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Modulation of human natural killer cytotoxicity by influenza virus and its subunit protein

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Summary. The influence of intact influenza virus and purified detergent solubilized haemagglutinin (HA) subunits from these viruses on human natural killer (NK) cell activity was examined. Effector cells incubated with whole influenza virus for 18 hr initiated the production of alpha interferon which was associated with the enhancement of NK cell activity. In contrast, purified influenza virus HA suppressed NK activity in a dose-dependent manner, when added at the onset of the cytotoxicity assay, or when used to pre-treated effector cells prior to assay for cytotoxicity against K562 target cells. Effector cells exposed to influenza HA for 90 min, washed and re-incubated in fresh medium for up to 18 hr, failed to regain their cytotoxicity. Suppression of NK cell cytotoxicity could not be ascribed to direct toxicity of HA preparations or residual detergent and preservative in these preparations. The augmented cytotoxicity of activated human effector cells was also susceptible to suppression by virus HA, and pretreatment of human PBL effector cells with HA for 90 min, prior to exposure to human alpha interferon caused NK effector cells to become refractive to the enhancing effects of HIFN. That direct interaction between influenza virus HA and effector cells was a requirement for suppression of activity was shown in experiments using Bromelain-released influenza HA, which would not be expected to bind to cells and which failed to suppress NK cell activity.

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INTRODUCTION

Spontaneous cytotoxicity has been observed in circulating lymphoid cells of human and many rodent species against a variety of established tumour cell lines, and this activity has been ascribed to a lymphocyte subpopulation designated natural killer (NK) cells (Herberman, Nunn & Lavrin, 1975; Herberman & Holden, 1978; Santoli, Trinchieri & Kopowski, 1978). The importance of NK cells as a surveillance mechanism against foreign cell types, including tumour cells and virus-infected cells, has been inferred, and NK cytolytic activity is augmented following treatment of effector cells with interferon (IFN) or IFN-inducers *in vitro* and *in vivo* (Trinchieri & Santoli, 1978; Einhorn, Blomgren & Strander, 1980).

Infection with many viruses results in IFN production and subsequent NK activation. For example, a rise in NK cell activity was observed shortly after infection of volunteers with influenza virus (Ennis *et al.*, 1981), and the severity of symptoms correlated with virus shedding, induction of IFN, and enhancement of NK cell activity. Recently Djeu *et al.* (1982) have reported the augmentation of human NK activity *in vitro* by influenza A and HSV-viruses; this augmentation was the result of induction of IFN

within the NK cell population. In contrast, other agents, including prostaglandins (Brunda, Herberman & Holden, 1980), cyclic nucleotides, histamine (Roder & Klein, 1979), phorbol ester and cholera toxin (Goldfarb & Herberman, 1981) are associated with the suppression of NK cell activity.

Only a few studies have been reported on the effect of viral antigens on spontaneous or IFN-augmented NK cell activity. Recently mumps virus and measles virus proteins and glycoproteins have been shown to enhance spontaneous cytotoxicity, although the observed increased cytotoxic activity following exposure of effector lymphocytes to viral glycoproteins may occur via an IFN-independent mechanism (Harfast *et al.*, 1980; Casali *et al.*, 1981).

We have investigated the effect of influenza virus and its subunit proteins on NK cell activity, and in contrast to the results of other investigations have observed impaired NK cell cytolytic activity following exposure to purified subunit haemagglutinin.

MATERIALS AND METHODS

Peripheral-blood lymphocytes

Peripheral blood was obtained from healthy male and female donors and citrate phosphate dextrose anticoagulent solution added. Leucocyte-enriched buffy coat was obtained by the addition of 10% dextran and sedimented at 37° for 30 min, and the mononuclear fraction was recovered by centrifugation on lymphocyte separation medium (Flow Laboratories Ltd, Irvine, Scotland). The recovered cells were washed 3 times in RPMI 1640 medium—10% newborn calf serum (RPMI-CS) and passed over nylon wool (Julius, Simpson & Herzenberg, 1973). Nylon-wool non-adherent (eluted) cells were recovered, washed 3 times in RPMI-CS, and the resulting cells used as effectors in NK assays.

Purification of influenza virus and preparation of HA

Influenza viruses were grown in embryonated hens' eggs and purified by standard techniques (Skehel & Schild, 1971). Approximately 20 mg of purified virus was disrupted by the addition of Triton X-100 detergent to a final concentration of 2% (v/v) for 15 min, and fractionated on 20%:50% (w/v) sucrose gradients using a SW41 rotor on a Beckman ultracentrifuge by centrifugation at 35,000 rev/min for 16 hr. Gradient fractions were examined for the presence of virus antigen by rocket immunoelectrophoresis (Oxford & Schild, 1977). Fractions were dialysed at room temperature for 4–5 days against phosphate-buffered saline (PBS) before being used. In certain experiments HA was removed from virus particles by digestion with bromelain.

The following viruses were used in the present studies: A/Brazil/11/78 (H1N1), A/England/77 (H3N2), A/Bangkok/79 (H3N2), A/Victoria/75 (H3N2), A/Hong Kong/1/68 (H3N2) and B/Lyon/79.

Target cells

The K562 leukaemic cell line free from mycoplasma contamination was used in the present study and grown in RPMI-CS medium as a suspension culture.

Human IFN

Human leucocyte (α) IFN was kindly supplied by Dr Karl Fantes, Wellcome Research Laboratories, Beckenham, Kent, England. This preparation was diluted to 1000 IU/ml and stored at −80°.

Interferon assay (cytopathic effect (CPE)-inhibition assay)

This assay is based on an indirect measure of the cell damage by the amount of vital dye taken up by the cells, and is described in detail elsewhere (Johnston, 1981). All IFN titres are given in $\log_{10}$ international units (IU/ml).

Four-hr chromium release assay

Cytotoxicity tests were performed as previously described (Hassan & Rees, 1980) in round-bottomed microtest plate wells (Falcon Microtest III Flexible assay plates, Becton Dickinson). The percent ^{51}Cr release was determined by the following formula:

$$\%\ ^{51}\text{Cr release} = \frac{(\frac{1}{2}\ \text{SN}) \times 2}{(\frac{1}{2}\ \text{SN}) + (\text{cell pellet} + \frac{1}{2}\ \text{SN})} \times 100,$$

(where SN = supernatant).

The percent cytotoxicity was calculated as follows:

$$\%\ \text{Cytotoxicity} = \frac{(\text{test release}) - (\text{spontaneous release})}{100 - (\text{spontaneous release})} \times 100.$$

The statistical significance of the results was determined by Student '*t*' test, and expressed in the usual manner: (*$P = < 0{\cdot}05$; **$P = < 0{\cdot}01$; ***$P = < 0{\cdot}001$).

Pre-treatment of effector cells with viral antigens and IFN

Peripheral-blood effector cells at a concentration of

Table 1. Interferon induction and enhancement of natural cytotoxicity by influenza type-A viruses

Exp. No.	Virus	% Cytotoxicity 50:1	25:1	12:1	Units of IFN/ml
	None	19·0	12·3	6·6	<2·3
1	A/Brazil/11/78	32·9***	18·0**	11·1**	22·9
	A/England/77	24·6***	16·4**	9·6*	165·9
	None	9·1	3·8	2·0	<2·3
2	A/Brazil/11/78	21·5***	13·1***	6·5**	61·6
	A/England/77	25·9***	16·9***	8·5**	870·9
	None	17·3	7·1	3·8	<2·3
3	A/Brazil/11/78	38·7	21·9***	10·2***	20·8
	None	36·3	16·0	7·8	<2·5
4	A/Victoria/75	46·9***	26·5***	23·4***	22·9
	A/Bangkok/79	62·4***	43·3***	25·0***	691·8

Effector cells (5×10^6/ml) were pre-incubated with influenza viruses (10 μg/ml) for 18 hr, followed by washing 3 times in RPMI-CS and tested in 4 hr ^{51}Cr-release test against K562 target cells.

Significant augmentation of NK cell activity determined by Student's '*t*' test: ***$P = <0{\cdot}001$; **$P = 0{\cdot}01$.

5×10^6 cells/ml were pre-treated with purified virus, virus haemagglutinin (HA) or IFN at 37° in a humidified 5% CO_2/95% air atmosphere. The time of incubation and experimental conditions is indicated for each experiment (Results section).

RESULTS

Induction of IFN and enhancement of NK activity by influenza virus

In the present study four influenza viruses (A/Brazil/11/78, A/Bangkok/79, A/Victoria/75, A/England/77) were tested *in vitro* for their ability to enhance NK cell cytotoxicity, and stimulate the production of IFN. Effector cells (5×10^6/ml) were pre-incubated with influenza virus (10 μg/ml) for 18 hr, the cells sedimented and supernatants collected for IFN estimation. Effector cells were then washed (3 times) in RPMI-CS and tested in a 4-hr ^{51}Cr-release assay against K562 target cells. Control effector cells were incubated in parallel in media alone. A significant ($P = <0{\cdot}05-<0{\cdot}001$) augmentation of the cytolytic activity was observed with all viruses tested (Table 1). Significant levels of IFN could be demonstrated in the culture supernatants, and in some instances enhanced NK cell activity correlated with the level of IFN produced. Virus concentration of 10 μg/ml was shown to be optimal for NK activation and IFN production since lower or higher concentrations (2–5 or 40 μg/ml) failed to induce maximum activation.

Experiments were performed to determine the time at which augmentation of natural cytotoxicity and IFN production by human PBLs occurred in response

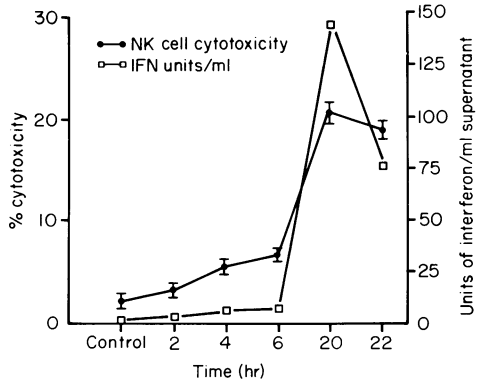

Fig. 1. Kinetics of IFN induction and NK cell activation by influenza virus. Effector cells were pre-treated with 10 μg/ml of influenza A/Brazil/11/78 virus for 2, 4, 6, 20 and 22 hr. The cells were centrifuged and supernatants were collected for IFN assay; the remaining cells were washed in RPMI-CS and tested in a 4-hr ^{51}Cr-release assay against K562 target cells.

to influenza A/Brazil/11/78. Effector cells (5×10^6/ml) were pre-treated with 10 g/ml of influenza A/Brazil/11/78 virus for 2, 4, 6, 20 and 22 hr, the culture supernatants were collected, and the cells washed ($\times 3$ in RPMI-CS) and assayed for cytotoxicity against K562 target cells. Interferon (IFN) was detected in culture supernatants within 2 hr of exposure to virus, which rose sharply at 20 hr (Fig. 1). There was a close correlation between IFN production and the augmentation of natural cytotoxicity; enhancement of natural killing was observd within 2 hr of virus pre-treatment, and more than a 3-fold increase was observed by 6 hr after treatment, reaching a maximum at 20 hr.

Treatment of human PBL with purified influenza HA

Following overnight exposure of human PBLs to subunit haemagglutinin (HA) antigen preparations from A/Victoria/75, A/Bangkok/79 and B/Lyon/79 influenza viruses, PBLs were recovered and assayed for cytolytic activity against K562 target cells. Lymphocytes incubated overnight at 37° without added HA demonstrated significant cytotoxicity ($P = <0{\cdot}01 - <0{\cdot}001$) in a 4-hr cytotoxicity assay, whereas in seven out of eight tests performed, pre-incubation of PBLs with purified influenza HA resulted in the absence of detectable natural killing (Table 2). Significant ($P = <0{\cdot}01 - <0{\cdot}001$) inhibition (80–100% reduction in cytotoxicity) of spontaneous cytotoxicity was shown in all experiments. Pre-treatment of PBLs with A/Victoria/75HA caused significant inhibition ($P = <0{\cdot}001$) of natural cytotoxicity at 1, 3 and 10 μg/ml (Fig. 2a). Similar dose–response curves were shown for A/Bangkok/79 and B/Lyon/79 HA. HA preparations incubated with effector cells overnight did not reduce the viability (as assessed by trypan-blue exclusion) of PBLs. The inhibitory effect of A/Victoria/75, A/Bangkok/79 and B/Lyon/79 HA on NK cell activity was found to be dependent upon

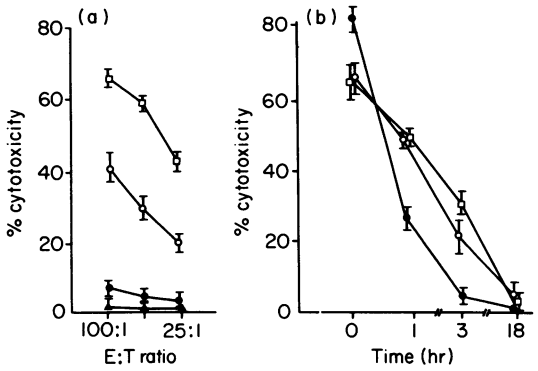

Fig. 2. (a) Dose–response curve—inhibition of spontaneous NK cell activity against K562 targets by influenza HA. A/Victoria/75; □ = control, ○ = 1 μg HA/ml, ● = 3 μg/ml, ▲ = 10 μg/ml. (Similar dose–response curves were shown for A/Bangkok/79 and B/Lyon/79 HA.) (b) Effect of time of exposure on human PBLs to purified HA from A/Victoria/75 (□), A/Bangkok/79 (●) and B/Lyon/79 (○). PBL effectors incubated at 37° with HA at 5 μg/ml.

Table 2. Effect of influenza virus HA on natural killing by human PBL

Exp. No.	HA	% Cytotoxicity: Control	% Cytotoxicity: HA-treated	% Inhibition
1	A/Victoria/75‡	11·5**	2·3	80·0**
2		12·9***	0	100***
3	A/Victoria/75§	65·7***	1·1	95·3***
4		64·3***	2·0*	95·7***
5	A/Bangkok/79‡	81·6***	0·9	98·8***
6		63·1***	4·6	88·5***
7	B/Lyon/79‡	39·9***	1·6	95·9***
8		41·7***	1·5	96·4***

† Effector cells treated overnight (18 hr) with influenza HA at 10 μg/ml, washed 3 times in RPMI-CS medium and tested for cytotoxicity against K562 targets at a 50:1 E:T ratio.

‡ HA obtained from National Institute for Biological Standards and Control.

§ HA obtained from Department of Virology, Medical School, University of Sheffield.

the time of exposure of effector cells to HA antigen. In experiments where the effector cells were washed thoroughly to remove residual HA antigen (×3 in RPMI) prior to testing, a significant reduction in natural killing ($P = <0{\cdot}01-<0{\cdot}001$) was observed following 1 hr pre-incubation with purified HA (Fig. 2b), and cytotoxicity was further decreased following incubation of PBLs for 3 hr ($P = <0{\cdot}001$), and 18 hr ($P = <0{\cdot}001$) with influenza HA.

Failure to recover NK cell activity following incubation of effector cells with influenza HA

Effector cells exposed to A/Bangkok/79 HA for 18 hr, were washed (×3 in RPMI-CS) and reincubated for a further 4-hr period in fresh medium prior to assay for cytolytic activity against K562 targets. The results show that PBLs exposed to A/Bangkok/79 HA failed to regain their original cytolytic activity following reincubation in fresh medium for 4 hr (Fig. 3a). A further experiment (Fig. 3b) was performed where effector PBLs were exposed to HA for 90 min, washed (×3 in RPMI-CS) and reincubated at 37°C for 18 hr in fresh medium; control PBLs were incubated in either medium alone, or in the presence of HA for 20 hr. The continued presence with HA caused complete abrogation of cytolytic activity, and pre-treatment of PBLs with HA for 90 min, followed by an 18-hr recovery period, failed to give recovery of cytolytic activity; the maximum cytotoxicity at 100:1 and 50:1 E:T ratio, was less than 15% that of control PBL activity (medium control) (Fig. 3b).

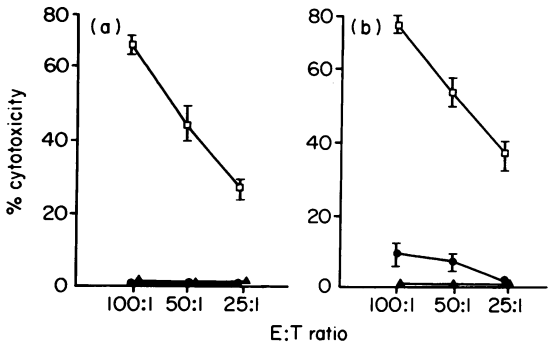

Fig. 3.(a) Cytotoxicity of human PBL effectors incubated with A/Bangkok/79 HA at 5 μg/ml concentration, followed by a second incubation in fresh medium for 4 hr prior to assay for cytolytic activity against K562 targets. Control (□), effector cells + HA for 22 hr (▲), effector cells + HA for 18 hr at 37° and 4 hr in fresh RPMI-CS medium (●). (b) Cytotoxicity of human PBL effectors incubated with A/Bangkok/79 HA at 5 μg/ml. Control (□) effector cells + HA for 90 min and incubated fresh medium 18 hr (●), effector cells + HA for 20 hr (▲).

Effect of influenza HA on IFN-boosted NK cell cytotoxicity

PBL effectors were incubated at 37° in the presence or absence of human α interferon (HIFN) (100 I.U./ml) and the cells washed (×3 in RPMI-CS medium) prior to assay. Control or IFN-treated effector cells were incubated with either A/Bangkok/79 or B/Lyon/79 HA (5 μg/ml concentration, 4-hr treatment prior to assay), and HIFN-treated and control PBLs minus HA were assayed for cytotoxicity in parallel. Both HIFN-treated and untreated NK effectors were highly susceptible to the inhibitory effect of purified HA and a significant ($P = <0{\cdot}001$) reduction in cytolytic activity was observed using A/Bangkok/79 (Fig. 4a); B/Lyon/79 HA gave similar results (not shown).

Modulation of HA-treated NK cell cytotoxicity by IFN

PBLs were incubated with A/Bangkok/79 and B/Lyon/79 HA at a concentration of 5 μg/ml for 90

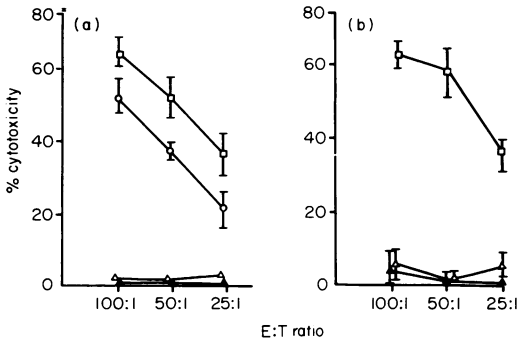

Fig. 4.(a) Effect of A/Bangkok/79 HA on HIFN-boosted and spontaneous human NK cell activity. HA used at a concentration of 5 μg/ml and added to PBL effectors for 4 hr at 37°. Effectors minus HIFN minus HA (○), effectors minus HIFN + HA (▲), effectors + HIFN minus HA (□), effectors + HIFN + HA (△). Similar results were obtained with B/Lyon/79 HA. (b) Effect of A/Bangkok/79 HA pre-treatment of human PBL effector cells on their ability to respond to stimulation by HIFN. Cytotoxicity control (□), effector cells pre-treated with HA (▲), effector cells pretreated with HA and incubated with HIFN (△). Effector cells were pretreated with HA at 5 μg/ml for 90 min at 37°, washed 3 times in RPMI-CS and incubated at 37° for 18 hr with HIFN at 100 I.U./ml, prior to assay for cytotoxicity against K562 targets. Similar results were obtained with B/Lyon/79 HA.

min at 37°, the cells were washed (×3 in RPMI-CS), and reincubated overnight (18 hr) at 37° in the presence or absence of HIFN (100 IU/ml), and subsequently tested for natural killing against K562 targets. Control PBLs incubated in medium alone showed significant cytolytic activity against K562 targets, whereas pre-treatment of effectors with HA for 90 min, followed by overnight incubation in fresh medium resulted in an almost complete abrogation of cytoxic activity (Fig. 4b). PBL effectors pre-treated with HA and reincubated in the presence of HIFN failed to show increased cytolytic activity above control (HA-treated only) levels. Thus, HIFN proved incapable of restoring the cytotoxicity of NK effectors previously exposed to influenza HA.

Effect of bromelain-released influenza HA on NK cells

The truncated HA obtained by bromelain digestion of whole virus is antigenically and structurally intact (Wrigley, Laver & Downie, 1977; Flanagan & Skehel, 1977; Brand & Skehel, 1972); however, the removal of the hydrophobic tail of the HA molecule by digestion with bromelain can prevent the attachment of the HA molecule to liposomes (Oxford, Hockley & Patterson, 1981), and it is unlikely that bromelain-released HA (BHA) would bind to the intact membrane of the cells. We investigated the effect of BHA on NK cytolytic activity. Effector cells were incubated at 37° in the presence of 2·5 μg/ml A/Bangkok/79 HA, or 10 μg/ml A/Brazil/11/78 and A/Hong Kong/1/68 BHA for 90 min. The cells were recovered, washed 3 times in RPMI-CS, and reincubated in fresh medium overnight prior to assay against K562 target cells. A/Bangkok/79 HA at 2·5 μg/ml reduced NK cytolytic activity, however in contrast 10 μg/ml BHA of A/Brazil or A/Hong Kong/1/68 failed to influence NK cell cytotoxicity (Table 3, Exp. 1). The lack of inhibitory effect

Table 3. Effect of bromelain-released influenza HA on human NK cell activity

Exp. No.	HA/BHA† (concentration)	E:T Ratio	% Cytotoxicity	% Inhibition
1	Control	50:1	19·4	
		25:1	20·1	
		12:1	10·5	
	A/Bangkok/79	50:1	3·3***	82·5
	HA	25:1	2·4***	88·11
	(2·5 μg/ml)	12:1	0·7***	93·3
	A/Brazil/11/78 BHA	50:1	23·1	—
	(10 μg/ml)	25:1	20·0	—
		12:1	11·9	—
	A/Hong Kong/1/68	50:1	19·8	—
	BHA (10 μg/ml)	25:1	19·8	1·4
		12:1	12·5	—
2	Control	50:1	47·8	
		25:1	32·9	
		12:1	21·7	
	A/Bangkok/79 HA	50:1	1·5***	96·8
	(2·5 μg/ml)	25:1	1·3***	96·1
		12:1	2·6***	88·0
	A/Brazil/11/78 BHA	50:1	48·6	—
	(50 μg/ml)	25:1	33·8	—
		12:1	20·6	5·1
	A/Hong Kong/1/68	50:1	45·9	3·9
	BHA (50 μg/ml)	25:1	30·5	7·3
		12:1	19·1	11·9

† Effector cells incubated with HA (haemagglutinin) or BHA (bromelain-released HA) for 90 min/37°, washed and incubated at 37° for 18–22 hr prior to assay.

*** $P = 0{\cdot}001$, representing a statistically significant reduction in cytotoxicity compared with control results.

was confirmed in a second experiment using A/Brazil/11/78 and A/Hong Kong/1/68 BHA at 50 μg/ml concentration (Table 3, Exp. 2).

DISCUSSION

There is now considerable evidence to suggest that virus infection, particularly infection with influenza virus, causes a marked alteration in host immunocompetence. The effect of influenza virus on lymphocytes was recently described by Faguet (1981), and Cate & Couch (1981). Depression in the numbers of circulating T lymphcytes in vaccinees (Faguet, 1981) and decreased *in-vitro* mitogen responsiveness have been reported (Jarstrand, Wasserman & Dahl, 1977; Manzella & Roberts, 1979). Depressed delayed hypersensitivity skin-test reactivity to recall antigens (Reed, Olds & Kisch, 1972) can occur, particularly in advanced disease (Cate & Couch, 1981), and macrophage and neutrophil disfunction has been shown (Roberts *et al.*, 1980; Abramson *et al.*, 1981). Alternatively, other limbs of the immune defence system may become activated during virus infection, and in particular NK cells show an increased cytolytic capacity as a result of *in-vitro* exposure to virus infected cells, and *in-vivo* virus infection (Santoli *et al.*, 1978; Welsh, 1981; Leung & Ada, 1981). Interferon activates NK cells, and its production may occur either as a result of virus infection of host cells, or the interaction of viral antigens with circulating lymphocytes (Welsh & Doe, 1980). Green *et al.* (1982) have reported interferon in acute-phase sera of 30 of 40 subjects with culture and/or serologically documented naturally acquired influenza A/Brazil/78 (H1N1) infection, and in the acute-phase sera of five of seven subjects with an influenza-like illness of undetermined aetiology. However, no statistical correlation was shown between IFN level and the course of the clinical illness.

In the present study the *in-vitro* effect of influenza virus and purified influenza haemagglutinin on human NK cells was evaluated. The activation of NK cells by influenza viruses was proportional to the virus concentration and time of exposure, and was accompanied by IFN induction. Naturally acquired influenza infection is accompanied by circulating IFN in titres comparable to those found in human recipients of passively administered, exogenously produced IFN-α (Greenberg *et al.*, 1976). More recently Djeu *et al.* (1982) have shown that the augmentation of NK activity by influenza A/PC virus appears to be caused through the induction of α-interferon (IFN) within the NK cell population itself. The results of the present study confirm that the IFN released from PBMC pre-treated with influenza virus is IFN-α (thermo and pH 2·0 stable). In contrast, Ennis & Meager (1981) have recently reported that purified HA preparations were not capable of inducing IFN.

Harfast *et al.* (1980) have reported that human peripheral-blood lymphocytes from healthy donors express enhanced natural cytotoxicity to target cells after a brief exposure to mumps virus *in vitro*; however, depletion of virus glycoprotein spikes by proteolytic enzymes resulted in abrogation of their ability to stimulate cytotoxicity. Natural-killer cell stimulation by mumps virus or its glycoproteins occurred without the generation of detectable levels of IFN in the culture medium, and Casali *et al.* (1981) have shown that the purified haemagglutinin and fusion glycoproteins of measles virus, either in soluble form or when inserted into artificial membranes, are capable of binding to human peripheral blood lymphocytes and enhancing cell-mediated cytotoxicity in a dose-dependent fashion. Viral glycoprotein-produced responses occurred in the absence of any detectable release of IFN into the culture medium, whereas NK activity due to whole virions was associated with IFN production. These studies (Harfast *et al.*, 1980; Casali *et al.*, 1981) demonstrate enhancement of NK cell cytotoxicity via IFN-dependent and -independent mechanisms.

In the present study purified influenza haemagglutinin (HA) suppressed NK cytotoxicity, and incubation (recovery) for up to 18 hr in fresh medium failed to restore the functional capacity of NK cells. This is unlike the reported suppression of NK activity with prostaglandins E_1 and E_2, where the continued presence of inhibitor is necessary for the maximum inhibition of NK cell activity (Brunda *et al.*, 1980). Furthermore, HA preparations were shown to be non-toxic to lymphocytes, and studies with bromelain-released HA (BHA) showed that HA binding to NK cells was a pre-requisite for inhibition to occur.

Tests (results not given) have shown that neither Triton X100 nor sodium azide suppresses NK cell cytolytic activity under the incubation conditions imposed here. Furthermore, extensive dialysis of HA preparations to remove possible contaminants did not alter the inhibitory activity of HA on NK function. We have recently observed that influenza HA causes enhanced cyclic AMP production from lymphocytes, (R. C. Rees, S. A. Ali & B. Brown, unpublished

results), and this is likely to have a significant influence in suppressing NK cytolytic capacity (Roder & Klein, 1979).

Although the implication of the HA results given here are, at present unclear, it is possible to speculate a role for HA inhibition during virus infection. Thus, inhibition of NK cytolytic activity by viral proteins may influence the balance between viral virulence, and the host's natural defence mechanism and determine the agression of the disease course.

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REFERENCES

ABRAMSON J.S., GIEBANK G.S., MILLS E.L. & QUIE P.G. (1981) Polymorphonuclear leukocyte disfunction during influenza virus infection in chinchillas. *J. infect. Dis.* **143,** 836.

BRAND C.M. & SKEHEL J.J. (1972) Crystalline antigen from influenza virus envelope. *Nature (Lond.)*, **238,** 145.

BRUNDA M.T., HERBERMAN R.B. & HOLDEN H.T. (1980) Inhibition of murine natural killer cell activity by prostaglandins. *J. Immunol.* **124,** 2682.

CASALI P., PATRICK-SISSONS J.G., BUCHMEIER M.J. & OLDSTONE M.B.B. (1981) *In vitro* generation of human cytotoxic lymphocytes by virus, viral glycoproteins induce non-specific cell-mediated cytotoxicity without release of interferon. *J. exp. med.* **154,** 840.

CATE T.R. & COUCH R.B. (1981) Lack of effect of influenza virus infection on induction and expression of delayed hypersensitivity. *J. infect. Dis.* **144,** 280.

DJEU J.Y., STOCKS N., ZOON K., STANTON G.J., TIMONEN T. & HERBERMAN R.B. (1982) Positive self-regulation of cytotoxicity in human natural killer cells by production of interferon upon exposure to influenza and Herpes viruses. *J. exp. Med.* **156,** 1222.

EINHORN S., BLOMGREN H. & STRANDER H. (1980) Interferon and spontaneous cytotoxicity in man. V. Enhancement of spontaneous cytotoxicity in patients receiving human leukocyte interferon. *Int. J. Cancer*, **26,** 419.

ENNIS F.A., BEARE A.S., RILEY D., SCHILD G.C., MENGER A., QIYI-HAU SCHOVATZ G. & ROOK H.H. (1981) Interferon induction and increased natural killer cell activity in influenza infections in man. *Lancet*, **ii,** 891.

ENNIS F.A. & MEAGER A. (1981) Immune interferon produced to high levels by antigenic stimulation of human lymphocytes with influenza virus. *J. exp. Med.* **154,** 1279.

FAGUET G.B. (1981) The effect of killed influenza virus vaccine on the kinetics of normal human lymphocytes. *J. infect. Dis.* **143,** 252.

FLANAGAN M.T. & SKEHEL J.T. (1977) The conformation of influenza virus haemagglutinin. *FEBS Lett.* **80,** 57.

GREEN J.A., CHARETTE R.P., YEH T.J. & SMITH C.B. (1982) Presence of interferon in acute and convalescent-phase sera of humans with influenza or an influenza-like illness of undetermined etiology. *J. infect. Dis.* **145,** 837.

GREENBERG H.B., POLLARD R.B., LUTWICK L.I., GREGORY P.B., ROBINSON W.S. & MERIGAN T.C. (1976) Effect of human leukocyte interferon and hepatitis B virus infection in patients with chronic active hepatitis. *N. Engl. J. Med.* **295,** 517.

GOLDFARB R.H. & HERBERMAN R.B. (1981) Natural killer cell reactivity: regulatory interactions among phorbol ester, interferon, cholera toxin and retinoic acid. *J. Immunol'* **126,** 2129.

HARFAST B., ORVELL C., ALSHEIKHLY A., ANDERSON T., PERLMANN P. & NORRBY E. (1980) The role of viral glycoproteins in mumps virus dependent lymphocyte-mediated cytotoxicity *in vitro*. *Scand. J. Immunol.* **11,** 391.

HASSAN Z.M. & REES R.C. (1980) Natural antitumour cytotoxicity of mouse lymph node cells; characterisation and specificity studies. *Europ. J. Cancer.* **16,** 1263.

HERBERMAN R.B. & HOLDEN H.T. (1978) Natural cell-mediated immunity. *Adv. Cancer Res.* **27,** 305.

HERBERMAN R.B., NUNN M.E. & LAVRIN D.H. (1975) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumours. I. Distribution of reactivity and specificity. *Int. J. Cancer*, **16,** 216.

JARSTRAND C., WASSERMAN J. & DAHL G. (1977) Peripheral blood lymphocyte populations in influenza patients. *Scand. J. infect. Dis.* **9,** 1.

JOHNSTON M.D. (1981) The characteristics required for Sendi virus preparation to induce high levels of interferon in human lymphoblastoid cells. *J. Gen. Virol.* **56,** 175.

JULIUS M.H., SIMPSON E. & HERZENBERG L.A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Europ. J. Immunol.* **3,** 645.

LEUNG K.N. & ADA G.L. (1981) Induction of natural killer cells during murine influenza virus infection. *Immunobiol.* **160,** 352.

MANZELLA J.P. & ROBERTS JR N.J. (1979) Human macrophage and lymphocyte responses to mitogen stimulation after exposure to influenza virus, ascorbic acid and hyperthermia. *J. Immunol.* **123,** 1940.

OXFORD J.S., HOCKLEY D.J. & PATTERSON S. (1981) The interaction of influenza virus haemagglutinin with phospholipid vesicles—morphological and immunological studies. *J. Gen Virol.* **52,** 329.

OXFORD J.S. & SCHILD G.C. (1977) Quantification of influenza structural proteins using rocket immunoelectrophoresis. *J. Gen. Virol.* **38,** 187.

REED W.P., OLDS J.W. & KISCH A.L. (1972) Decrease skin-test reactivity associated with influenza *J. infect. Dis.* **125,** 398.

ROBERTS JR N.J., DIAMOND M.E., GORDON D. JR, SIMONS R.L. & STEIGBIGEL R.T. (1980) Mitogen responses and interferon production after exposure of human macrophages to infectious and inactivated influenza viruses. *J. Med. Virol.* **5,** 17.

RODER J.C. & KLEIN M. (1979) Target-effectors interaction in the natural killer cell system. IV. Modulation by cyclic nucleotides. *J. Immunol.* **123,** 2785.

SANTOLI D. & TRINCHIERI G. & KOPROWSKI H. (1978) Cell-mediated cytotoxicity against virus infected target cells in humans. II. Interferon induction and activation of natural killer cells. *J. Immunol.* **121,** 532.

SKEHEL J.J. & SCHILD G.C. (1971) The polypeptide compositions of influenza A viruses. *Virology*, **44,** 396.

TRINCHIERI G. & SANTOLI D. (1978) Antiviral activity induced by culturing lymphocytes with tumour derived virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J. exp. Med.* **147,** 1314.

WELSH R.M. (1981) Do natural killer cells play a role in virus infections? *Antiviral Research*, **1,** 5.

WELSH R.M. JR., DOE W.F. (1980) Cytotoxic cells induced during lymphocyte choriomeningitis virus infection of mice: natural killer cell activity in cultured spleen lymphocytes concomitant with T-cell-dependent immune interferon production. *Infect. Immun.* **30,** 473.

WRIGLEY N.G., LAVER W.G. & DOWNIE J.C. (1977) Binding antibodies to isolated haemagglutinin and neuraminidase molecules of influenza virus observed in the electron microscope. *J. Mol. Biol.* **109,** 405.