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A Small Fraction of Cells Communicates the Maximal Interferon Sensitivity to a Population (40622)

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It is generally accepted that the action of interferon results from a direct effect of the molecule on each responding cell. This concept has recently been questioned by our demonstration of the transfer of interferon-induced viral resistance between both heterologous cells (1, 2) and human cell lines (3). This phenomenon required close cell proximity and is thought to represent cellular communication which may be mediated by a secondary messenger molecule(s) (4). Based on these findings, we have proposed that by means of this transfer process the natural mechanism of interferon protection may include action on cells not in contact with interferon but near the interferon-responding cell. Consistent with this idea was our predicted finding that the rate and degree of interferon action was determined by the cell density (5); as the cell density was lowered interferon action decreased and reached a minimum when the majority of cells were not in contact with one another. One explanation is that the most sensitive cells in a heterogeneously responding population determine the overall response only under conditions of high cell density where they contact less sensitive cells. This idea has been tested by cloning cells to determine the response of the individually reacting cells and their contribution to the response of the whole culture. We found a marked heterogeneity between cells in their sensitivity to interferon and their ability to transfer resistance. Reconstitution experiments indicate that only a minor fraction of cells determines the interferon response of the population.

Materials and methods. Cell culturing and cloning. Mouse L-929 cells in Eagle's minimal essential medium (MEM) with 10% fetal calf serum were passaged 1 to 10 biweekly. For cloning, L cells were suspended in MEM supplemented with 10% fetal calf serum, nonessential amino acids, and 25 m*M* Hepes buffer. Cells (20/ml) were distributed at 5 ml/flask into a series of Falcon tissue culture flasks (area, 25 cm^2). They were incubated undisturbed at 37° for 10 days at which time the medium was replaced with mouse interferon or fresh medium. Following interferon treatment, 10^5 plaque-forming units (PFU) of vesicular stomatitis virus (VSV) was added to each flask (approximate input multiplicity of infection was 20 PFU/cell). After overnight incubation at 37°, surviving clones were stained with crystal violet and counted. The cloning efficiency of L cells was 70–100%.

For the establishment of cloned L-cell lines, 0.1 ml/well of a cell suspension (30 cells/ml) was added to each well of Falcon Micro Test II tissue culture plates. After 10 days incubation in 4% CO_2 at 37°, wells containing a single clone were visually identified. About 4 days later, the cells from these wells were removed with trypsin and placed in Falcon tissue culture flasks (25 cm^2) for 2 weeks then into 75-cm^2 flasks. Three days later they were assayed for interferon sensitivity. Highly resistant or sensitive cloned cells were then used in cell mixing experiments.

Interferon. Interferon was produced by infection of mouse L cells with a lentogenic strain of Newcastle disease virus (NDV) as previously described (6).

Sensitivity of L-cell clones to interferon was determined either by a microyield reduction assay (1×10^5 cells/well) (1) or a slight modification of the method of microplaque reduction using methylcellulose overlay instead of carboxymethylcellulose (7.5×10^4 cells/well) (7). The VSV challenge dose of these two assays was 3 PFU/cell and 25–30 PFU/well, respectively. Interferon titers are expressed in terms of the NIH research reference mouse interferon as assayed on our parental L-929 cells.

Results. Heterogeneity of the response to interferon. Cell cloning was employed to establish whether there is variation in the inter-

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feron response of individual L cells within a population. Figure 1 shows that when a low density of L cells is plated such that each cell forms a clone, the number of clones protected against virus challenge increased with the length of interferon treatment. Likewise, at a given time, there is an increase in the number of protected clones with increasing concentrations of interferon. These data show that individual L cells differ in the amount of interferon to which they respond as well as the time required for the response. These findings strongly suggest marked differences in the interferon response of individual L cells.

To determine the degree of variation, cell lines were established from individual L cells. Representative dose–responses for three of these cloned L-cell lines are shown in Fig. 2. At the extremes, clones differed by 10-fold in their sensitivity to interferon and almost 100-fold in the maximum degree of protection against virus. Table I summarizes the results of the interferon responses of 18 L-cell clones. Clearly, there is a large heterogeneity among individual L cells in both the sensitivity to and maximum protection afforded by interferon.

Transfer of interferon-induced viral resistance between cloned L cells. If the transfer of viral resistance occurs between individual cells of a cell line, then this should be demonstrable with cloned cells derived from the parent population.

When cloned L cells of "high" (clone 2) and "low" (clone 1) sensitivity to interferon were cocultivated in a 1-to-1 ratio the response of the mixed population approached that of the "high" responding clone (Fig. 3). The virus yields from noninterferon-treated cells of "high" and "low" responding clones

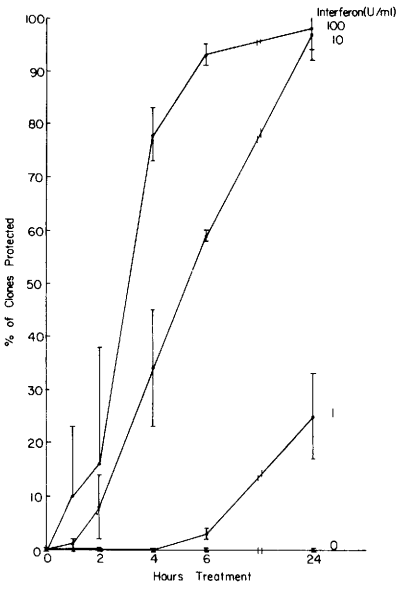

FIG. 1. Variation in interferon protection of L-cell clones against VSV.

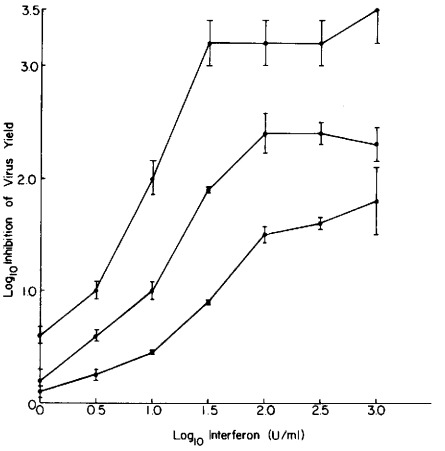

FIG. 2. Representative interferon dose–responses of three cloned L-cell lines. Data are presented as the mean (three replicates) and 95% confidence limits.

TABLE I. VARIATION OF THE INTERFERON DOSE–RESPONSES OF CLONED L CELLS

Clone	Interferon dose (U/ml) $\log_{10}$ inhibition of VSV yield[a]					
	1	3	10	30	100	300
A	0.03	0.19	0.37	0.92	1.5	1.6
B	0.20	0.26	0.76	1.5	1.7	1.9
C	0.08	0.88	1.5	2.6	2.7	2.9
D	0.03	0.74	1.8	2.3	2.5	2.8
E	0	0.70	1.8	2.5	2.5	2.8
F	0.09	1.2	1.7	2.6	2.8	3.3
G	0.20	0.45	1.5	1.8	2.1	1.9
H	0.07	0.68	1.7	2.8	2.8	2.6
I	0.10	0.28	0.92	1.5	2.3	2.2
J	0.10	0.85	2.0	3.2	3.2	3.3
K	0	0.44	1.5	2.2	2.6	2.4
L	0	0.43	1.2	1.9	2.2	2.2
M	0.42	0.88	2.0	2.7	2.8	73.5
N	0	0.17	1.2	1.8	2.2	2.5
O	0	0.4	1.0	1.9	2.4	2.4
P	0.36	0.78	1.8	2.3	2.3	2.5
Q	0.15	1.2	1.9	2.4	2.6	2.5
R	0	0.47	1.1	1.9	2.5	2.5

[a] Differences of 0.5 $\log_{10}$ are significant at $P < 0.05$.

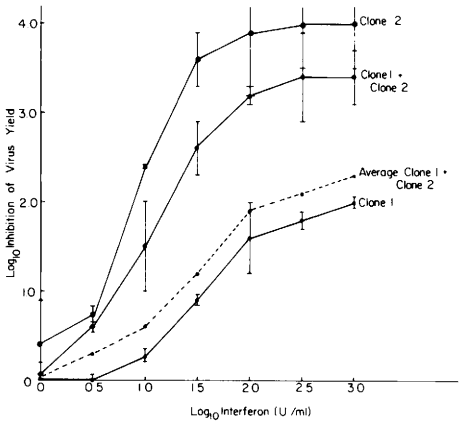

FIG. 3. Transfer of interferon-induced viral resistance, between cloned L cells. Data are presented as the mean (three replicates) and 95% confidence limits.

(alone or mixed) were the same and the yield from interferon-treated "high" responding cells was insignificant compared to equivalently treated "low" responding cells. Thus if transfer had not occurred, in the presence of interferon the yield should have been 50% of the yield from interferon-treated "low" responding cells. In terms of the maximum amount of protection confered by interferon, there was no significant difference between the mixed cells and the "high" responding cells. This observation has been repeated with three different clones. The increase in sensitivity ranged from 3- to 30-fold and the increased maximum degree of protection ranged from 5- to 30-fold. Based on these data, it seems that individual cells within a heterogeneous population can transfer interferon-induced viral resistance amongst themselves.

The fraction of cells which determines the interferon response of the population. Since the preceding experiments demonstrated that transfer of viral resistance can occur within a population of cells, it is important to determine what fraction of cells controls the response of the population. This was investigated by comparison of the interferon response of the parent L-cell population, 40 L-cell clones, and a reconstituted L-cell population. In a plaque reduction assay, the response of the reconstituted population (equal numbers of each cloned cells) was not significantly different from the parental population, while both were four to five times more sensitive than the average of the 40 individual clones (Fig. 4). This indicates that transfer occurs within L cells and can be demonstrated with a plaque reduction assay. It also appears that the reconstituted population closely approximates the parental population.

A compilation and analysis of this data (Table II) shows that with 1 and 3.3 U/ml of interferon the response of the population is determined at most by 10 and 30% of the cells, respectively. Whether all of these clones transfer is not known at present. At 10 and 33 U/ml almost all the cells respond equally (100% inhibition). Thus, the transfer of viral resistance can play an important role in the action of interferon.

Discussion. We have proposed that the natural action of interferon does not require a direct effect of the molecule on each cell. This proposition stemmed from our previous demonstrations of the transfer of interferon-induced viral resistance between cells (1–3). Supportive of this was the finding that interferon action was determined by the cell density (5). At a cell density where the majority of cells in a population could not contact one another there was a precipitous drop in inter-

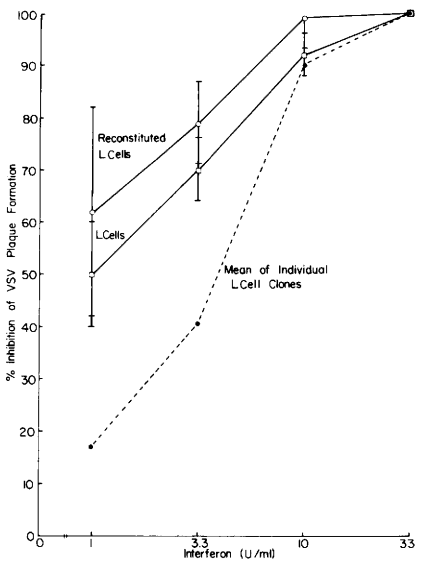

FIG. 4. Interferon dose–responses of "parental" L cells, a reconstituted L-cell population, and the mean response of 40 L-cell clones. Data for the "parental" L cells and the reconstituted L-cell population are presented as the mean (three replicates) and 95% confidence limits.

TABLE II. THE FRACTION OF CELLS WHICH DETERMINES THE INTERFERON RESPONSE OF THE POPULATION

Interferon (U/ml)	Percentage inhibition of VSV plaque number, number of clones			Percentage inhibition of VSV plaque number		Percentage of clones at or above reconstituted population's percentage inhibition
	0–35	31–60	61–99	Mean of clones	Reconstituted population	
1	35	1	4	17	62	10
3.3	25	3	12	40	79	30
10	2	2	36	90	99	78
33	1	0	39	98	100	98

feron activity. This cell-proximity effect was proposed to result from variation in interferon sensitivity between individual cells in the population and an inability of the most sensitive, first-responding cells to transfer their viral resistance to less sensitive, slower-responding cells when they were not in contact.

In this study is was shown by the cloning of individual L cells that there is a very marked heterogeneity among individual cells in both their sensitivity to and maximum degree of protection afforded by interferon. Further, cloned L cells of "high" interferon sensitivity can transfer their viral resistance to clones of "low" sensitivity. By studying the interferon response of individually reacting clones and a reconstituted parental population of cells, it was found that as few as 10% of these cells can determine the response of the population. These findings strongly support our interpretation of the cell proximity effect and our contention that the action of interferon does not require a direct effect of the molecule on each responding cell.

Our earlier demonstrations of the transfer of interferon-induced viral resistance employed cells of different animal species (1, 2). The observation of transfer among cells within a population from a single species suggests that the transfer process is operational *in vivo*. This is all the more likely since the cell proximity effect occurs with primary mouse embryo cells as well as diploid human fibroblasts (which are similar to normal cells *in vivo*) (5). This means that the natural mechanism of interferon protection probably includes action on cells near the interferon-responding cell. This process would amplify the interferon response since sensitive, fast-responding cells would transfer resistance to less sensitive, slower-responding cells which constitute the bulk of the population. The observation that a small fraction of cells (10%) determines the response of the population shows that the transfer process plays a highly important role in the action of interferon.

There are many similarities between interferon and polypeptide hormones (8). For instance, interferon acts at the cell membrane (9, 10). It has been proposed that, as with polypeptide hormones, the interferon-cell membrane interaction produces a secondary messenger molecule(s) which induces the antiviral state (1, 11–13). Based on our previous data a likely mechanism for the transfer of interferon-induced viral resistance may be gap junctional transfer of the putative secondary messenger(s). Shortly after our demonstration of cell-to-cell communication of interferon activity, a similar finding with polypeptide hormones was reported (14). This leads to the intriguing possibility that as with interferon there may be marked heterogeneity among "sensitive" cells in their response to polypeptide hormones and only a small number of cells determine the response of the population (or *in vivo* the tissue). If this is correct then direct cell-to-cell communication represents a novel mechanism for the amplification of hormone or hormone-like activity.

Summary. Cloned L cells were shown to be very heterogeneous in their time and dose–responses to mouse interferon. Sensitive cells transferred interferon-induced viral resistance to less sensitive cells making them equally sensitive. A small fraction of cells was found to determine the overall response of the "parental" L-cell population. Based on these findings a new mechanism of interferon action on cells not in contact with interferon was proposed. The implications of this proposal to polypeptide hormone action are discussed.

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