PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

4. The Effect of Interferon on the Expression of Human Cell-Surface Antigens

O. R. Burrone and C. Milstein

Phil. Trans. R. Soc. Lond. B 1982 **299**, 133-135
doi: 10.1098/rstb.1982.0116

support the notion that IFN activates macrophages. Early and late phases of spreading were both stimulated. The mechanism of this action remains speculative, but it may be associated with mechanisms involved in enhanced expression of foreign surface as well as Fc receptors on IFN-treated macrophages, and the enhanced phagocytosis mediated by these receptors.

This study has been supported by grants from the Norwegian Society for Fighting Cancer and the Norwegian Cancer Society.

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4. The effect of interferon on the expression of human cell-surface antigens

By O. R. Burrone and C. Milstein, F.R.S.
M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

We have studied the effect of highly purified human interferon-α (the NK2-IFN preparation of Secher & Burke (1980)) on the expression of surface antigens by the human cell line Molt 4. Cytofluorimetric analysis with monoclonal antibodies revealed an increase in the membrane expression of HLA-A, B and C but not of other surface antigens. This effect has previously been observed with other human lymphoid cells (Fellous *et al*. 1979; Heron *et al*. 1968). The increase in the binding of fluorescent antibody is due to an increase in the number of HLA molecules in the membrane. This was demonstrated in an experiment in which surface proteins were iodinated after treatment with interferon. The difference in the content of HLA molecules could then be visualized on polyacrylamide gel electrophoresis of the immuno-purified surface antigens. In addition, a band corresponding to 16000 M_r protein, absent from the surface of several human cell lines, has been found to be induced after IFN treatment.

The effect of IFN-α on the synthesis of HLA and β2m was studied by pulse-labelling treated and untreated cells with [^{35}S]methionine for 3 h. The cells were then lysed, and the lysates used for immunoprecipitation with monoclonal antibody for HLA and with rabbit anti-human β2m serum. The immunoprecipitates were then analysed by SDS-polyacrylamide gel electrophoresis. A several-fold increase in the amount of newly synthesized HLA and β2m molecules was clearly observed. These results demonstrate that it is the rate of synthesis of HLA and β2m molecules that has been affected by the interferon treatment, thus leading to the enhanced expression of the antigens on the membrane, and to an increase in the secretion of free β2m.

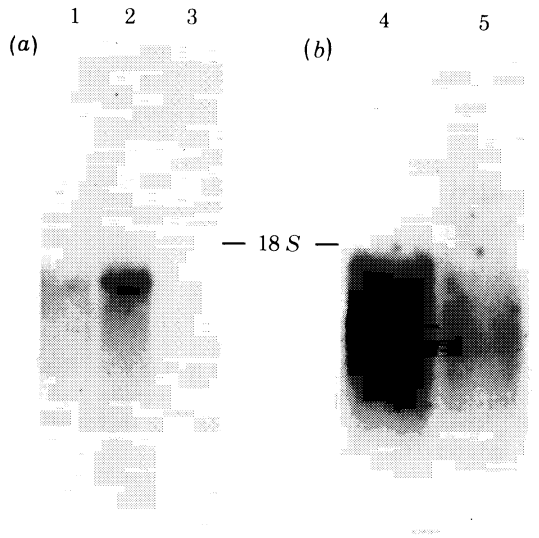

FIGURE 1. HLA-A, B and C-related mRNA sequences present in Molt 4 cells. Total cytoplasmic mRNA from non-treated and IFN α-treated (2000 u ml^{-1}, 10 days) Molt 4 cells were electrophoresed on an agarose gel, blotted onto nitrocellulose paper and hybridized to ^{32}P-labelled DNA from a clone containing HLA-B sequences. Experiment (*a*): lanes 1 and 2, 0.7 μg and 2 μg of mRNA from IFN-α-treated cells; lane 3, 2 μg of mRNA from non-treated cells. Experiment (*b*): lane 4, 2 μg mRNA from IFN-α-treated cells; lane 5, 10 μg mRNA from non-treated cells. IFN units calibrated with reference to the MRC 69/19 standard.

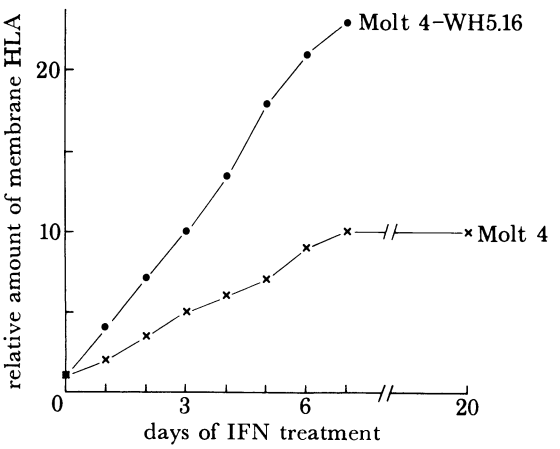

FIGURE 2. Kinetics of IFN-α-induced increase in HLA expression. Cells from clone Molt 4-WH5.16 and wild-type Molt 4 were incubated with IFN-α at a concentration of 2000 u ml^{-1} for different times, labelled with the McAb W6/32 and fluorescein-conjugated second antibody and analysed in the fluorescence-activated cell sorter. The relative amount of membrane HLA-A, B and C was calculated as the ratio of each fluorescence intensity value divided by the fluorescence intensity of the wild-type Molt 4 cells in the absence of IFN.

The increased rate of HLA synthesis is due to an increase in the amount of HLA mRNA present in the cell after interferon treatment. This was demonstrated by hybridizing poly(A)-containing RNA of interferon treated cells with ^{32}P-labelled DNA from a clone containing specific sequences coding for HLA-B (kindly provided by A. K. Sood (Sood *et al.* 1981)). As is shown in figure 1, a very substantial increase, which we estimate to be of the order of 10 to 20 times in the amount of messenger RNA, occurs as a result of long-term interferon treatment.

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A number of studies of HLA expression after IFN treatment have been done by immunofluorescence analysis, using the fluorescence-activated cell sorter and the monoclonal antibody W6/32. As shown in figure 2, the accumulation of HLA surface protein increases steadily and roughly linearly for up to 6 days. By continuous sorting of cells with the highest content of HLA after the interferon treatment, we have derived a population of cells that show an enhanced response to interferon treatment relative to the original Molt 4 cells. From this population, a clone was isolated, and the time response to interferon treatment in comparison with the wild-type Molt 4 cells is also shown in figure 2. It can be seen that both curves start at the same point. The increases in the amount of HLA-A, B and C on the surface of the mutant clone is about twice the rate of wild-type Molt 4. The maximum value at 6 days in the mutant cells is about twice than in the wild-type cells.

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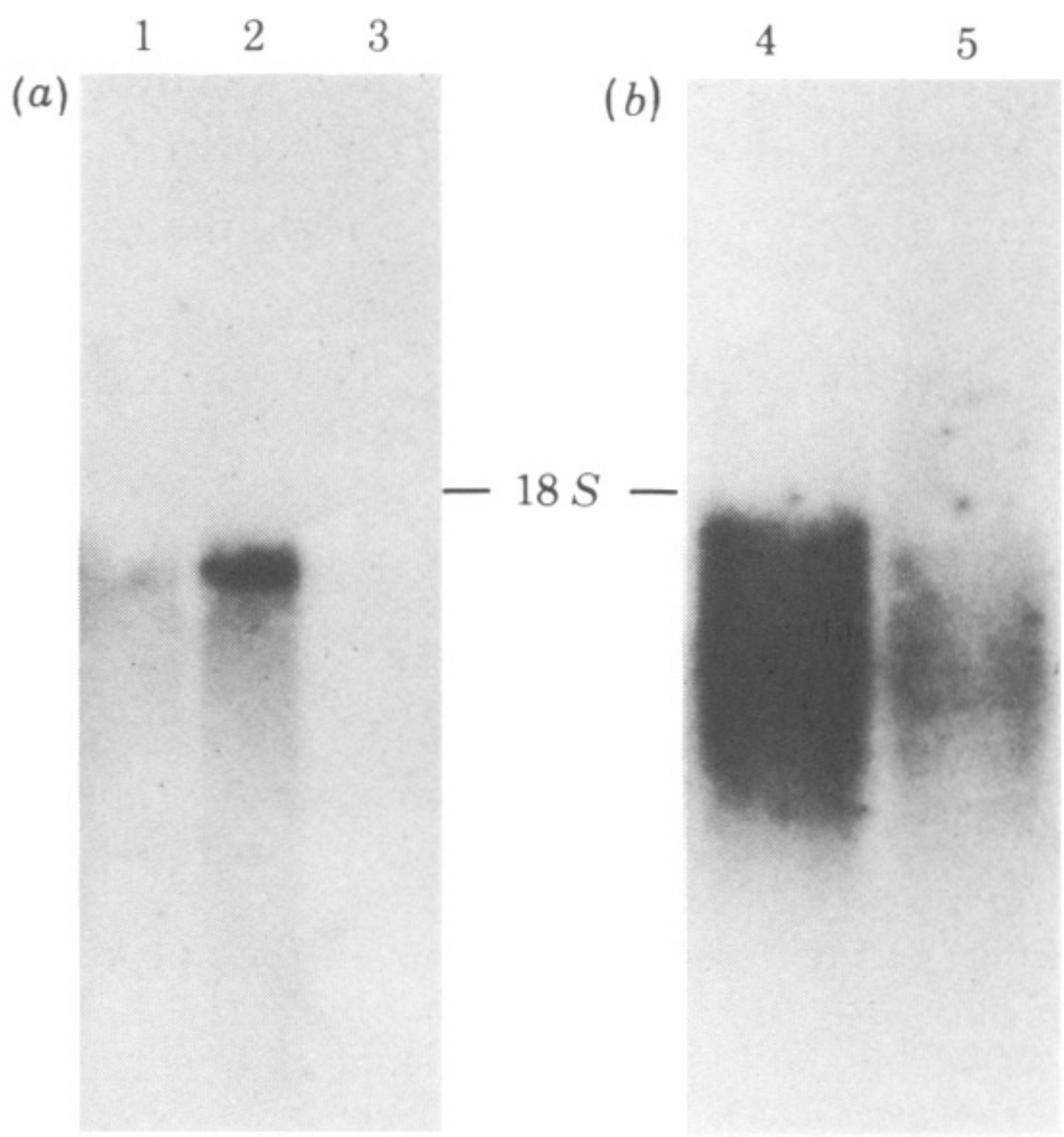
5. Analysis of antiviral state in RD114 and A204 cells after interferon treatment

By Y. Tomita, J. Nishimaki, F. Takahashi and T. Kuwata
Department of Microbiology, School of Medicine, Chiba University, Chiba 280, *Japan*

Interferon exerts antiviral and various non-antiviral activities on cells. For the development of the antiviral state in cells against exogenous infection of cytolytic viruses such as vesicular stomatitis virus and encephalomyocarditis virus (EMCV), induction of 2′–5′-oligo(A) synthetase or double-stranded (ds)-RNA-dependent protein kinase, or both, has been shown to have a crucial role (Revel 1979). However, in some cases, the antiviral effects of interferon are virus-specific (Nilsen *et al.* 1980; Samuel & Knutson 1981). Recently, different effects of mouse interferon on retrovirus production and on EMCV replication in a clonal cell line from NIH/3T3 cells were reported (Czarniecki *et al.* 1981). The inhibition of retrovirus production by interferon occurs at a late stage of virus growth before maturation and release of the virus from the cell surface (Friedman 1977; Billiau 1977). It may be correlated with some non-antiviral actions of interferon on cell membrane such as the inhibition of cell fusion induced by inactivated retrovirus (Tomita & Kuwata 1979) or Sendai virus (Tomita & Kuwata 1981). In the present study, we compared the effects of interferon on retrovirus and EMCV replication, and cell fusion in rhabdomyosarcoma cells chronically infected with retrovirus. Productions of [^{3}H]uridine-labelled retrovirus from HuIFN-α treated and untreated control cells were estimated by sucrose gradient centrifugation. As shown in figure 1, productions of baboon endogenous retrovirus (M7) from A204 cells and feline endogenous retrovirus (RD114) from RD114 and RD114-C1 cells (a subclone of RD114 cells) were in each case highly sensitive to the antiviral action of HuIFN-α. ^{3}H-labelled viruses released from interferon-treated A204 and



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