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The Results of Controlled Observations on the Prophylaxis of Influenza with Interferon

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The results are reported of experimental investigations and epidemiological observations on the use of human leucocyte-produced interferon for influenza prophylaxis. Field trials with interferon were carried out during the outbreak of Hong Kong influenza in the USSR in January and February 1969. These covered about 14 000 people in comparable interferon- and placebo-treated groups and achieved an effectiveness of between 56.3% and 69.2% according to the age-group studied.

The importance is shown, not only of artificially introduced interferon, but also of the interferon naturally produced by a subject in response to infection. The degree of such production varies both with each individual's capacity to form interferon and with the capacity of a particular strain of Hong Kong virus to elicit the productive response. The author suggests that isolated virus strains be subdivided into interferon-positive (I+) and interferon-negative (I−) strains according to the presence or absence of this phenotypic character.

EARLIER STUDIES

Following the report of Gresser (1961) on the possibility of interferon production by human blood leucocytes treated *in vitro* with Sendai or measles viruses, we began to study this method, bearing in mind the necessity of providing a homologous interferon preparation for medical purposes.

In the course of this study some conditions for optimal interferon production were determined, including a favourable cell concentration and the use of Newcastle disease virus, strain H, as the best inducer among the many viruses tested. Leucocytes were supplied by the Institute of Haematology and Blood Transfusion from specially chosen healthy donors. A quantity of 500 ml of donor blood yielded about 50 ml of leucocyte mass which contained from 6×10^6 to 20×10^6 leucocytes per ml. The dose of virus used in interferon production was 10 to 100 $CPED_{50}$ (50% cytopathic effect dose) of Newcastle disease virus per leucocyte. The activity of the leucocyte-produced interferon was assayed in strains of diploid cells of human embryonic skin-muscle tissue; vesicular stomatitis virus, Indiana strain, was used as the indicator virus.

After numerous preliminary experiments, together with Dr T. A. Bektemirov we began in 1965 to study the effects of human leucocyte interferon on volunteers in experimental influenza, using for challenge A2 virus strains employed for production of live influenza vaccines.[2] Volunteers were treated with different doses of interferon by the intranasal route, using dispersion at different periods before challenging with a virus dose equal to 10^6 ID_{50} for chick embryos. The effects were determined by clinical examination of the volunteers for their general condition, by reisolation of the virus from the upper respiratory tract, and by following the antibody level in their blood sera.

The experiments showed that the minimum interferon dose for prophylactic effect was not less than 128 units,[3] and that the resistance induced was greatest during the first 24 hours, lasting for no more than 48 hours, after which a further administration of interferon was required. The most pronounced prophylactic effect in volunteers was obtained with 256 units of interferon repeatedly introduced before challenge (Solov'ev et al., 1966;

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[2] The experiments were carried out in the laboratory of the Department of Virology, Central Institute for Post-Graduate Training of Physicians, Moscow.

[3] For details of the method, see Bektemirov & Rappoport (1967).

Solov'ev, 1968). In these experiments altogether 375 volunteers took part; the results were supported later by similar observations by Dr A. N. Slepuškin [1] on an additional 131 volunteers, using our interferon. All this work unmistakably indicated the activity of the preparation in preventing experimental influenza as well as its complete safety for man after multiple intranasal administration.

In September 1967 our interferon was used for prophylactic purposes by Dr V. M. Bolotovskij and Dr L. N. Nefedova during an outbreak of A2 influenza among 474 schoolchildren. Their observations (Bolotovskij et al., 1968) corroborated the results obtained in volunteers and provided the basis for the Ministry of Public Health of the USSR to sanction more extensive application of the leucocyte interferon, and for us to set up a special laboratory for production of the preparation at the Virology Department, N. F. Gamaleja Institute for Epidemiology and Microbiology.[2]

In 1968 the effect of interferon was studied among population groups totalling 3100 persons of different ages, including the newborn, in different epidemiological situations, involving both A2 influenza (localized outbreaks) and other respiratory virus infections. Again a positive conclusion was arrived at from the results obtained by the physicians who carried out interferon prophylaxis and the complete safety of the preparation was conclusively proved.

STUDIES WITH HONG KONG INFLUENZA

Field trials

Very extensive field trials were carried out during the recent influenza epidemic caused by the Hong Kong virus. In Moscow the outbreak of Hong Kong influenza took place in January and February 1969, lasted for 40 days, and showed no important differences in the clinical course of the disease from the epidemics of preceding years. Virological investigations, however, showed an unusually high percentage (up to 80%) of virus isolations from washings of patients' upper respiratory tract as well as some clear differences between the characteristics of the isolates and those of their predecessors.

About 14 000 people of different ages were included in the interferon prophylaxis trials in population groups with high, medium, and low incidences of the disease. All observations in 1969, just as in previous years, were conducted under controlled conditions, with administration of a placebo to control groups of comparable age and size.[3]

The upper part of Table 1 summarizes the results among adults, who were given interferon daily for 5 days from the time that the first cases of influenza were recorded in each of the groups. It will be seen that the degree of protection in the interferon-treated groups, as compared with the controls, was 56%.

The middle portion of Table 1 summarizes the results of observations among schoolchildren 7–12 years of age in Moscow. Interferon was used daily for from 3 to 7 days from detection of the first cases of influenza. The degree of protection afforded was 69%.

The lower part of Table 1 summarizes the results of interferon administration in communities of children from 2–6 years of age in Donetsk in the Ukraine. Some 59% of these children were protected.

To illustrate the dynamics of the epidemic process in the different comparable groups, graphs are presented showing the effectiveness of human leucocyte interferon among adults given interferon at the height of the outbreak (Fig. 1) and before the outbreak (Fig. 2) of Hong Kong influenza.

Mechanism of protection

As to the mechanism of protection by interferon introduced in the upper respiratory tract, we believe that the effect of the introduced preparation is not infrequently combined with the action of interferon produced by cells of the respiratory organs. It is likely that the maximum prophylactic effectiveness of artificially introduced interferon is attained when infection of an individual occurs and a latent infectious process develops in consequence of the protection afforded by the introduced interferon. Multiplication of the virus, although not giving rise to symptoms of the disease, serves as the inducer for interferon production by cells of the mucous membranes of the respiratory tract. This possibility is borne out by the fact that a considerable proportion

[1] Laboratory of Epidemiology of Influenza, D. I. Ivanovskij Institute of Virology, Moscow (personal communication).

[2] O. V. Barojan, Director of the Institute; V. D. Solov'ev, Chief, Virology Department; V. P. Kuznecov, Chief, Laboratory of Interferon Biosynthesis, Moscow.

[3] The majority of the trials and observations were organized and conducted by the Central Institute of Epidemiology, Ministry of Public Health, USSR (Professor A. A. Sumarokov, Director; Dr V. M. Bolotovskij, in charge of the trials); results of these trials are shown in Table 1. Similar data obtained by the Gamaleja Institute (Dr I. N. Gajlonskaja) on a further 4163 subjects have not been included in that table.

TABLE 1

EFFECTIVENESS OF ADMINISTRATION OF HUMAN LEUCOCYTE INTERFERON AGAINST HONG KONG INFLUENZA

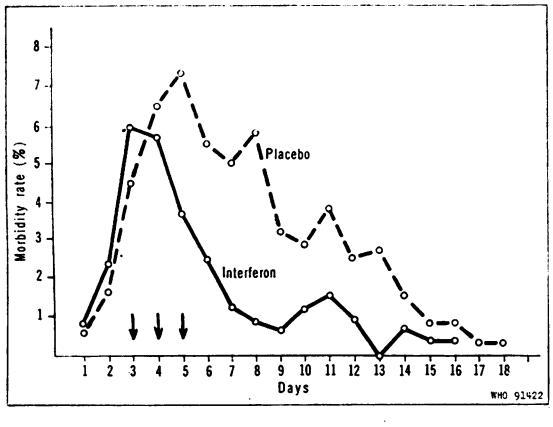
Preparation	No. in study	Morbidity		Standard error	Effectiveness [a]	
		No. of cases	%		*K*	*E* (%)
Adults (Moscow)						
Interferon	2 994	231	7.7	±0.49	2.3	56
Placebo	3 129	551	17.6	±0.68		
Children 7–12 years old (Moscow)						
Interferon	1 917	119	6.2	±0.55	3.2	69
Placebo	2 055	413	20.1	±0.88		
Children 2–6 years old (Donetsk)						
Interferon	463	22	4.8	±0.99	2.4	59
Placebo	454	53	11.7	±1.5		

[a] $K = \frac{\text{morbidity in the placebo group } (b)}{\text{morbidity in the interferon group } (a)}$

$E = \frac{100\,(b-a)}{b}$

FIG. 1

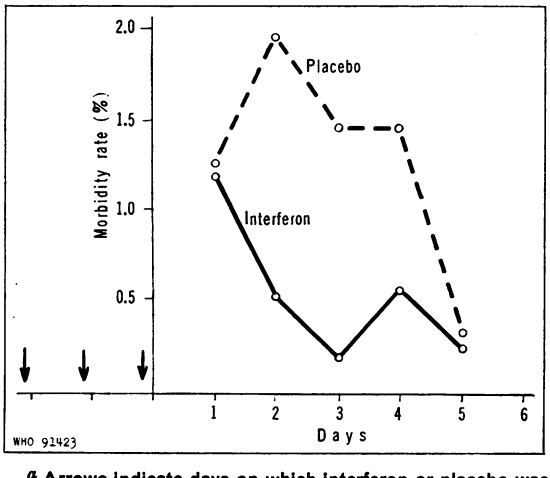
MORBIDITY AMONG ADULTS GIVEN INTERFERON (374 SUBJECTS) OR PLACEBO (382 SUBJECTS) *AT THE HEIGHT* OF THE OUTBREAK OF HONG KONG INFLUENZA [a]



[a] Arrows indicate days on which interferon or placebo was administered.

FIG. 2

MORBIDITY AMONG ADULTS GIVEN INTERFERON (320 SUBJECTS) OR PLACEBO (317 SUBJECTS) *BEFORE* THE OUTBREAK OF HONG KONG INFLUENZA [a]



[a] Arrows indicate days on which interferon or placebo was administered. The vertical scale indicates the start of the outbreak.

TABLE 2
INTERFERON FORMATION BY HONG KONG INFLUENZA VIRUS STRAINS

Interferon-negative (I−) virus strains					Interferon-positive (I+) virus strains				
Virus strain No.	Haemagglutinating activity (reciprocal titre)	Chick-embryo ID_{50}	Neuraminidase activity (μg neuraminic acid/ml virus suspension/min)	Interferon in serum (reciprocal titre)	Virus strain No.	Haemagglutinating activity (reciprocal titre)	Chick-embryo ID_{50}	Neuraminidase activity (μg neuraminic acid/ml virus suspension/min)	Interferon in serum (reciprocal titre)
1	160	10^{10}	7.8	0	2	320	10^{10}	13.1	128
9	640	10^{12}	2.5	0	15	640	10^{8}	10.6	128
13	160	10^{12}	6.4	0	23	320	10^{8}	6.7	128
17	80	10^{9}	5.0	0	84	160	10^{12}	11.2	128
19	640	10^{5}	2.3	0	86	40	10^{11}	7.3	128
31	80	10^{9}	4.8	0	36	160	10^{5}	6.7	128
68	320	10^{6}	9.9	0	50	320	10^{7}	23.7	128

(up to 50%) of interferon-treated persons *who did not contract the disease* developed 4-fold or greater increases in antibody levels, indicating that they underwent latent infections.

It has been shown in special investigations both in animals of various species and in man, that the degree of interferon production varies with the individual. According to our data, between 12% and 30% of persons fail to produce interferon.

Hong Kong virus as inducer

In this connexion, of great interest are experiments which prove that different strains of Hong Kong influenza virus isolated during the 1969 outbreak likewise differ in their ability to induce interferon-formation. Some 46 strains tested in this respect after 2 passages in chick embryos all belonged to the same serotype and grew well after allantoic inoculation of 9-day-old embryos and had comparable haemagglutination titres. When these strains were injected intravenously into mice in equal doses, 18 strains appeared to be poor interferon-inducers, 20 were good interferon-inducers and the remaining 8 were intermediate.

When examined under the electron microscope, all the strains showed a similar morphology: spherical virions and filamentous forms with a predominance of the latter (see Fig. 4).

Seven strains with minimal capacity to produce interferon (I−) and 7 with a maximal capacity to do so (I+) were used for more detailed study of their properties (Table 2). The interferon-positive (I+) strains were found to be also more active in neuraminidase activity.

Strains 1 (I−) and 2 (I+) were subjected to heating at 56°C for 60 minutes; no difference was found in thermostability of haemagglutinins at the end of that time; however, in comparative titration for infectivity to chick embryos, strain 1 (I−) was found noticeably more thermolabile (Fig. 3).

FIG. 3
RELATIVE THERMOSTABILITY OF I+ AND I− STRAINS OF HONG KONG INFLUENZA VIRUS

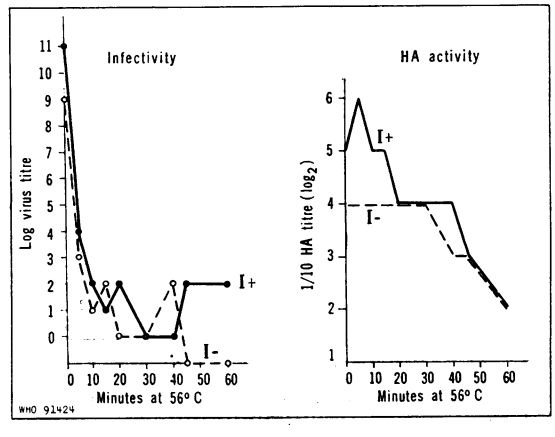


FIG. 4

FILAMENTOUS PARTICLE OF I− STRAIN (A) AND SPHERICAL VIRION OF I+ STRAIN (B)

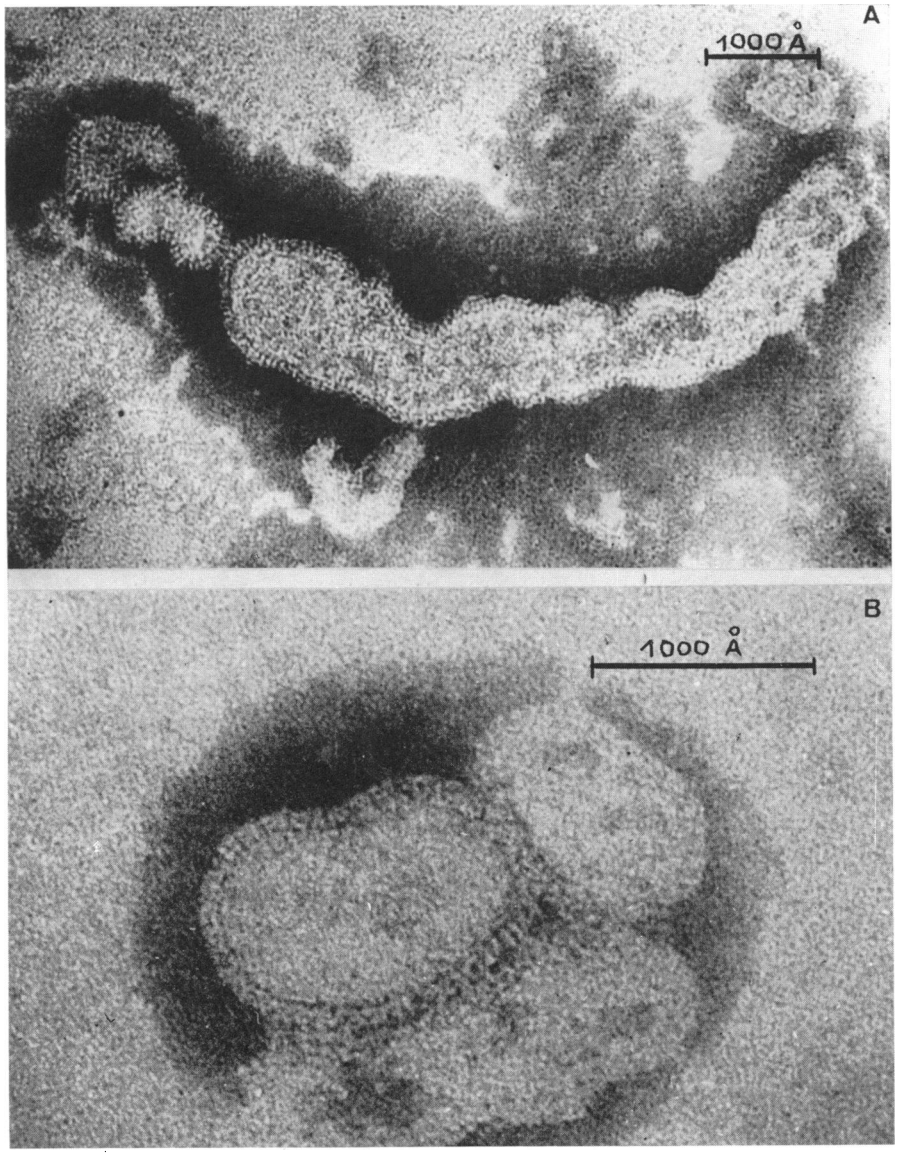


TABLE 3
INTERFERON FORMATION IN MAN BY 2 STRAINS OF HONG KONG INFLUENZA VIRUS

Volunteer	Antihaemagglutinins		Virus in nasal washings in 24 hours	Interferon in nasal washing in 24 hours		Clinical picture in 48–72 hours
	Before infection	After infection		Human embryo fibroblasts	Diploid cells	
			I− virus			
I.M.	40	40	10^2	0	16	Normal
P.M.	80	320	10^4	0	0	Influenza; 38.6°C
A.L.	160	320	10^2	0	0	Influenza; 38.1°C
O.N	40	80	0	4	16	37.1°C (1 day)
M.L.	80	160	10^2	0	4	Influenza; 38.3°C
			I+ virus			
P.L.	80	40	0	4	16	Normal
B.N.	160	40	0	0	8	Normal
M.A.	160	80	0	0	4	Normal
B.A.	80	160	0	4	8	Normal
A.P.	160	160	10^3	8	16	37.1°C (1 day)

The same 2 strains of the virus were also tested for interferon production in man; for this purpose they were given intranasally to 2 groups of volunteers, of 5 persons each, in a dose of 10^6 ID_{50} (for chick embryos), 0.5 ml in volume. All the volunteers had had Hong Kong influenza 2½ months earlier, and we considered that there was little likelihood of repeated disease as a result of further infection. The actual findings proved different, however, and strain 1/Moscow/69 (I−) of Hong Kong influenza virus produced typical disease after an incubation period of 48–72 hours.[1] In these persons, virus was detected in nasal washings 24 hours after infection and very small amounts of interferon were found in some of these washings. The volunteers in the other group, who were given I+virus (strain 2/Moscow/69), showed quite a different picture: disease was practically absent, while interferon was found in the nasal washings in every case without exception. The results with these groups of volunteers are shown in Table 3.

Conclusions

The following preliminary conclusions may be drawn from this work with volunteers:

[1] The disease in volunteers was suppressed the following day by massive administration of concentrated interferon.

(1) The interferon-inducing property of freshly isolated strains of Hong Kong influenza virus constitutes a rather important phenotypic character.

(2) Interferon-negative (I−) strains seem to have a considerable pathogenicity for man.

(3) Strains of Hong Kong influenza virus that have elevated neuraminidase activity but are interferon-positive (I+) are less pathogenic for man, which argues against involvement of the enzyme in the degree of invasiveness of the virus.

(4) In the light of the reported experimental findings, the general conclusion may be drawn that analysis of the effectiveness of human leucocyte interferon in influenza prophylaxis should be made on the basis of controlled observations taking into account not only epidemiological factors but also the specificity of antiviral action of both exogenous and endogenous interferon and information on the manner in which the organism forms interferon and on its capacity to do so.

DISCUSSION

Several factors may be considered to influence the effect of leucocyte interferon: the dose, the frequency and method of administration, the general

character and specific features of the infectious process, and the sensitivity of the virus against which the interferon is used. It seems that with influenza the preparation answers the purpose for which it is intended as we are convinced by the experiments carried out in volunteers. By strictly controlled observations on volunteers given a definite dose of virus and a definite quantity of interferon we succeeded in obtaining the necessary correlation of results, allowing us to draw a positive conclusion as to the suitability of this method of prophylaxis and to lay the basis for its possible adoption in practice.

Epidemiological investigations carried out by various experts into the prophylactic action of interferon during influenza outbreaks between 1967 and 1969 have confirmed our earlier findings. During the epidemic of Hong Kong influenza, the average decrease in morbidity among the population groups in which the interferon was used amounted to 61.6%. It should be noted here that the preparations used, both interferon and placebo, were coded, which increases the objective value of the results obtained.

The leucocyte interferon seems to act not only independently, but not infrequently in association with the action of interferon formed endogenously by cells of the respiratory tract in response to a latent infection. Hence the importance of evaluating the capacity of individuals to form interferon, thus bringing to light any subjects in the population who are unable to do so.

A remarkable feature was the discovery of heterogeneity among strains of Hong Kong influenza virus, which were clearly different in their interferon-inducing activity. The 46 strains investigated could almost be divided into 2 nearly equal groups according to the presence or absence of such activity. Interferon-positive (I+) strains had high neuraminidase activity but weak pathogenicity for man; in the interferon-negative (I−) strains the enzyme activity was lower but they caused typical influenza infection in inoculated persons. Two strains tested, one I+ and the other I−, differed also in thermostability. This finding is of interest not only in itself but also because it offers a new opportunity for detailed study of the pathogenesis of influenza infection.

We consider that there are sufficient grounds to recommend human leucocyte-produced interferon as one of the means of influenza prophylaxis. The method is absolutely harmless, simple and convenient, and should be applied where there is an immediate threat of infection, that is, as a means of emergency prophylaxis. It can both be valuable on its own and serve as a supplementary method to increase the protective value of vaccination.

The investigations of Lavrov et al. (1968) have shown the practical interest of combining interferon with amantadine, since the former blocks the reproductive cycle of the virus while the latter prevents the virus from penetrating the cell.

There are also prospects for the further improvement of leucocyte interferon by purification and concentration. In our laboratory, partial purification of the preparation from components with a molecular weight of less than 5000 was undertaken on a production scale, using a filtration procedure with Sephadex G25. The interferon purified by this method and concentrated to 1000 units/ml was used during the outbreak of Hong Kong influenza for treating 147 influenza patients. According to the physicians in charge, the results were promising, provided that instillations of interferon were made within the first 2 days of the disease. It is expected that this concentrated preparation may be more effective for prophylactic use than the interferon previously used.

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