

ENHANCEMENT OF IgE-MEDIATED HISTAMINE RELEASE FROM HUMAN BASOPHILS BY VIRUSES: ROLE OF INTERFERON

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A variety of immunologic and immunopathologic processes are known to operate during the course of a viral infection (1). Despite a vast literature on mechanisms of humoral and cellular immunity in viral infections and considerable information on the replication of viruses in cells of the immune system, there are still few experimental data on the existence or importance of immediate hypersensitivity (allergic) reactions in viral diseases.

Clinical studies dating back almost 40 yr have suggested that upper respiratory tract infections can precipitate or potentiate attacks of bronchial asthma (2-17). Several reports also have shown that viral infections are associated with recurrence of wheezing in asthmatic patients (2, 3, 5, 7-16). Still other studies have shown that viral infections (e.g., influenza) can increase bronchial sensitivity to drugs such as methacholine (4, 6, 9). By viral isolation and serologic techniques, a variety of viruses including respiratory syncytial virus, parainfluenza virus, corona virus, and rhinoviruses have been identified in patients with asthma (3, 5, 8, 10-16).

The release of histamine from leukocytes (basophils) of allergic individuals after stimulation with ragweed antigen E or anti-IgE has proved to be a reliable *in vitro* model for immediate hypersensitivity reactions (18-20). The present investigation using this *in vitro* model was initiated to study the capacity of several common viruses to release histamine from leukocytes.

Materials and Methods

Reagents. Tris buffer was purchased from Sigma Chemical Co., St. Louis, Mo. Human serum albumin (HSA)¹ was obtained from Behring Diagnostics, American Hoechst Corp., Somerville, N. J. Ragweed antigen E and human leukocyte interferon prepared with Sendai virus were obtained from the Research Reagent Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. Human fibroblast interferon, prepared

¹ *Abbreviations used in this paper:* AbN/ml, antibody nitrogen/ml; Adeno-1, adenovirus type 1; Antigen E, ragweed antigen E; EDTA, ethylenediaminetetraacetic acid; EID₅₀, 50% egg infectious doses; FBS, fetal bovine serum; HSA, human serum albumin; HSV-1, herpes simplex virus, type 1; Influenza A, influenza virus, type A (PR-8 strain); MEM, minimum essential medium; moi, multiplicity of input (infection); Poly I:Poly C, poly-riboinosinic acid:poly-ribocytidilic acid; PBS, phosphate-buffered saline; PFU, plaque-forming units; PRK, primary rabbit kidney; Sendai, parainfluenza virus, type 1 (Sendai); SFV-7, simian foamy virus, type 7; TCID₅₀, 50% tissue culture infectious doses; VSV, vesicular stomatitis virus.

with poly I:poly C, was obtained from the Laboratory of Viral Diseases, NIAID. Mouse interferon induced by NDV in C-243-3 mouse cells was obtained from Bionetics, Kensington, Md. Anti-human IgE (Fc specific) was kindly provided by Dr. K. Ishizaka, Johns Hopkins University, Baltimore, Md.

Buffers and Media. Both Eagle's minimum essential medium (MEM) and RPMI 1640 medium were supplemented with penicillin (50 U/ml), streptomycin (50 $\mu\text{g}/\text{ml}$), fungizone (0.125 $\mu\text{g}/\text{ml}$), and 2% or 10% fetal bovine serum (FBS) heat inactivated at 56°C for 30 min. Primary rabbit kidney (PRK) cells were maintained in MEM with 2% FBS.

Tris A-buffered saline (Tris A, pH 7.4) contained 120 mM NaCl, 5 mM KCl, 24 mM Tris, and 0.03% HSA. Tris A-EDTA is Tris A with 4 mM EDTA and Tris ACM is Tris A with 1 mM Ca^{++} and 0.5 mM Mg^{++} .

Virus Preparation. Herpes simplex virus type 1 (HSV-1), adenovirus type 1 (Adeno-1), and simian foamy virus type 7 (SFV-7) were propagated and assayed in PRK cells. Influenza virus type A (PR-8) and parainfluenza virus type 1 (Sendai) were propagated and assayed by inoculation of the allantoic fluid of embryonating hen's eggs. Virus pools, uninfected PRK cell controls, and allantoic fluids were centrifuged at 2,000 rpm for 10 min to remove cell debris. The supernatant fluids were centrifuged at 30,000 rpm for 2 h and the pellets were resuspended in Dulbecco's phosphate-buffered saline (PBS) to 1/10 the original volume. Single virus pools containing the following titers were used throughout the study: HSV-1, 1.3×10^8 plaque-forming units (PFU)/ml; Adeno-1, 5.6×10^7 tissue culture infectious doses₅₀ (TCID₅₀)/ml; SFV-7, 1×10^8 TCID₅₀/ml; Influenza A, 3×10^9 egg infectious doses₅₀ (EID₅₀)/ml; and Sendai, 3×10^{10} EID₅₀/ml.

Leukocyte Preparation. Human peripheral blood was mixed with 2.25 ml of Dextran (Dextran 6% wt/vol in saline, Abbott Laboratories, North Chicago, Ill.) supplemented with 0.25 ml of 15% glucose and 1.0 ml of 0.1 M EDTA per 10 ml of blood. The mixture was allowed to settle at room temperature for about 90 min. The leukocyte-rich plasma was removed and the cells were sedimented at 1,200 rpm for 8 min at 4°C and suspended in Tris A-EDTA. Removal of red blood cells was accomplished by a 30-s hypotonic shock. After sequential washes in Tris A-EDTA and RPMI medium, the leukocytes were resuspended in RPMI medium containing 2% FBS (21).

Histamine Release Reaction. Leukocyte suspensions, containing about 5×10^6 cells in 0.5 ml, were incubated with either an equal volume of infectious virus or the equivalent amount of UV-inactivated virus or the appropriate control media. Except where stated, a virus:cell ratio of 1:1, expressed as the multiplicity of infection (moi), was used. After incubation at 37°C in a 5% CO₂ atmosphere for 2 h, the cells were washed, resuspended in RPMI medium, and incubated for an additional 22 h at 37°C. All experiments were performed at least twice and in each experiment reactions were set up in duplicate. Controls consisted of media, uninfected PRK cells, or allantoic fluid prepared and diluted in the same manner as the virus pools.

At the end of the 24-h incubation period, the leukocytes were sedimented by centrifugation at 1,100 rpm for 8 min and the supernatant fluids were saved for histamine assay or interferon titration. The cells were then washed once with Tris A and resuspended with an appropriate amount of Tris ACM. Duplicate cell samples in 0.1 ml vol were incubated at 37°C with an equal volume of serial dilutions of ragweed antigen E or anti-human IgE. After 45 min the tubes were transferred to an icebath and 0.3 ml of cold Tris ACM was added. The cells were centrifuged at 1,700 rpm for 15 min at 4°C and the supernatant fluids were assayed for histamine. Total cellular histamine content, expressed as "completes," was determined by lysis of leukocytes with 3% perchloric acid. The histamine released into the supernatant fluids of the "blanks" which contained cells and Tris ACM did not exceed 10% of the total cellular histamine.

Histamine Assay. Histamine was assayed by an automated fluorometric technique (22, 23). Percent histamine release was calculated by the formula: % release = $100 (e_i - \bar{b}/\bar{c} - \bar{b})$, where e_i = fluorometric readings for experimental supernates, \bar{b} = mean of blanks, and \bar{c} = mean of completes. The data were analyzed by either the standard t test for two means, or by paired t tests when two histamine release curves were compared.

Interferon Assay. Interferon activity in the supernatant fluids of leukocyte cultures was assayed by a modification of the microplate method developed by Green et al. (24). In brief, monolayers of WISH cells in microplates were incubated for 5 h at 37°C with serial twofold dilutions of interferon samples in a 0.1-ml vol. The cells were then challenged with 30–50 PFU of VSV. At the end of 1 h, the virus inoculum was removed and the cells were washed and refed with 0.1 ml of MEM supplemented with 2% FBS and 0.75% methylcellulose. After 18 h at 37°C, the cells

TABLE I
Histamine Content of Supernatant Fluids in Leukocyte Cultures after Incubation with HSV-1, Influenza A, and Adeno-1 for 24 h

Virus*	Treatment of virus	Number of leukocyte donors	Number of tests performed	Histamine release (%)‡	
				Control	Virus
HSV-1	None	5	7	5.2 ± 0.5	5.8 ± 0.4§
HSV-1	UV	5	6	6.1 ± 0.4	6.9 ± 0.4§
Influenza A	None	6	12	5.0 ± 0.7	5.2 ± 0.6§
Influenza A	UV or heat	4	7	5.5 ± 0.6	5.8 ± 0.3§
Adeno-1	None	2	3	5.1 ± 0.6	5.2 ± 0.4§

* moi of 0.1 or 1.0.

‡ Mean ± SE.

§ $P > 0.1$ (standard t test for percent histamine release between control media and virus).

were fixed with ethanol and stained with Giemsa's solution and viral plaques were counted. The interferon titer was expressed as the reciprocal of the highest dilution that reduced VSV plaque counts by 50%.

Results

Inability of Viruses to Release Histamine from Leukocyte Cultures. The initial experiments were designed to see whether the incubation of viruses with leukocytes caused the release of histamine. Peripheral blood leukocytes obtained from both allergic and nonallergic human donors were incubated with HSV-1, Influenza A, and Adeno-1 at an moi of 0.1 or 1.0. After 24 h of incubation at 37°C in a 5% CO₂ atmosphere, the supernatant fluids were assayed for their histamine content. As seen in Table I, the amount of histamine released into the supernatant fluids was not altered by the addition of either infectious or inactivated viruses to the leukocyte cultures. There also was no appreciable change in the cellular histamine content (data not shown).

Effect of Viruses on Histamine Release Induced by Anti-IgE

INFECTIOUS VIRUS. Leukocytes were incubated with infectious viruses or control media for 24 h and then challenged with anti-IgE for 45 min and the amount of histamine released into the supernatant fluids was determined. The results of a typical experiment with the cells from one individual are shown in Fig. 1. Leukocytes that had been treated with HSV-1 showed enhanced histamine release when exposed to anti-IgE ($P < 0.001$). At the optimum anti-IgE concentration, the cells cultured with control media released 20% histamine whereas cells cultured with HSV-1 released 36% (Fig. 1*a*). Similarly, leukocytes incubated with Influenza A and then challenged with anti-IgE showed enhanced histamine release ($P < 0.001$). At optimum concentrations of anti-IgE, cells treated with control allantoic fluids released 22% histamine, whereas cells treated with Influenza A released 51% histamine (Fig. 1*b*). Incubation of cells with Adeno-1 also resulted in a statistically significant increase in histamine release ($P < 0.001$), although the absolute enhancement was not as pronounced as with the other viruses (Fig. 1*c*).

UV-INACTIVATED VIRUS AND LATEX BEADS. Leukocytes were preincubated with infectious or UV-inactivated virus. After 24 h of incubation the cells were

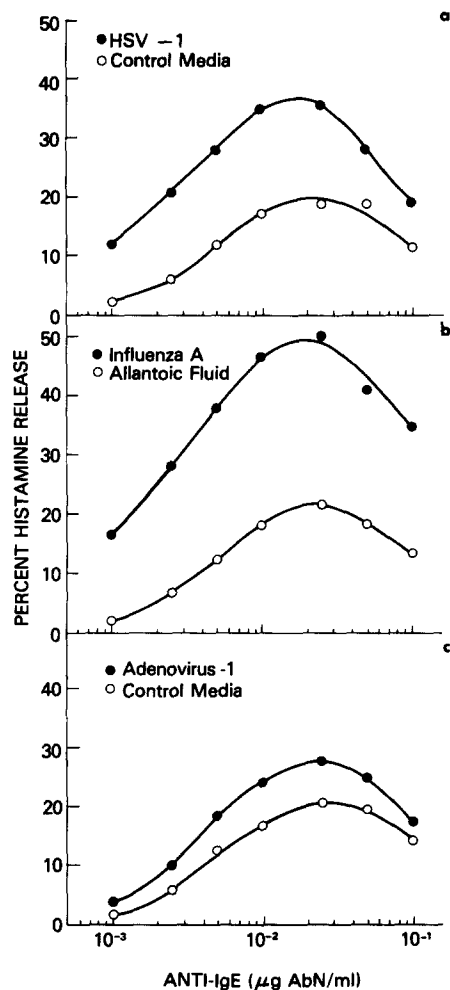


FIG. 1. Enhanced histamine release from leukocytes incubated with infectious viruses. Leukocytes were incubated for 24 h with HSV-1, Influenza A, or Adeno-1. The cells were then washed and the amount of histamine released after challenge with different concentrations of anti-IgE was determined.

challenged with different concentrations of anti-IgE. The data in Fig. 2 show that leukocytes incubated with either infectious or UV-inactivated virus released significantly more histamine than leukocytes exposed to control media ($P < 0.001$). Paired t tests revealed no significant difference between the curves generated by infectious vs. UV-inactivated virus ($P > 0.1$). In subsequent histamine release experiments, UV-inactivated viruses were used.

To see if the increase in histamine release was due to activation of phagocytosis, leukocytes were incubated with latex beads for 24 h. Analysis of the supernatant fluids after incubation of the leukocytes with anti-IgE failed to reveal any enhancement of histamine release (data not shown).

DOSE-RESPONSE STUDIES. The relationship between the concentration of virus (HSV-1) incubated with leukocytes and the resulting increase in histamine

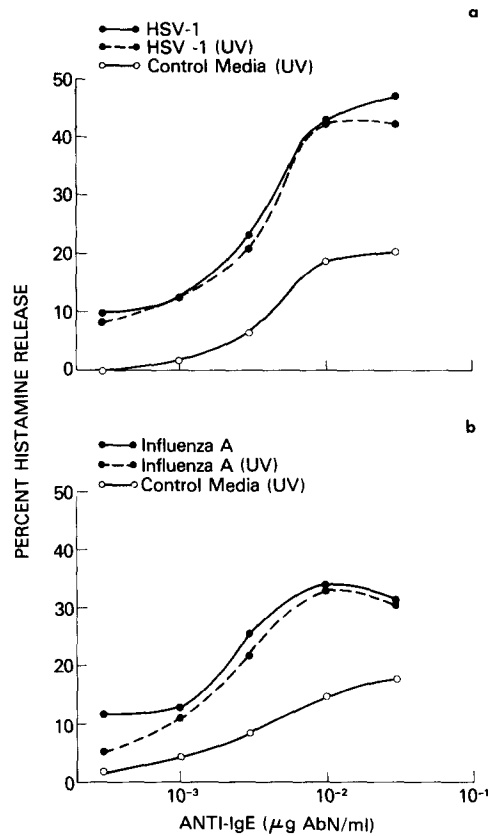


FIG. 2. Enhancement of histamine release by infectious or UV-inactivated viruses. Leukocytes were incubated for 24 h with virus or control media. The cells were then washed, challenged with different concentrations of anti-IgE, and the amount of histamine released was determined.

release is shown in Fig. 3. A significant enhancement of histamine release ($P < 0.001$) was observed when the ratio of virus to leukocytes (moi) was 0.01 or greater. At an moi of 0.001 there was no significant increase in histamine release ($P > 0.1$).

Relationship between Histamine Release and Interferon Activity. The capacity of viruses to enhance histamine release after challenge with ragweed antigen E or anti-IgE was tested with leukocytes from different donors. The data in Table II show that HSV-1 enhanced histamine release in six out of seven donors ($P < 0.001$). Leukocytes from the seventh donor released high amounts of histamine on challenge with anti-IgE and the presence of virus did not result in further enhancement.

The demonstration that both infectious and UV-inactivated virus enhanced histamine release indicated that viral replication was not required, and raised the possibility that enhancement might be immune specific. To study this possibility, the relationship between the level of anti-HSV neutralizing antibody and histamine release was investigated. Preliminary experiments (data not shown) revealed that exposure of leukocytes from anti-HSV-1 negative

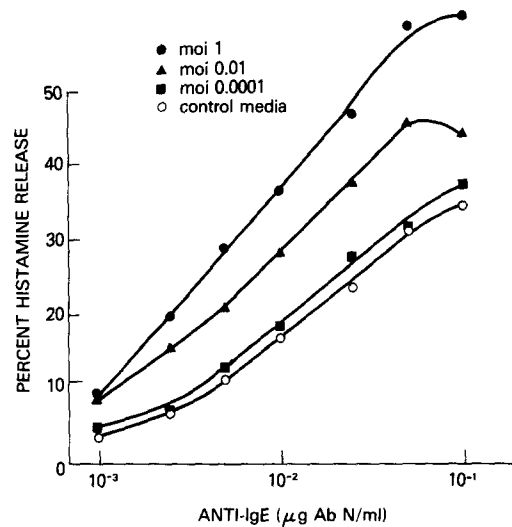


FIG. 3. Relationship between virus concentration and histamine release. Leukocytes were incubated with different concentrations of UV-inactivated HSV-1 and at the end of 24 h challenged with anti-IgE.

TABLE II
Relationship between Enhanced Histamine Release and Interferon Activity in Supernatant Fluids from Leukocytes Incubated with HSV-1

Donors	Treatment of Leukocytes		Histamine release (%)		<i>t</i> value‡	Interferon titer (U/0.1 ml)	
	Stimulant	Concentration*	Control	Virus		Control	Virus
1	Antigen E	5×10^{-3}	45.9	78.8	26.49§	<10	320
2	Antigen E	3×10^{-3}	38.6	61.4	12.24§	<10	20
3	Anti-IgE	1×10^{-1}	14.1	51.5	9.13§	<10	320
4	Anti-IgE	1×10^{-1}	9.2	43.4	4.44§	<10	160
5	Anti-IgE	1×10^{-1}	24.0	43.6	7.75§	<10	40
6	Anti-IgE	3×10^{-2}	19.5	36.3	9.84§	NT¶	NT
7	Anti-IgE	1×10^{-1}	77.4	71.3	-1.26**	<10	<10

* In each case the histamine release curve was determined with seven different concentrations of ragweed antigen E or anti-IgE. The data presented is the stimulant concentration that gave the highest histamine release. Antigen E concentration in $\mu\text{g/ml}$; anti-IgE concentration in $\mu\text{g AbN/ml}$.

‡ Histamine release curves (virus vs. control) were compared by the paired *t* test by using data from the seven different concentrations of each stimulant.

§ $P < 0.001$ ($df = 13$).

|| UV-inactivated virus was used at an moi equivalence of 1 in all cases except that of donor no. 6 in which infectious virus was used.

¶ Not tested.

** $P > 0.1$ ($df = 13$).

individuals to UV-inactivated HSV-1 resulted in enhanced histamine release. The observation that inactivated virus was capable of enhancing histamine release in nonimmune individuals tended to exclude an immune-specific mechanism and led us to look at the possible role of virus-induced interferon.

Supernatant fluids from leukocyte cultures that had been incubated with virus (HSV-1) or control (PRK) media (Table II) were assayed for interferon activity. The supernatant fluids of control cultures contained no interferon activity. In contrast, in five out of six cases tested, the supernatant fluids from cultures exposed to virus contained interferon activity ranging from 20 to 320 U/0.1 ml. The only exception was donor no. 7 whose leukocytes showed no increase in histamine release, and the interferon titer in the supernatant fluids was less than 10 U/0.1 ml.

Time Course of Enhanced Histamine Release: Relationship to Interferon Activity in Supernatant Fluids. To determine the minimum length of time between exposure of leukocytes to virus and development of enhanced histamine release, leukocytes were exposed to UV-inactivated HSV-1 for 2 h. The viral inoculum was removed and the cells were washed and incubated at 37°C. At 4, 8, 16, 24, and 48 h after initial exposure to virus, the leukocytes were challenged with anti-IgE. The data in Fig. 4 show significant enhancement of histamine release beginning at 8 h after exposure of cells to virus ($P < 0.001$). This effect increased progressively up to 48 h.

To see if the enhancement of histamine release was related to the presence of interferon, supernatant fluids collected at the different times were assayed for interferon activity. In all control cultures there was less than 10 U interferon/0.1 ml. In contrast, the interferon titer in the supernatant fluids from cultures treated with HSV-1 rose to 20 U by 8 h and was 320 U/0.1 ml by 24 h.

Interferon Induction and Enhanced Histamine Release by Different Viruses. The data in Table III show that histamine release is enhanced by HSV-1, Influenza A, and Sendai virus, but not by SFV-7. Between 20 and 160 U of interferon/0.1 ml were detected in the supernatant fluids from cultures treated with HSV-1, Influenza A, and Sendai virus, while less than 2 U of interferon/0.1 ml was found in supernatant fluids from cultures treated with SFV-7.

Enhancement of Histamine Release by Supernatant Fluids from Leukocyte Cultures. The previous experiments showed a correlation between enhancement of histamine release and the presence of interferon in the supernatant fluids. To test whether interferon-containing supernatant fluids could transfer the capacity to enhance histamine release, supernatant fluids from cultures 24 h after exposure to UV-inactivated HSV-1 or PRK control media were added to fresh leukocytes. The interferon titer in the supernatant fluids from the HSV-1-treated cultures was 320 U/0.1 ml, while the interferon titer in the supernatant fluids from the PRK control cultures was less than 10 U/0.1 ml. These supernatant fluids were incubated with fresh leukocytes for 24 h and the cells were then washed and challenged with ragweed antigen E and the amount of histamine released was determined. The data in Fig. 5a demonstrate that the supernatant fluids from the HSV-1-treated cultures markedly enhanced the release of histamine ($P < 0.001$).

To rule out the possibility that residual virus, rather than interferon, was responsible for the enhancement of histamine release, leukocyte cultures were stimulated with a nonviral interferon inducer. Cultures were exposed to poly I:poly C (20 μ g/ml) for 2 h, washed, reincubated for 22 h, and then exposed to ragweed antigen E. The data in Fig. 5b show that treatment with poly I:poly C

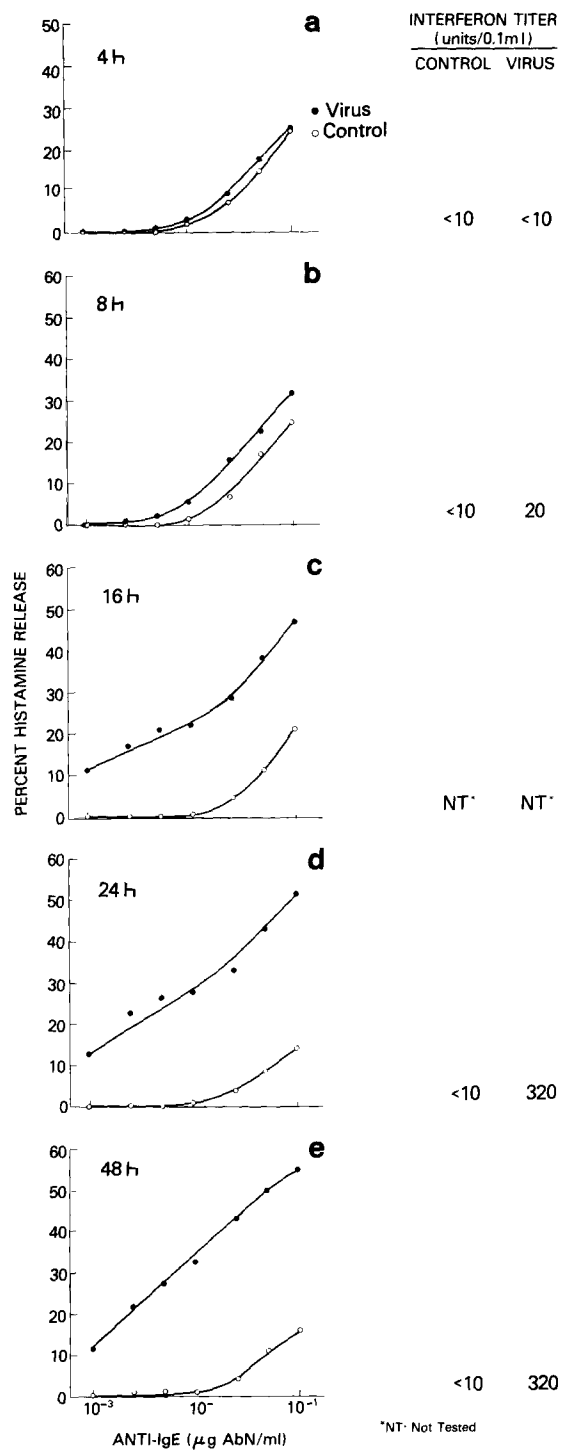


FIG. 4. Temporal relationship between the development of enhanced histamine release and interferon activity in the supernatant fluids. Leukocytes were incubated with UV-inactivated HSV-1 for 2 h. Cells were then washed and incubated in fresh medium. At 4, 8, 16, 24, and 48 h after initial exposure to virus, supernatant fluids were collected and assayed for interferon activity. The cells were then challenged with anti-IgE and histamine release was determined.

TABLE III
Interferon Induction and Enhanced Histamine Release by Different Viruses

Viruses*	Histamine release (%)‡		<i>t</i> value	Interferon titers (U/0.1 ml)	
	Control	Virus		Control	Virus
HSV-1	38.6	61.4	12.24§	<2	20
Influenza A	33.6	56.7	10.91§	<2	160
Sendai	33.6	54.5	9.76§	<2	160
SFV-7	24.8	26.7	2.83	<2	<2

* Leukocytes were exposed to UV-inactivated viruses at an moi of 0.1 or an equivalent dilution of control media (PRK media or allantoic fluid).

‡ Leukocytes were challenged with nine different concentrations of ragweed antigen E; for statistical analysis the entire histamine release curves were compared by utilizing the paired *t* test. The data presented are the percent histamine release at the optimal ragweed antigen E concentration (3×10^{-3} $\mu\text{g/ml}$).

§ $P < 0.001$ ($df = 17$).

|| $P > 0.1$ ($df = 17$).

resulted in an increase in histamine release ($P < 0.001$). The supernatant fluids from these cultures contained 40 U of interferon/0.1 ml, while the supernatant fluids from leukocyte cultures treated with control media contained no interferon.

Enhancement of Histamine Release by Interferon. The effect of standard reference interferon on the enhancement of histamine release is illustrated in Fig. 5c. Cells were incubated with either leukocyte or fibroblast interferon for 24 h and then challenged with ragweed antigen E. Both preparations of interferon enhanced the release of histamine ($P < 0.001$).

The effect of different concentrations of leukocyte interferon on histamine release is illustrated in Fig. 6. The data show that as the concentration of interferon was increased, the capacity of the cells to release histamine was correspondingly enhanced. Statistically significant enhancement was found with 80 U or more of interferon per milliliter ($P < 0.001$), but not with 8 U/ml ($P > 0.01$).

To see if the antiviral activity of interferon could be dissociated from the factor responsible for enhancement of histamine release, interferon samples were treated by: (a) dialysis (pH 2.0 for 24 h); (b) heat (65°C for 30 min); or (c) trypsin (0.002% trypsin for 1 h at 37°C). The treated samples were then tested for their ability to enhance histamine release and for their antiviral activity. The data in Table IV show that trypsin destroyed both the antiviral and the histamine-enhancing activity of the samples, while treatment with pH 2.0 or heat failed to destroy either activity.

Since interferon is known to be species specific, the effect of mouse interferon on the release of histamine from human leukocytes was examined (Fig. 7). Statistical analysis of the histamine release curves by paired *t* tests showed that mouse interferon produced significantly less enhancement of histamine release ($P > 0.01$) than human interferon ($P < 0.001$).

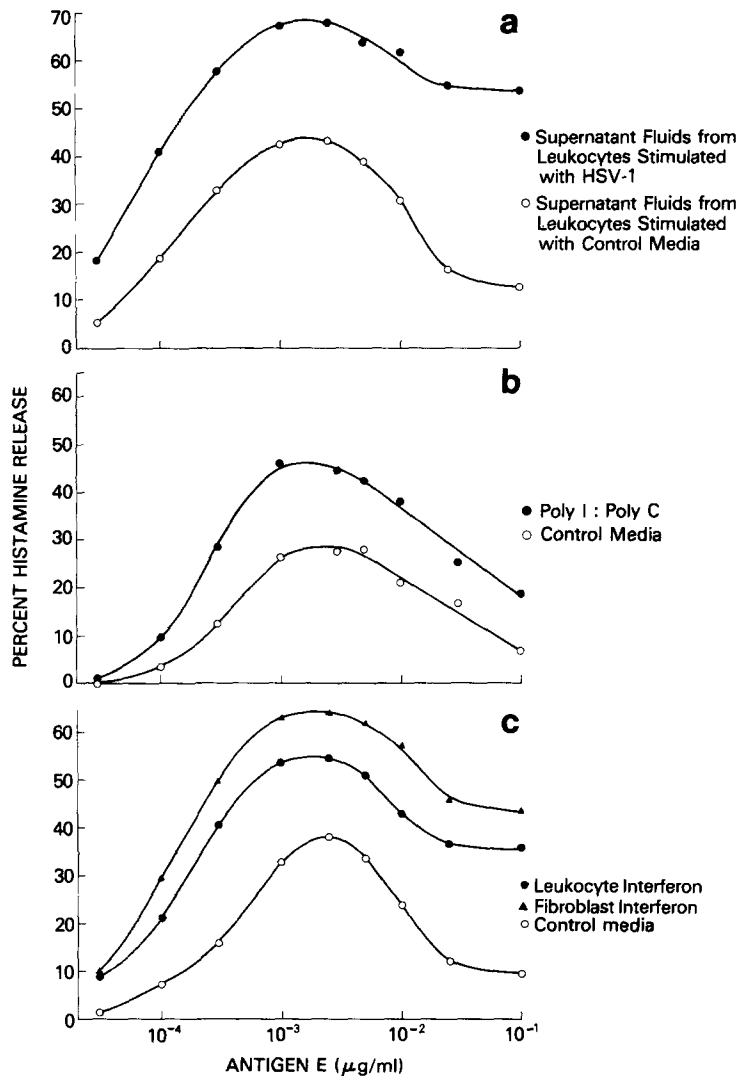


FIG. 5. Enhancement of histamine release by interferon. Leukocytes were incubated with: (a) supernatant fluids from leukocyte cultures exposed to HSV-1 (320 interferon U/0.1 ml) or PRK control media (<10 interferon U/0.1 ml); (b) poly I:poly C (20 $\mu\text{g}/\text{ml}$); (c) human leukocyte interferon (80 U/0.1 ml) or human fibroblast interferon (100 U/0.1 ml). After 24 h of incubation, leukocytes were challenged with ragweed antigen E and histamine release was determined.

Discussion

The present study showed that incubation of HSV-1, Influenza A, or Adeno-1 viruses with human peripheral blood leukocytes failed to release histamine into the supernatant fluids. If, however, these leukocyte cultures were first incubated with virus and then challenged with ragweed antigen E or anti-IgE, a marked enhancement of histamine release occurred. Both infectious and nonin-

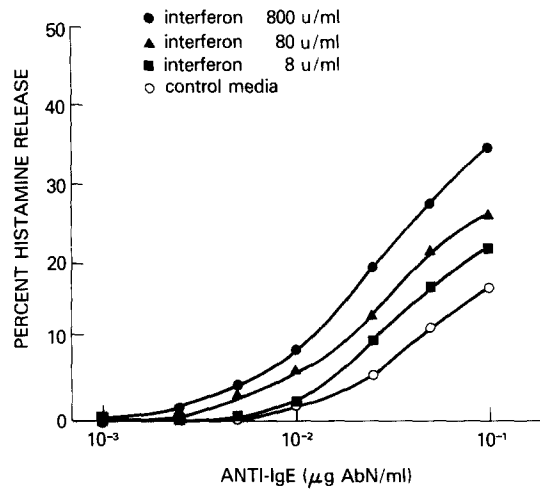


FIG. 6. Effect of interferon concentration on histamine release. Leukocytes were incubated with 8, 80, or 800 U/ml of standard leukocyte interferon. After 24 h of incubation, cells were washed and challenged with anti-IgE, and histamine release was determined.

TABLE IV
Relationship between Antiviral and Histamine-Enhancing Activity of Preparations Containing Interferon

Material	Treatment	Interferon titer (U/0.1 ml)		Histamine release (%) [*]		<i>t</i> value ‡
		Control Media	Interferon	Control Media	Interferon	
Leukocyte interferon	None	<5	80	12.6	26.2	8.82§
	Dialysis (pH 7.2)		80		26.7	9.03§
	Dialysis (pH 2.0)		80		31.5	9.37§
	Heat (65°C, 30 min)		40		19.9	7.17§
Fibroblast interferon	None	<5	100	14.4	35.0	8.87§
	Trypsin	<5	<5	21.5	22.3	2.76

* Leukocytes were incubated with either control media (RPMI medium with 2% FBS) or interferon preparations that were treated in different ways. After 24 h, the cells were washed and challenged with seven different concentrations of anti-IgE. The histamine release with the optimal anti-IgE concentration (3×10^{-2} µgAbN/ml) is presented.

‡ Histamine release curves (interferon vs. control) were statistically analyzed by the paired *t* test by using data from all seven concentrations of anti-IgE.

§ $P < 0.001$ ($df = 13$).

|| $P > 0.01$ ($df = 13$).

fectious viruses were effective. Examination of the culture fluids revealed that the enhancement of histamine release was associated with a soluble factor that had the properties of interferon. Several lines of evidence indicate that interferon, in fact, was responsible for the enhancement of histamine release. First, all stimulants that enhanced histamine release (i.e., viruses and poly I:poly C) also induced interferon. In contrast, stimulants that did not enhance histamine

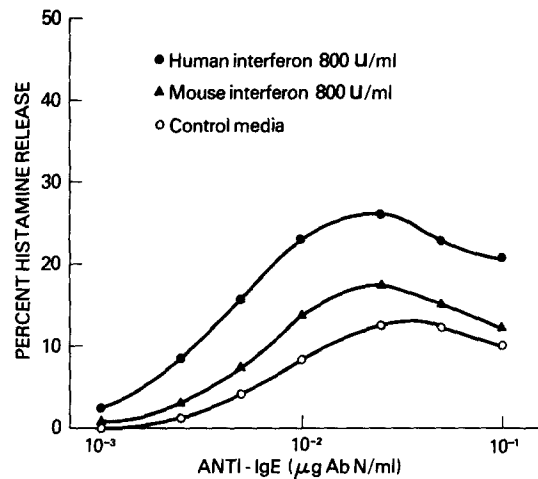


FIG. 7. Species specificity of interferon. Leukocytes were incubated with 800 U/ml of human or mouse interferon. After 24 h of incubation, cells were washed and challenged with anti-IgE and histamine release was determined.

release (Simian foamy virus, PRK cell control, allantoic fluids, RPMI medium) did not induce interferon (25). Second, there was a direct relationship between both the time of appearance and the amount of interferon in the culture fluids and the degree of histamine enhancement. Third, all preparations of human interferon, regardless of the tissue of origin (i.e. leukocytes or fibroblasts), were effective. Controls not containing interferon and heterologous interferon (i.e. mouse) were ineffective. Fourth, the soluble factor responsible for the enhancement of histamine release could not be dissociated from the antiviral activity of interferon by standard physicochemical means. Whether the enhancement of histamine release by interferon requires new RNA and protein synthesis (e.g., the reputed "antiviral protein" of interferon) is presently under investigation.

Since the basophils in human peripheral blood represent less than 1% of the total leukocyte population, the majority of the virus-induced interferon in leukocyte cultures almost certainly comes from the other cell types (26). In an individual not immune to a particular virus, interferon could be nonspecifically induced by the interaction of that virus with a variety of cell types (e.g., lymphocytes, macrophages, fibroblasts). In an individual immune to a particular virus, interferon could be specifically induced by the interaction of that virus with immune lymphocytes (26). The resulting interferons, regardless of origin, could then act on the basophils to enhance histamine release.

How interferon enhances histamine release is not clear. It is known that in preparations of human leukocytes, histamine is present only in basophils. These basophils have specific receptors for IgE and such receptors are not present on other leukocytes (19, 27). The release of histamine is triggered by the bridging of two IgE molecules on the basophil surface (28). The reaction can be initiated by either ragweed antigen E or anti-IgE and the two mechanisms appear identical (29). The release of histamine from the cell is a secretory process and does not result in cell death. This secretory process is modulated by the level of intracellular cyclic AMP, and agents such as isoproterenol, prostaglandin E, cholera

toxin, or theophylline, which raise intracellular levels of cyclic AMP, will inhibit the release of histamine. Agents which either decrease cyclic AMP levels (e.g., imidazole, α -adrenergic compounds, $\text{PGF}_{2\alpha}$), or increase cyclic GMP levels (e.g., exogenous cyclic GMP, carbamylcholine) have been reported to enhance histamine release from lung mast cells (30, 31), but thus far not from peripheral basophils (32). Microtubules and microfilaments also play a role in this secretory process; colchicine, which inhibits microtubular aggregation, inhibits the release reaction. Heavy water, which in many systems promotes microtubular aggregation, enhances histamine release and also reverses the action of colchicine (33, 34). Cytochalasin B, an agent known to disrupt microfilaments, also will enhance the IgE-mediated histamine release reaction (34-36). Whether or not interferon enhances histamine release by altering cyclic nucleotide levels, promoting microtubule aggregation, disrupting microfilaments, or some other mechanism remains to be determined.

The demonstration here that interferon enhances histamine release represents a hitherto unrecognized biological role for interferon. This points to the possibility that the induction of interferon may be one of the cofactors responsible for precipitating or potentiating attacks of bronchial asthma during viral infections (2-17). Recent evidence from several laboratories indicates that interferon can suppress the growth of certain tumor cells and alter the functional response of cells of the immune system (for review, see reference 26). If it turns out that interferon acts on a common metabolic pathway (e.g., cyclic nucleotides or microtubule aggregation), interferon may modulate a variety of other biological processes.

Summary

Human leukocytes maintained in culture are induced to release histamine when exposed to ragweed antigen E or anti-IgE. Leukocyte cultures incubated with virus (i.e. HSV-1, Influenza A, and Adeno-1) but not exposed to ragweed antigen E or anti-IgE fail to release histamine. If, however, leukocyte cultures are first exposed to virus and then to ragweed antigen E or anti-IgE, significant enhancement of histamine release occurs. Both infectious and inactivated virus enhance histamine release and the degree of enhancement is related to the concentration of virus and the length of the incubation. Tissue culture fluid harvested 8 h after exposure of leukocytes to virus contains a soluble factor which is capable of enhancing histamine release when added to fresh leukocyte cultures. This factor has all the properties of interferon including species specificity and cannot be dissociated from the antiviral activity of interferon. Moreover, both known inducers of interferon (poly I:poly C) and standard preparations of interferon are capable of enhancing histamine release. The enhancement of histamine release by interferon represents a new biological role for interferon.

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