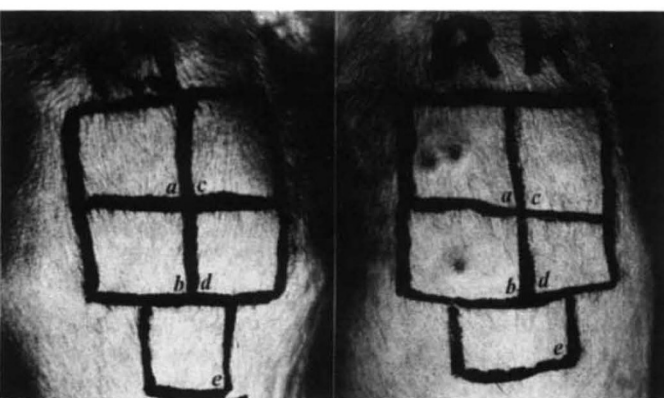# Antiviral effect of interferon *in vivo* may be mediated by the host

INTERFERON has antiviral effects both *in vivo* and *in vitro*. Viral replication is inhibited in interferon-treated cells *in vitro* because the reproductive cycle of the virus is inhibited at the transcriptional or translational level, depending on the virus–cell system studied[1]. This direct inhibition of viral replication has been assumed to also be the mechanism by which interferon exerts its antiviral effect *in vivo*. We report here results that indicate protection by interferon against viral infection *in vivo* without inhibition of the viral replication of the same virus *in vitro*.

To test the antiviral effect of human leukocyte interferon (HLI) *in vivo*, six rhesus monkeys were infected intradermally with different doses of vaccinia virus (strain of the Rijks Instituut voor de Volksgezondheid, RIV)[2] of which three were treated daily with HLI administered intramuscularly. The HLI was prepared as described previously[3]. The preparation used in these experiments had a specific activity of 2.1×10^6 units per mg protein. In control monkeys the lesions reached their maximum at day 7. Daily intramuscular injections of 5×10^5 units HLI per kg body weight during 8 d completely inhibited the development of the typical vaccinia-induced skin lesions (Fig. 1). No skin lesions were detected during the observation period of 4 weeks following infection. However, we were unable to correlate this distinct *in vivo* effect of HLI with a corresponding inhibition of the cytopathogenic effect (CPE) of the same virus on diploid rhesus monkey skin fibroblasts or on Rous sarcoma virus transformed human fibroblasts (RSb cells). Up to 10^4 units of HLI failed to protect the cells against the CPE of vaccinia virus (RIV strain), although the CPE of vesicular stomatitis virus (VSV) was effectively inhibited (Table 1). Surprisingly, vaccinia virus was resistant to interferon in our *in vitro* system, but the same results were obtained *in vitro* with the vaccinia virus strain WR and with smaller challenge doses (10^{-3} TCID$_{50}$)[4]. Also no anti-vaccinia activity could be detected in the sera of the monkeys 4 h after the first injection with HLI. At this time, the sera showed the maximum titre of antiviral activity when tested against VSV. Similar experiments performed with human diploid skin fibro-

Table 1 *In vitro* inhibition of the cytopathogenic effect (CPE) of vaccinia virus by human leukocyte interferon (HLI) and serum from rhesus monkeys treated with HLI

| Preparation | RSb cells | | Skin fibroblasts from rhesus monkeys | |
	VSV	Vaccinia virus	VSV	Vaccinia virus
HLI, 10^4 units ml^{-1}	10,000	<1	10,000	<1
Pre-serum*	<10†	<10	ND	ND
Post-serum*	350†	<10	ND	ND

Rous sarcoma virus transformed human fibroblasts (RSb) or monkey skin fibroblasts were plated in microtitre plates, grown to confluency (4×10^4 cells per well) and treated with serial dilutions of HLI containing 10^4 units per ml of rhesus monkey sera (before and after injection of 5×10^5 units HLI kg^{-1}). After overnight incubation, the supernatant was removed and cells were infected with vesicular stomatitis virus (VSV) or vaccinia virus (multiplicity of infection 0.25 TCID$_{50}$ per cell). The incubation was terminated when untreated infected control cells showed more than 90% CPE. The cells were stained with crystal violet. Both viruses were always tested simultaneously in the same microtitre plate and an interferon standard preparation was included. The activity of interferon is expressed as the reciprocal of the highest dilution giving 50% protection. The titres are given as reference units (tested against standard preparation 69/19).
* Before and after 4 h after injection of rhesus monkeys with 5×10^5 units of HLI per kg.
† Mean titre of three monkeys. ND, not determined.

blasts and rhesus monkey kidney cells showed identical results. There was also no inhibition of the production of infectious vaccinia virus (unpublished results).

These findings indicate that interferon can be effective *in vivo* against a virus which is insensitive to its antiviral action in various cell types. Likewise, it has been shown by others[5] that interferon can inhibit *in vivo* the growth of tumour cells which are resistant to its growth inhibitory action *in vitro*. Interferon may activate several defence systems of the host, for example, the cytotoxicity of the natural killer cells and the macrophages[6,7]. Perhaps such 'aggressive' cells can selectively destroy virus-infected cells *in vivo*.

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Fig. 1 *In vivo* effect of human leukocyte interferon (HLI) in rhesus monkeys infected intradermally with vaccinia virus. Monkeys were treated with HLI from day −1 to day 7 at 5×10^5 units kg^{-1} i.m. They were vaccinated intradermally at day 0 with the following preparations: 10^7 TCID$_{50}$ ml^{-1} vaccinia virus (*a*); 10^6 TCID$_{50}$ ml^{-1} vaccinia virus (*b*); 10^5 TCID$_{50}$ ml^{-1} vaccinia virus (*c*); 10^7 TCID$_{50}$ ml^{-1} vaccinia virus, subjected to UV- and heat-inactivation (*d*); NaCl (*e*). For each dilution, 0.05 ml were injected at three sites. The vaccination sites 12 d after infection are shown in a rhesus monkey treated with HLI (left) and in a control monkey (right). Three monkeys were treated with interferon and three served as controls.