PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

3. Morphological Parameters of the Effect of Interferon on Mouse Peritoneal Macrophages

M. Degre, H. Rollag and Elisabeth Kjeldsberg

Phil. Trans. R. Soc. Lond. B 1982 **299**, 131-133
doi: 10.1098/rstb.1982.0115

3. Morphological parameters of the effect of interferon on mouse peritoneal macrophages

By M. Degré, H. Rollag and Elisabeth Kjeldsberg
National Institute of Public Health, Department of Virology and The Wilhelmsens Institute of Bacteriology, Rikshospitalet, University of Oslo, Oslo, Norway

Several macrophage functions are modulated by treatment with homologous interferon (IFN). For example, phagocytic activity of mouse peritoneal macrophages (MPM) is enhanced by moderate concentrations of mouse fibroblast interferon (MuIFN-β) (Rollag & Degré 1981). Spreading of freshly seeded macrophages on glass surfaces is stimulated by macrophage-activating agents (Mörland & Kaplan 1977), IFN inducers (Rabinovitch *et al.* 1977) and IFN (Schultz *et al.* 1978). We report here quantitative data on effect of MuIFN-β on the spreading of MPM *in vitro*.

Cells were seeded on glass surfaces in Eagle's MEM, and spreading was examined after incubation at 37 °C for various periods by phase-contrast light microscopy (p.c.m.). Cells, fixed in 2.5 % glutaraldehyde, were scored as round or spread, at least 200 cells in each preparation (Rabinovitch & De Stefano 1973).

Two phases of macrophage spreading *in vitro* can be distinguished. The early phase occurs within 1 h of seeding and it does not require new membrane synthesis, while the late phase of spreading requires synthesis of new membrane (Rabinovitch & De Stefano 1973). To study the early phase, mice were injected intravenously with 2×10^4 units† of MuIFN-β, 100 haemagglutinating units of Newcastle disease virus (NDV) or mock preparation. MPM were harvested the next day, seeded and examined after 1 h incubation. Spreading was enhanced by both IFN and NDV. The percentage of spreading cells was nearly doubled compared with cells obtained from control mice or mice given mock preparation (38.2 % and 35.6 % compared with 18.6 %).

MPM from unstimulated mice were seeded with or without the presence of MuIFN-β to study the late phase of spreading. IFN enhanced spreading time- and dose-dependently. Maximal difference (45.6 % compared with 36.4 %), produced by 100 units ml^{-1} or more of IFN, was observed after 3–5 h incubation. The effect was species-specific, and so no effect was seen after treatment with similar concentrations of human leucocyte IFN, and it was neutralized by anti MuIFN-β globulin.

In similar experiments the extent of spreading was examined by scanning electron microscopy. By this method it is possible to observe minor and earlier morphological developments than by p.c.m. Cell cultures were prefixed in 2.5 % glutaraldehyde, post fixed in 0.1 % osmium tetroxide, dehydrated in alcohol and critical point dried in carbon dioxide. The specimens were coated with platinum. The findings confirmed that MuIFN-β activates macrophage spreading (figure 1). After only 2 h of incubation, marked differences were observed in the number of spreading cells. No qualitative differences could be seen between IFN-treated and control MPM.

† Units are with reference to standard no. G-002-504-501 from the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

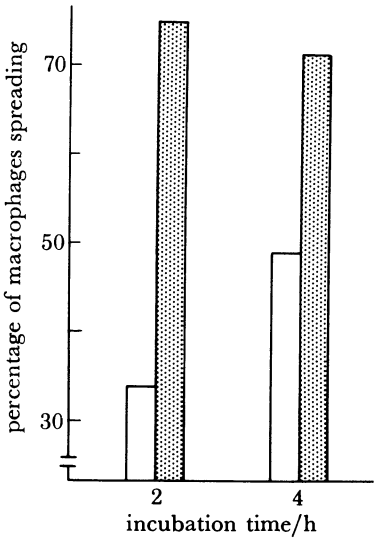

FIGURE 1. Spreading of mouse peritoneal macrophages on a glass surface after incubation *in vitro* with 10^3 units ml^{-1} of MuIFN-β (stippled columns) or a mock IFN preparation (open columns) for 2 or 4 h. Scanning electron microscope observation.

TABLE 1. EFFECT OF INTERFERON TREATMENT ON THE RELATIVE AREA OCCUPIED BY MOUSE PERITONEAL MACROPHAGES ON A GLASS SURFACE

treatment	incubation period/h	number of MPMs measured	mean area	s.e.m.	
	experiment 1				
culture medium	5	87	100	5	
MuIFN–β, 100 u ml^{-1}	5	77	112	6	
MuIFN-β, 100 u ml^{-1} + anti-IFN globulin	5	50	106	8	
HuIFN-α, 100 u ml^{-1}†	5	38	101	8	
	experiment 2				
culture medium	24	71	100	4	
MuIFN-β 100 u ml^{-1}	24	90	153	6	$p < 0.001$‡
MuIFN-β, 5×10^4 u ml^{-1}	24	20	142	8	$p < 0.001$‡
MuIFN-β, 100 u ml^{-1} + anti-IFN globulin	24	75	107	5	$p < 0.001$§
HuIFN-α, 100 u ml^{-1}	24	45	98	8	

† 100 u ml^{-1} tested in human embryo fibroblast cells.
‡ Difference from controls.
§ Difference from cells treated with MuIFN-β, 100 u ml^{-1}.

The surface area occupied by the spreading macrophages on glass surfaces was measured on p.c.m. photomicrographs, by using a digital planimeter. Treatment of cells with MuIFN-β increased the surface area (table 1). This effect appeared after prolonged incubation of cells with IFN.

The results indicate that macrophages spread more rapidly and after prolonged incubation to a larger size, defined as surface area, under influence of homologous IFN. Since spreading generally accompanies other parameters of activation (Mörland & Kaplan 1977), our results

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.

References

Mörland, B. & Kaplan, G. 1977 Macrophage activation *in vivo* and *in vitro*. *Expl Cell Res.* **108**, 279–288.
Rabinovitch, M. & DeStefano, M. J. 1973 Macrophage spreading *in vitro*. *Expl Cell Res.* **77**, 323–334
Rabinovitch, M., Manejias, R. E., Russo, M. & Abbey, E. E. 1977 Increased spreading of macrophages from mice treated with interferon inducers. *Cell. Immunol.* **29**, 86–95.
Rollag, H. & Degré, M. 1981 Effect of interferon preparations on the uptake of non-opsonized *Escherichia coli* by mouse peritoneal macrophages. *Acta path. microbiol. scand.* B **89**, 153–159.
Schultz, R. M., Chirigos, M. A. & Heine, U. I. 1978 Functional and morphological characteristics of interferon treated macrophages. *Cell Immunol.* **35**, 84–91.

---

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.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES