Antiviral Research, 1 (1981) 179–183
Elsevier/North-Holland Biomedical Press

IN VIVO IMMUNE STIMULATION BY INTERFERON DURING VIRAL INFECTION

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(Received 22 April 1981; accepted 10 June 1981)

Treatment of Rhesus monkeys with human leukocyte interferon prevents the development of skin lesions after intradermal infection with vaccinia virus. The treatment does not prevent the development of immunity to vaccinia. Inactivated vaccinia virus, which is non-immunogenic in untreated monkeys, induced immunity under interferon treatment, indicating that interferon had an immune-stimulating effect.

interferon immune system

INTRODUCTION

Interferon can be an efficient antiviral agent in vivo, especially when applied prophylactically. The exact mechanism of this in vivo antiviral activity is not yet known. Apart from direct interactions with cells resulting in a resistance to viral infection, interferon exerts other biological effects which may contribute to its antiviral activity in vivo [3]. The relative importance of the different effects of its antiviral activity in vivo is poorly understood. Some of the reported effects, e.g. immunosuppression, are even in contradiction with an efficient antiviral activity in vivo. However, immunosuppression by interferon has been established by studying its effect on transplant survival [6] on delayed type hypersensitivity to red blood cells [2], and on other artificial systems, rather than by investigating its effect on immunity to viral infections where it may play a more physiological role.

We reported earlier that human leukocyte interferon protected Rhesus monkeys against intradermal vaccinia infection [8, 11], although it did not protect monkey cells against vaccinia infection in vitro. Thus, the in vivo effect appeared to be mediated by the host. To investigate the role of the immune system in this host-mediated effect, we studied the production of antibodies and the extent of protection against vaccinia virus in interferon-treated Rhesus monkeys and in controls. The results of this study are reported here. They constitute evidence that interferon efficiently stimulates the immune system in vivo during viral infection.

0166-3542/81/0000-0000/$02.50

EXPERIMENTAL

The human leukocyte interferon employed in this study was prepared as previously described [1]. It had a specific activity of 10^6 international units · mg^{-1} protein.

Source, propagation and titration (in plaque-forming units, p.f.u.) of the vaccinia virus (RIV strain) were as described earlier [5]. For the experiment presented in Table 2, the virus was inactivated by heat and UV Light. No residual live virus could be found by cell culture. Antibodies to vaccinia virus were determined by a haemagglutination inhibition assay in the laboratory of Dr. J. van der Logt (University of Nijmegen).

Rhesus monkeys (*Macaca mulatta*) bred at the TNO Primate Center (Rijswijk, The Netherlands) and weighing 1.5–3 kg were used. The monkeys were kept in quarantine from 2 weeks before the start until 2 weeks after completion of the experiments. The animals of the interferon-treated groups received 500,000 units · kg^{-1} intramuscularly on day -1 and day 0. On day 0, the animals of groups A and B of Table 1 were infected with 0.05 ml vaccinia virus (10^8 p.f.u. · ml^{-1}) at nine sites on the chest. Groups A and B of Table 2 were injected on day 0 with 0.05 ml heat- and UV-inactivated vaccinia virus (10^8 p.f.u. · ml^{-1} before inactivation). On day 28, all monkeys were revaccinated with live vaccinia virus as described for day 0. In both experiments, three animals (group C) served as controls for the revaccination procedures. Seven days after the first and second vaccinations, the skin lesions were scored under code by two independent observers, using an arbitrary scale of 0–4 based on the appearance and severity of papules and pustules. The statistical significance of the protection afforded by interferon and the vaccination was investigated by the Mann–Whitney U-test [9]. In the experiment represented in Table 1, blood was taken on day 0 and day 28 for titration of antibodies to vaccinia virus.

Table 1 shows that 500,000 units · kg^{-1} human leukocyte interferon on day -1 and day 0 completely protected Rhesus monkeys against the development of skin lesions. However, the development of immunity against the virus, as determined by the antibody reaction and protection against a reinfection with vaccinia, was the same as in the untreated animals which developed skin lesions after the first vaccination. In man, intracutaneous virus growth with subsequent development of skin lesions is regarded as essential for the development of immunity to vaccinia virus after primary vaccination. To determine whether this is also true for Rhesus monkeys, we injected such animals with inactivated vaccinia virus and infected them with live vaccinia virus 4 weeks later. The results of this experiment are shown in Table 2. The five animals (group B) that were injected with inactivated vaccinia were not protected when injected with live vaccinia 4 weeks later. The mean lesion score 7 days after live vaccinia infection was not significantly reduced when compared with controls that were not pretreated with inactivated virus. This shows that, in Rhesus monkeys under normal conditions, the vaccinia virus also has to replicate with subsequent development of skin lesions to induce immunity. A possible explanation for the results shown in Table 1 is that interferon, although diminishing the amount of viral antigen by inhibition of virus replication, enhances the

TABLE 1

The effect of interferon on the development of immunity after intradermal vaccinia virus infection in Rhesus monkeys

Experimental group	No. of animals	Treatments		Lesion scores[a] 7 days after		Antibody titer[b] on	
		Intramuscular interferon injections	Vaccination on days	first vaccination	second vaccination	day 0	day 28
A	6	500,000 units · kg^{-1} (days –1 and 0)	0 and 28	0.8[c] (1.2)	0.5[c] (0.2)	<20	93 (61)
B	6	None	0 and 28	3.8 (1.3)	0.0[c]	<20	80 (0.0)
C	3	None	28	–	3.6 (0.2)	n.d.[d]	n.d.

[a] Average score (S.D.).
[b] Logarithmic mean of reciprocals of end-point dilution (S.D.).
[c] Comparison with corresponding control groups: $P < 0.05$.
[d] Not done.

TABLE 2

The effect of interferon on the development of immunity after intradermal injection of inactivated vaccinia virus in Rhesus monkeys

Experimental group	No. of animals	Intramuscular interferon injections	1st vaccination (day 0)		2nd vaccination (day 28)	
			Vaccine	Lesion score[a]	Vaccine	Lesion score[a]
A	6	500,000 units · kg^{-1} (days –1 and 0)	Inactivated	0.3 (0.1)	Live	1.1 (1.5)[b]
B	5	None	Inactivated	0.0	Live	3.0 (0.8)[c]
C	3	None	None	–	Live	4.0 (0.0)

[a] Mean lesion score (S.D.).
[b] Comparison with group C: $P < 0.05$.
[c] Comparison with group C: $P > 0.05$.

immune reaction to the reduced antigenic stimulus, thus leading to a normal level of immunity. This immune-enhancing effect is quite likely, as interferon proved to be capable of stimulating an immune reaction to inactivated virus. Indeed, Rhesus monkeys injected with inactivated virus, while being treated with interferon, had a significantly reduced lesion score 7 days after live vaccinia infection (Table 2, group A) when compared with untreated controls. Conceivably, this immune-enhancing effect of interferon makes it possible that, even though the antigenic stimulus is diminished by inhibition of virus replication, immunity to the virus can still be acquired. It is unlikely that the protection against vaccinia challenge 4 weeks after viral infection under interferon treatment is caused by non-specific resistance. Indeed, the protective effect of human interferon in Rhesus monkeys lasts less than 10 days, not 4 weeks [7]. Although further studies are necessary to provide evidence that the immune system is mediating the reported effect, the most likely explanation seems to be that interferon has an immune-stimulative effect. The effect of interferon reported here, which has also been described for the interferon inducer, polyriboinosinic–ribocytidylic acid [4, 10], could extend the applicability of interferon in man. It is conceivable that interferon would prove useful as an immune adjuvant to improve the effectiveness of certain poorly immunogenic vaccines, e.g. hepatitis B and rabies vaccines.

ACKNOWLEDGEMENTS

This study was supported by the Nierstichting Nederland (grant c197). The authors would like to thank Professor Dr. H. Balner for his critical comments on the manuscript, Dr. A.C. Ford for correcting the English text, and D. v.d. Velden for typing the manuscript.

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