SPECIFICITY OF INTERFERON

BY

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Cells treated with heat-inactivated influenza virus have the property of interfering with the growth of other viruses. Isaacs and Lindenmann (1957) gave the name "interferon" to the substance responsible for this interference. Substances with similar properties have since been found in many virus-host cell systems (Ho and Enders, 1959). There is some evidence (Isaacs and Hitchcock, 1960) that interferon may be in part responsible for recovery from virus infection. Interferon is non-specific in its action against viruses, that produced by influenza virus, for example, having been shown to be active against members of the myxovirus, enterovirus, pox virus, and arbor virus groups.

Burke (1961) has shown that interferon is a protein of molecular weight approximately 62,000 and that it is either non-antigenic or only poorly so. It is relatively stable to heat and changes in pH. It is active on virus-infected cells not only *in vitro* but also *in vivo*, as has been shown for vaccinia virus infections of the skin of rabbits (Lindenmann *et al.*, 1957; Isaacs and Westwood, 1959) and of the eyes of rabbits (Cantell and Tommila, 1960), and for Bunyamwera virus in mice (Hitchcock and Isaacs, 1960). Interferon production *in vivo* has been shown to occur also in mice infected by influenza virus (Isaacs and Hitchcock, 1960) and for vaccinia infection of rabbits' skin (Nagano and Kojima, 1958). It thus has physical and biological properties that make it of potential value as a broad-spectrum viral antibiotic.

However, it has been found that interferon prepared from cells of the species of animal used in the test system is more effective than interferon prepared in a foreign species. This has been shown to be the case both *in vivo* and *in vitro* (Isaacs and Westwood, 1959; Sutton and Tyrrell, 1961). It would clearly be preferable on the grounds of safety and economy to use for man interferon prepared from non-human, and preferably non-primate, cells. It was therefore decided to reinvestigate how interferon preparations made in various different types of cell affected the development of vaccinia lesions in animals.

Methods

Chick interferon was prepared as previously described (Andrews and Dudgeon, 1961). Human-amnion, monkey-kidney (rhesus and cynomolgus), and rabbit-kidney interferons were made by infecting monolayer cell-cultures with the Melbourne strain of influenza virus inactivated by ultra-violet light, as described for the preparation of chick interferon. After three hours at 37° C. the virus was replaced by Earle's saline, and the final culture was harvested after 24 hours at 37° C. Control preparations were similarly made, but without infecting the cells with irradiated influenza virus. The possibility that interferon was produced in the control cell-cultures by a latent virus producing no degeneration of the cultures was not excluded, but none appeared to have been present to any great extent, as judged by the results. (We have previously observed partial inhibition of vaccinia in the rabbit's skin with one control preparation. The inhibition was intermediate between that produced by a known sample of interferon and the "unprotected" vaccinia control.) Interferon-containing solutions were dialysed against citrate buffer at pH 2 for 24 hours and then adjusted to pH 7.2, as recommended by Isaacs and Burke (1958).

FIG. 1

FIG. 2

FIG. 1.—Skin of rabbit showing sites of injection of interferon and vaccinia lesions. A, Chick interferon (M 1). B, Chick interferon control. C, Monkey interferon control. D, Rabbit interferon control. E, Medium 199. F, Calf interferon. G, Chick interferon (G 10). H, Monkey interferon (C 9). I, Rabbit interferon (G 12). J, Vaccinia control.

FIG. 2.—Skin of cynomolgus monkey showing sites of injection of interferon and vaccinia lesions. A, Chick interferon (G 5). B, Chick interferon (G 29). C, Cynomolgus monkey interferon (G 21). D, Rhesus-monkey interferon (G 32). E, Rabbit interferon (G 12). F, Vaccinia control.

Concentration of Interferon.—Some interferon preparations were concentrated approximately 10 times. This was achieved either by freeze-drying or by osmodialysis with 40% polyethylene glycol ("carbowax" 20,000 M or 4,000 M). Polyethylene glycol, especially the material of average molecular weight 4,000, diffused through the dialysis bags into the interferon, but did not invalidate its measurement either *in vitro* or *in vivo*.

Animal Experiments

Three experiments, each on six rabbits, were carried out. Interferon (0.1 ml.) was injected intradermally into the depilated flanks of the rabbits at five or six sites on each side. Vaccinia virus (20% suspension of infected chick chorio-allantoic membranes) was used as a challenge virus. Either immediately or 24 hours later, 0.1 ml. was inoculated at the same sites. The lesions were examined daily, and the diameter of the erythema was measured in millimetres. The mean diameters for six rabbits recorded on the stated (usually sixth) day after challenge with vaccinia virus are given in the tables. Necrosis and subsequent scab formation were recorded on an arbitrary scale, + indicating for each rabbit the usual degree of necrosis seen in untreated areas of rabbit skin challenged with vaccinia. There was a slight variation in susceptibility to vaccinia from rabbit to rabbit.

The single experiment with a cynomolgus monkey was performed in a similar manner, except that the challenge virus was a 50% suspension of chick chorio-allantoic membranes. The rhesus monkeys available were found to be relatively resistant to vaccinia given intradermally.

Results

In the first experiment, as shown in Table I and also in Fig. 1, it was found that the rabbit-kidney interferon and the chick interferon (G 10) greatly reduced the area of erythema and oedema when given 24 hours before challenge. The rabbit interferon completely suppressed necrosis in all six rabbits, and the other interferons restricted the necrosis to two out of the six rabbits, compared with the vaccinia controls, in which necrosis developed in five out of six. The interferon controls produced a slight but perceptible protective effect.

The results of the second experiment are shown in Table II. All the interferon preparations given 24 hours before challenge suppressed necrosis, and the rabbit-kidney interferon was the most effective preparation. The cynomolgus-monkey interferon and one batch of chick interferon (G 29C), and also the homologous interferon, markedly both reduced the area of erythema and suppressed necrosis when given 24 hours before

TABLE I.—Effect at 6 Days of Species of Origin of Interferon on Vaccinia Virus Infections of the Skin of Rabbits

	Species of Origin of Interferon given 24 Hours before Challenge						Controls given 24 Hours before Challenge			
	Chick M 1	Chick G 10	Calf	Monkey ¹ C 9	Rabbit ² G 12	None	Medium 199	Chick	Monkey ¹	Rabbit
Necrosis. Each + represents a necrotic lesion in one rabbit	++	++	++	++	0	++++	++++	+	++	++
Diameter of lesions (mm.)	5	2	6	13	3.5	12.5	12	7	10	8

¹ Cynomolgus monkey. ² Concentrated $\times 5$ by polyethylene glycol.

TABLE II.—Effect at 6 Days of Time of Administration and Species of Origin of Interferon on Vaccinal Lesions

		Species of Origin of Interferon				
		Control	Chick ¹ G 5	Chick ² G 29C	Rabbit ³ G 12	Monkey ⁴ G 21B
Interferon given 24 hours before challenge	Necrosis	+++++	0	0	0	0
	Diameter of lesions (mm.)	13.3	12	4.2	2.4	3.6
Interferon and vaccinia given simultaneously	Necrosis	+++++	+++++	+++	0	0
	Diameter of lesions (mm.)	15.6	18	6.1	5.7	8.1

¹ Concentrated $\times 10$ by freeze-drying. ² Concentrated $\times 10$ by polyethylene glycol. ³ Concentrated $\times 5$ by polyethylene glycol. ⁴ Cynomolgus monkey.

TABLE III.—Effect at 6 Days of Time of Administration and Species of Origin of Interferon on Vaccinal Lesions

		Species of Origin of Interferon					
		Control	Chick G 10	Monkey ¹ G 32	Monkey ² G 21B	Human Amnion G 35	Rabbit G 12
Interferon given 24 hours before challenge	Necrosis	++++	0	0	0	0	0
	Diameter of lesions (mm.)	18.8	5.1	5.1	4.1	3.4	0
Interferon and vaccinia given simultaneously	Necrosis	++++	++	+++	+	++	0
	Diameter of lesions (mm.)	20.1	17.6	20.8	11.7	17.4	4.3

¹ Rhesus monkey. ² Cynomolgus monkey.

TABLE IV.—Effect of Species of Origin of Interferon on Vaccinal Virus Infections of the Skin of a Cynomolgus Monkey

		Species of Origin of Interferon given 24 Hours before Challenge					
		Control	Chick ¹ G 5	Chick ² G 29C	Monkey ³ G 21	Monkey ⁴ G 32	Rabbit ⁵ G 12
8 days	Lesion	Pustule	—	Pustule	—	—	Pustule
	Diameter of erythema (mm.)	14	0	12	0	0	14
10 "	Lesion	Erythema with central necrosis and large scab	—	Erythema with central necrosis and large scab	—	Erythema with small scab	Erythema with central necrosis and large scab
	Diameter of erythema (mm.)	11	0	12	0	6	12

¹ Concentrated $\times 10$ by freeze-drying. ² Concentrated $\times 10$ by polyethylene glycol. ³ Cynomolgus monkey. ⁴ Rhesus monkey. ⁵ Concentrated $\times 5$ by polyethylene glycol.

challenge. The rabbit and cynomolgus-monkey interferons were also effective when given simultaneously with the vaccinia virus.

The third experiment showed that chick, rhesus, cynomolgus, human-amnion, and rabbit interferons were all active in suppressing the lesions when given 24 hours before challenge (Table III). Necrosis was observed only in the vaccinia controls. When the interferons were given at the same time as the challenge the homologous interferon gave good and the cynomolgus interferon slight protection.

Observations on the development and subsequent remission of the lesions confirmed these findings.

The results of the single experiment with a cynomolgus monkey are shown in Table IV, and also in Fig. 2, where it will be seen that the homologous interferon and the heterologous chick interferon (G 5) were highly effective in inhibiting vaccinal lesions. The rhesus-monkey interferon was rather less active.

Attention is called to the fact that the chick interferon (G 5) was found to be minimally active in rabbits (Table II) and that the rabbit (G 12) and the chick interferons (G 29C) were active in rabbits but inactive in the cynomolgus monkey.

Discussion

Isaacs and Westwood (1959) have described the superiority of rabbit over chick interferon in the inhibition of vaccinal lesions in the rabbit skin. Sutton and Tyrrell (1961) have shown in tissue-culture systems that interferon prepared in homologous cells is more effective than that prepared in heterologous cells. They also found that this species specificity in the action of interferon was not absolute, and, in particular, that rhesus-monkey interferon was the one most active on heterologous cells, including human cells. The work described here confirms these observations to some extent. Thus the rabbit and monkey (cynomolgus) interferons were most active in rabbits and a monkey, respectively. However, significant inhibition of vaccinal lesions in the skin was produced by some preparations of heterologous interferon. This observation could be important if interferon proves effective in human virus infections and might open the way to use of non-human cells in preparing interferon intended for therapy in human subjects. At present the evidence adduced here, like that of Sutton and Tyrrell (1961), suggests that monkey cells are a source of interferon that could reasonably be used in experimental work on man, but the choice of the best cell for producing interferon for use in man must await the results of further tests and clinical trials.

Summary

Interferon prepared from various species of cells has been found to inhibit the development of vaccinal lesions in the skins of rabbits and a monkey. Though the interferon prepared in homologous species of cells has the greatest effects, significant inhibition was produced by some interferons prepared from heterologous cells.

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EPIDEMIOLOGY OF ACUTE INTUSSUSCEPTION

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The north-east region of Scotland is a very suitable area in which to carry out incidence studies. About 90% of all the surgical emergencies occurring in the population of 425,000 are dealt with in the Aberdeen general and special hospitals. When the fringe counties of Kincardineshire and Morayshire are excluded the percentage of referrals approaches 100%. This is especially true of an acute abdominal condition such as intussusception. Several detailed studies on the incidence of intussusception have been carried out in densely populated and industrial areas of Britain (Morrison and Court, 1948; MacMahon, 1955; Court and Knox, 1959; Smith, 1960), but little has been known about its incidence in a less densely populated and mainly rural area. The present investigation was designed to provide such information.

The Investigation

Clinical Material.—The case records of all acute intussusceptions occurring in Aberdeen City, Aberdeenshire, and Banffshire during the years 1950–9 were studied. There were 145 cases, 140 being of the so-called idiopathic type. All except three had been treated at the Royal Aberdeen Hospital for Sick Children; smaller peripheral hospitals were visited to ensure that no cases were missed. From the data collected the incidence in the population at risk, the seasonal incidence, the age-and-sex distribution, and the family histories of the children affected were determined.

Incidence

During the decade under review there were 66,144 live births in the three areas designated above. The 145 intussusceptions occurring during this period therefore give an overall incidence of 2.19 per 1,000 live births (Table I). When the incidences in the city and in the two county areas are compared, it is found that the incidence is greater in the rural districts (2.55 per 1,000 births compared with 1.81 in the city). This difference is statistically significant ($\chi^2=4.131$; D.F. 1; $0.02 < P < 0.05$). The permanent address of the mother has been