-H12 HeLa cells infected with is-1 (△) and is+ (▲). (a) Virus yield after no IFN pretreatment; (b) virus yield following 24 h pretreatment with 10 units/ml human α/β IFN.

Table 2. Induction of IFN by mengovirus in F-H12 and NOR cells*

<table>
<thead>
<tr>
<th>Line</th>
<th>M.o.i.</th>
<th>Virus</th>
<th>Dilution†</th>
<th>Titre (u/ml)</th>
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<td>IFN control (40 u/ml)</td>
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<td>–</td>
<td>50 40 30 25 15 5</td>
<td>40</td>
</tr>
<tr>
<td>pH-treated IFN control (20 u/ml)</td>
<td>–</td>
<td>–</td>
<td>35 25 15 9 0 0</td>
<td>10–20</td>
</tr>
<tr>
<td>pH-treated IFN control (20 u/ml) and 10⁶ is-1</td>
<td>–</td>
<td>–</td>
<td>35 25 15 9 0 0</td>
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</tr>
<tr>
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<td>is-1</td>
<td>0 0 0 0 0 0</td>
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</tr>
<tr>
<td></td>
<td>is+</td>
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<td>0</td>
<td></td>
</tr>
<tr>
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<td>1:0</td>
<td>is-1</td>
<td>5 3 0 0 0 0</td>
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<td>is+</td>
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</tr>
<tr>
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<td>30 20 5 0 0 0</td>
<td>5–10</td>
</tr>
<tr>
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<td>is+</td>
<td>0 0 0 0 0 0</td>
<td>0</td>
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</tr>
<tr>
<td>F-H12‡</td>
<td>0:1</td>
<td>is-1</td>
<td>0 0 0 0 0 0</td>
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<tr>
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<td>1:0</td>
<td>is-1</td>
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<td>0:5–1:0</td>
</tr>
<tr>
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<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:0</td>
<td>is-1</td>
<td>25 15 5 0 0 0</td>
<td>5–10</td>
</tr>
<tr>
<td></td>
<td>is+</td>
<td>0 0 0 0 0 0</td>
<td>0</td>
<td></td>
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</table>

* F-H12 cells in microtitre trays were pretreated for 24 h with inactivated supernatant fluid from infected cells and then challenged with is-1 at an m.o.i. of 4. See Methods.
† Values expressed as percentage cells surviving at 24 h after challenge.
‡ Cells pretreated with 20 u/ml IFN prior to infection.
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Fig. 6. Ribosomal RNA cleavage products detected in is-l- or is+-infected F-H12 cells (lanes 1 to 6) and NOR cells (lanes 7 to 12). All samples were pretreated with 20 units/ml α/β IFN except those in lanes 1 and 7. All samples were infected at an m.o.i. of 20 p.f.u./cell except lanes 1, 2, 7 and 8. Samples in lanes 3 and 9 (is-l-infected) and 4 and 10 (is+-infected) were harvested at 10 h after infection and samples in lanes 5 and 11 (is-l-infected) and 6 and 12 (is+-infected) were harvested at 12 h after infection. The positions of cleavage products 1 and 2 are indicated with arrows.

alone did not activate the endonuclease, as can be seen in the uninfected control lanes. Similarly, without IFN pretreatment, cleavage products were never detected.

Karyotypes

The F-H12 and NOR lines appeared to have very similar karyotypes. However, based on an analysis of 21 spreads, the F-H12 line showed a mode of 67 and a secondary mode of 65 chromosomes/cell. Based on 22 spreads, the NOR line showed a mode of 66 and a secondary mode of 64. The extra chromosome in the F-H12 line appeared to be one of the four small chromosomes that comprise the F and G series.

Discussion

The F-H12 line represents the most IFN-responsive cells which can be selected from the H1 parental line by means of the is-l mutant. With this system, F-H12 was observed to be up to 100 times more sensitive to IFN than the parental line. Yet, in general, F-H12 is not particularly responsive to IFN since wild-type mengovirus, wild-type and thymidine kinase-negative strains of herpes simplex virus type 1 and vesicular stomatitis virus (which is generally highly sensitive to IFN), grow no differently in this line than in the unselected NOR line. Furthermore, even using is-l, the F-H12 line is no more responsive to IFN than the standard human diploid fibroblast line, FS11 (Weissenbach et al., 1979). Nevertheless, it is apparent that some aspect of the IFN-mediated antiviral state is altered in the F-H12 line since there are marked differences in the cell survival assay (Fig. 2), and in the kinetics of is-l replication in protected cells (Fig. 5). Simon et al. (1976) suggested that is-l responds to a different IFN-mediated host function than is+, and demonstrated that the 'is' phenotype is recessive in mixed infection with is+. Fout & Simon (1983) have proposed that mouse L cells have two classes of antiviral activity: one which acts on both is+ and is-l (AVA-1) and one which acts on only is-l (AVA-2). AVA-2 appears to be a previously uncharacterized, separate IFN-mediated activity. The IFN-responsive phenotype of the F-H12 line is specific for is-l and therefore (quite possibly) AVA-2. This system should prove useful for identifying specific aspects of the IFN-induced antiviral state.
Selection of interferon-responsive HeLa cells

The cell survival assay provided a more quantitative and sensitive measure of IFN activity than the standard c.p.e. determination. In this assay, the number of surviving clones was determined 7 days following infection in cells protected by different levels of IFN. In some respects, the method is too sensitive since the NOR line had a tendency to show 30 or 40 surviving clones at one IFN level and partial confluence at the next. This is probably because it grows more rapidly than the F-H12 line (25 h compared to 31 h). The extra division will reduce the maximum number of clones that can be counted at 7 days by a factor of two.

Is-1, but not is+ can induce IFN in mouse L cells pretreated with IFN (Marcus et al., 1981; Simon et al., 1984). However, the amount induced varies widely from line to line. Is the F-H12 phenotype due to IFN induction? As seen in Table 2, slightly higher concentrations of IFN are induced in the NOR line than in F-H12. Furthermore, induction only occurred in cells pretreated with at least 10 units/ml α/β IFN. Hence, it cannot explain the observed difference between the lines.

Ribosomal RNA cleavage was observed in protected cells, but not until 10 to 12 h post-infection. This is in contrast to the results of Rice et al. (1985) who found that encephalomyocarditis virus induced degradation of the 18S subunit 6 h post-infection. This may be related to the relatively slow growth of the virus in our system (Fig. 4 and 5). However, our results are consistent with the findings of Cayley et al. (1982) and Silverman et al. (1982) who find that in spite of the high endogenous levels of (2-5)Aₙ synthetase in HeLa cells, no rRNA is degraded unless cells are both treated with IFN and infected with virus. It is evident that differences in IFN responsiveness in our HeLa lines cannot be accounted for by the degree of activation of the (2-5)Aₙ system.

Investigations into the basis of the increased IFN responsiveness of our F-H12 HeLa line are continuing. The ease with which the mutant was isolated suggests that the mutation was a single-step event. Although it is possible that a point mutation was involved, a gain or loss of an entire chromosome seems more likely. While karyotypic analyses are too preliminary to point to a specific chromosome, an attractive possibility is chromosome 21, which contains the genes of IFN receptors. However, this would suggest that F-H12 should respond equally to all viruses and this is not the case. Furthermore, binding assays indicate that all of our lines have the same number of receptors. The molecular basis for AVA-2 remains to be determined, but studies in L cells indicate that there is a direct attack on the viral RNA. Similar studies in the F-H12 line should prove to be informative.

The cell survival assay using 50 clones as the endpoint has been used to measure IFN in human serum generously supplied by Dr M. Krim (Sloan-Kettering Institute). The results were up to fivefold more sensitive than the standard IFN assay using human diploid fibroblasts. This is not enough improvement to merit replacement of the standard c.p.e. assay which can be read in 24 h. However, we selected our F-H12 line from a relatively IFN-insensitive parental line. Our selection procedure and assay methods might be useful in generating a 'super-sensitive' cell line from a more IFN-responsive parent.

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REFERENCES


