Mouse Interferon Receptors: A Difference in Their Response to α and β Interferons

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SUMMARY

The interferon receptors of C3H/10T½ mouse cells respond differently to α and β interferons under certain conditions. If C3H/10T½ cells which have been maintained in logarithmic growth phase are exposed to trypsin or Pronase immediately before they are treated with mouse interferons, they evince an antiviral response to α interferon but not to β interferon. In contrast, contact-inhibited C3H/10T½ cells, L-929 mouse cells or human HEL cells lost the ability to respond to both α and β interferons after treatment with trypsin or Pronase. When L-929 cells are incubated at 37 °C following exposure to these proteolytic enzymes, they completely regain their ability to respond to mouse β interferon within 2 h. These observations suggest that the receptors for α and β interferons are different in their topographical distribution in C3H/10T½ cells.

Receptors for α and β interferon can be distinguished from receptors for γ interferon by several criteria, whereas the behaviour of α receptors closely resembles that of β receptors. For example, homospecific α and β interferons compete with one another for receptor sites on both mouse (Aguet & Blanchard, 1981) and human (Branca & Baglioni, 1981; Yonehara et al., 1983) cells respectively. Human γ interferon competes with neither α nor β human interferons (Branca & Baglioni, 1981; Aguet et al., 1982), although in the reverse experiment, β interferon will compete with γ interferon, but α interferon will not (Anderson et al., 1982). The antiviral and antigrowth activities of both α and β interferons are inhibited by gangliosides but neither of these activities of γ interferon are so inhibited (Ankel et al., 1980). Estimates of the number of α or β receptor sites per cell vary between 350 for human diploid and 550 for human trisomic fibroblasts (Epstein et al., 1982), 1000 for mouse L-1210S cells (Aguet & Blanchard, 1981) and 5000 for human lymphoblastoid cells (Branca & Baglioni, 1981). The location and distribution of receptors depend upon the method of cultivation of the cells. Electron micrographs show that mouse β interferon receptors in L-929 cells grown in suspension are in coated pits or on coated membranes (Kushnaryov et al., 1983). When these cells are cultivated in monolayers, however, receptors are sparse and are neither in coated pits nor coated membranes (Kushnaryov et al., 1982). Both the synthesis and degeneration of the receptors have been estimated to have a half-life of 2 h (Aguet & Blanchard, 1981; Sakaguchi et al., 1982). To assess the possible structural differences between the receptors for α and β interferons, we studied the sensitivities of these receptors to proteolytic enzymes.

The L-929 and HEL cells and vesicular stomatitis virus (VSV), Indiana serotype, were those we employed previously (Sakaguchi et al., 1982). For this study the virus was again plaque-purified thrice in L-929 cells and after growth in secondary chick fibroblasts, was stored at −70 °C. The C3H/10T½ cell line was a kind gift from our late colleague, Dr Charles Heidelberger. These cells must be maintained (Reznikoff et al., 1973a) in the logarithmic growth phase because after they become confluent, there are alterations not only of their morphology but also of their susceptibility to chemical transformation and other traits (Reznikoff et al., 1973b). They were passaged every 10 days, 2 x 10⁵ cells being seeded into a
32 oz bottle containing Eagle's basal medium (BME) and 10% heat-inactivated foetal calf serum (FCS; Flow Laboratories). The mouse L-929 and human embryonic lung (HEL) cells, however, were grown to confluency at each passage. Four interferons were obtained from Lee BioMolecular Research Laboratories, Inc. (San Diego, Ca., U.S.A.): mouse α (containing contaminating β), mouse β, human α and human β. They contained respectively $1.7 \times 10^6$, $7.6 \times 10^7$, $1.1 \times 10^5$, and $2.3 \times 10^5$ international reference units (IU)/mg of protein. Antisera neutralizing α and β mouse and human interferons respectively were kindly provided by the late Dr Kurt Paucker (Medical College of Pennsylvania, Philadelphia, U.S.A.). Trypsin (Difco) or Pronase (Calbiochem) concentrations were 3 mg/ml in all experiments reported here, 10-fold higher than that capable of abolishing the antiviral response to mouse β interferon. We routinely added DNase (100 μg/ml; Sigma) to alleviate cell clumping. Trypsin or Pronase treatment was carried out at 37 °C for 15 min in serum-free Hanks' balanced salt solution containing neither Mg$^{2+}$ nor Ca$^{2+}$ (HBS-). Proteolysis was stopped by washing the cells four times in 10 ml of HBS-/10% FCS at 4 °C. Except for plaque counts (Sakaguchi et al., 1982), we used cell suspensions for all procedures.

To determine the kinetics of receptor synthesis, cells were exposed to trypsin or Pronase, incubated at 37 °C for varying periods, treated with 18 000 IU/ml of interferon for 2 h in an ice-bath, washed five times with ice-cold HBS- to remove unadsorbed interferon and reacted at 37 °C for 15 min in the presence of homologous anti-interferon antibody to inhibit the possible re-binding of eluted interferon. The interaction between interferon and functional receptors was thus limited to the 2¼ h pulse. The cells were then infected with VSV. At ice-bath temperature, cells were incubated with relatively high concentrations of interferon for at least 2 h to attain a desirable degree of eventual antiviral protection (data not shown). While we verified the report (Brouty-Boy6, 1977) that at lower interferon concentrations, C3H/10T½ cells are more sensitive to interferon than L-929 cells when incubation is done at 37 °C, this differential effect was absent or slight at ice-bath temperature with the relatively high concentrations we employed (data not shown). We used 18 000 IU/ml for the rest of the experiments to make stringent tests of the ability of trypsinized or Pronase-treated cells to respond to interferon. Controls showed that when such cells were treated with interferon in the presence of 10% foetal calf serum, their virus output was the same as that of cells treated with interferon in HBS- serum-free medium.

The marked antiviral sensitivity of L-929 cells to mouse β interferon at high concentration was completely abolished by prior exposure of the cells to Pronase without ensuing incubation at 37 °C (Fig. 1). This shows that no detectable receptor synthesis occurred during subsequent incubation of the cells with interferon for 2 h at 4 °C. An antiviral response was already discernible within 15 min after treatment with anti-interferon antibody at 37 °C was complete, indicating that proteolysis has been halted and that β interferon receptor replacement at 37 °C had already begun. Receptor synthesis reached a plateau within approximately 2 h. We found similar kinetics of receptor regrowth in both trypsinized and Pronase-treated cells.

Pronase-treated C3H/10T½ cells resembled L-929 cells in that they were insensitive to mouse β interferon, but in contrast to L-929 cells, were capable of a strong antiviral response to mouse α interferon (Fig. 2). This result suggested that the receptors for α and β interferons are different in their topographical distribution in C3H/10T½ cells under certain conditions. We found the same pattern when these two cell lines were treated with trypsin instead of Pronase. If the C3H/10T½ cells were not kept strictly in logarithmic growth phase by cultivating them in accord with the 10-day reseeding protocol of Reznikoff et al. (1973a) and were instead allowed to form confluent monolayers, they no longer responded to α interferon following enzymic digestion (data not shown). Replication of VSV in Pronase-treated, contact-inhibited human HEL cells was likewise suppressed by neither α nor β human interferons (data not shown).

It is postulated that α and β interferons bind to the same receptor because both α and β interferons can be inhibited by gangliosides (Ankel et al., 1980; Kushnaryev et al., 1983) and by each other (Aguet & Blanchard, 1981; Branca & Baglioni, 1981; Yonehara et al., 1983). The spectrum of biological activities of different human α interferons varies in different cells (Weck et al., 1981), however, and binding and activity studies of the α interferons (Hannigan et al., 1983) and of hybrid molecules derived from α interferons (Streuli et al., 1981; Rehberg et al.,...
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Fig. 1. Regrowth of mouse interferon β receptors in L-929 cells. A pellet of $1 \times 10^7$ cells was (○, △) or was not (□, interferon control; Δ, virus control) treated with 3 mg/ml Pronase in 10 ml of serum-free HBS$^-$ for 15 min at 37 °C; washed four times at 4 °C in HBS$^-$/10% FCS to stop proteolysis; incubated in HBS$^-$/10% FCS at 37 °C for various periods; incubated with (○, △) or without (□, Δ) 18 000 IU/ml of interferon in HBS$^-$/10% FCS for 2 h at 4 °C; washed in HBS$^-$/10% FCS five times at 4 °C; treated with homologous anti-interferon antibody for 15 min at 37 °C, and plated in BME/2% FCS in a 35 mm dish ($1 \times 10^6$ cells). After incubation at 37 °C for 18 h, VSV (multiplicity 0.01) was adsorbed for 1 h, and cells were washed twice, incubated a further 24 h at 37 °C, frozen and thawed. The supernatant fluid was assayed by plaque titration in L-929 monolayers for VSV yield.

Fig. 2. Antiviral response of (a) C3H/10T½ and (b) L-929 mouse cells which were (cross-hatched bars) or were not (open bars) exposed to Pronase immediately before treatment with α or β mouse interferons respectively. Experimental conditions were otherwise as described in Fig. 1.

1982) suggest that interferon molecules possess two independent binding sites. Furthermore, γ interferon binds to cells previously exposed to α interferon, but not to those treated with β interferon (Anderson et al., 1982). Our data provide further evidence that the receptors for α and β interferons in a given cell might differ.

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REFERENCES


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