

Influenza A virus enhances allergic histamine release

Effect of neuraminidase

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Histamine release was examined in leukocyte suspensions from patients allergic to house dust mite, grass pollen, birch pollen or cat dander. Influenza A virus was found to enhance the antigen-induced mediator release, but did not cause release of histamine from the cells *per se*. Also histamine release induced by anti-IgE in cell suspensions from normal individuals was enhanced by virus. The potentiating effect of influenza A virus might be due to neuraminidase on the surface of virus, since a similar effect was caused by a purified neuraminidase obtained from *Vibrio cholerae*, and the effect of virus as well as the neuraminidase was completely abolished by a potent neuraminidase inhibitor. The synergistic enhancement in IgE-mediated histamine release by virus could be of significance for the conversion from latent to manifest asthma.

Key words: allergic asthma; antigen; basophils; histamine release; influenza A virus; neuraminidase; neuraminidase inhibitor; potentiation; sialic acid.

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CLINICAL ASPECTS

The results of our experiments showed that basophil histamine release caused by specific antigen in leukocyte suspensions from house dust mite-, cat dander- and pollen allergic patients was enhanced in the presence of influenza A virus. Since viral infections in the upper respiratory tract have frequently been shown to precipitate or exacerbate attacks of bronchial asthma, the enhancement of mediator release by virus might play a role in the conversion from latent to manifest asthma.

Upper respiratory tract infections often provoke or exacerbate attacks of bronchial asthma (4, 9, 10, 15) and it is therefore possible that virus and bacteria play an important role in airway hyper-reactivity. In the present study we have examined the influence of influenza A virus on IgE-mediated histamine release from human basophils.

mite, grass pollen, birch pollen or cat dander. All patients had a positive skin prick test and positive basophil histamine release to these allergens, and their asthma was controlled by local β_2 -agonists and/or local steroids. Furthermore, five normal individuals (age 28-52 years) were included.

MATERIAL AND METHODS

Patients

The study included 17 patients (age 24 to 55 years) with bronchial asthma, possibly in connection with hay fever, and with a well-documented history of allergy to either house dust

Material

The following allergens were used: house dust mite (*Dermatophagoides pteronyssinus*), grass pollen (*Phleum pratense*), cat dander and birch pollen (*Betula verrucosa*), all Soluprick-SQ 3HEP from ALK, Denmark.

Rabbit-antihuman IgE (Σ -chain), 400,000 IU/ml was from Behringwerke AG, FRG.

Human influenza A virus A/Caen/1/84 (H3N2) was obtained as previously described (3). Non-virus-infected allantoic fluid centrifuged 30 min at 1500 *g* and dialysed against isotonic phosphate buffer (pH 7.4) was used as the viral medium in control experiments. *Vibrio cholerae* neuraminidase was from Behringwerke AG, FRG. The activity of the virus as well as the purified neuraminidase preparation was estimated according to (8, 11). Under our experimental conditions the activity of 1 ng viral protein/ml corresponded to 5 μ U/ml of the purified neuraminidase. The neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid was purchased from Sigma, St. Louis, USA.

Preparation of cell suspension

From each person blood was drawn by venipuncture, anti-coagulated with EDTA in a final concentration of 0.01 M and mixed with double volume of 0.9% NaCl. Leukocytes with approx. 2% basophilocytes were obtained by Ficoll-Hypaque gradient centrifugation (13), washed twice, and suspended in a glucose-free Tris-AMC buffer containing 25 mM Tris at pH 7.6, 0.12 M NaCl, 5 mM KCl, 0.6 mM CaCl₂, 1.1 mM MgCl₂, and 0.3 mg of human serum albumin/ml (13).

Histamine release

Histamine release experiments were performed by incubation of 40 μ l leucocyte suspension for 40 min at 37°C with 5 μ l of diluted allergen or anti-IgE and 5 μ l glucose-free Tris-AMC buffer. The release of histamine was assayed spectrophotometrically by determination of the residual histamine in the washed cell sediment (13). Only release of more than 10% of the total histamine content in the sample was considered significant, since the spontaneous release of histamine amounted to up to 10%. A potentiating effect of influenza A virus or *V. cholerae* neuraminidase on the histamine release was examined by replacing the 5 μ l Tris-AMC buffer by virus or neuraminidase, both used in 10-fold dilutions. Some experiments also included 0.2 mM neuraminidase inhibitor 2-deoxy-2,3-dehydro-

N-acetylneuraminic acid, pH 7.4.

A direct histamine releasing capability of influenza A virus or the *V. cholerae* neuraminidase was examined by incubating 40 μ l cell suspension with 5 μ l Tris-AMC buffer and 5 μ l virus or *V. cholerae* neuraminidase used in 10-fold dilutions. To examine whether the potentiating effect of virus was related to a specific antigen-antibody reaction, three control experiments were performed with cells from allergic persons by removing the IgE molecules from the cell surface (14) prior to the incubation of the cells with allergen and virus.

RESULTS

The influence of influenza A virus on anti-IgE-induced histamine release was examined by stimulating cells from five normal individuals with anti-IgE. Final dilutions of 10⁻⁵ to 10⁻⁴ were used to obtain a low release of histamine (< 25%). When virus was included in the samples, the mediator release was enhanced by 55 to 150% in experiments with cells from four of the five persons. When the cells were stimulated with anti-IgE alone, the histamine release was found to be 11.4 $\pm$ 2.89% (mean $\pm$ SEM). The combination of anti-IgE and influenza A virus resulted in a maximal histamine release of 25.6 $\pm$ 2.98% (mean $\pm$ SEM) in experiments with cells from the four responding subjects. The peak was obtained in final concentrations of the virus corresponding to the range of 10⁻³ to 1.0 ng viral protein/ml (Fig. 1). In cell suspensions from the normal individuals, influenza A virus did not induce histamine release *per se*. When virus was replaced by the viral medium, allantoic fluid, no enhancement of anti-IgE-induced histamine release was obtained in 10 examined persons.

To examine whether influenza A virus could enhance antigen-induced histamine release in allergic persons, similar experiments were performed with cells from patients allergic to house dust mite, grass pollen, birch pollen or cat dander (Table 1). When the cells were exposed to virus and the specific allergen to which the patient was sensitized, the mediator release was enhanced by 35 to 130%. Representative examples of experiments with cells from persons

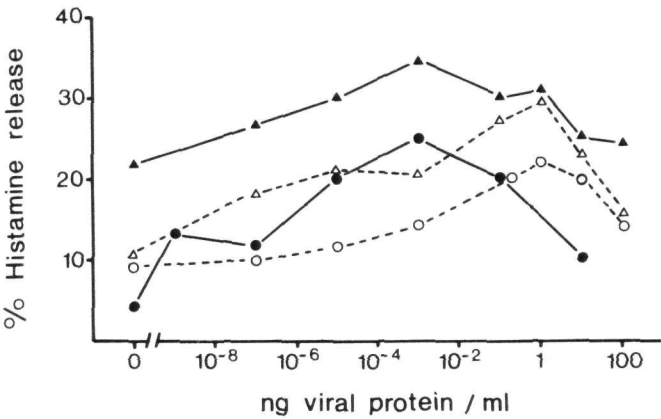


Fig. 1. Potentiation by influenza A virus of histamine release induced by anti-IgE in leukocyte suspensions from 4 normal individuals. Duplicate determinations. Note that the virus *per se* caused no release of histamine.

allergic to house dust mite (Fig. 2A) and persons allergic to grass pollen (Fig. 2B) are shown. A similar potentiating effect of virus was seen in experiments with cells from persons allergic to birch pollen or cat dander (Table 1). In all these experiments virus enhanced the histamine release significantly ($P < 0.01$ by Student's t-test) in 13 of the 17 subjects when comparing samples with and without virus. The peak was obtained in final concentrations of virus corresponding to the range of 10^{-5} to 1.0 ng viral protein/ml (median 10^{-3} ng viral protein/ml). When the histamine release induced by specific allergen alone exceeded 25 %, inclusion of virus seldom resulted in an enhancement of the mediator release. For this reason, an allergen-induced histamine release of < 25 % was desired. In cell suspensions from the allergic persons, influenza A virus did not induce histamine release *per se*.

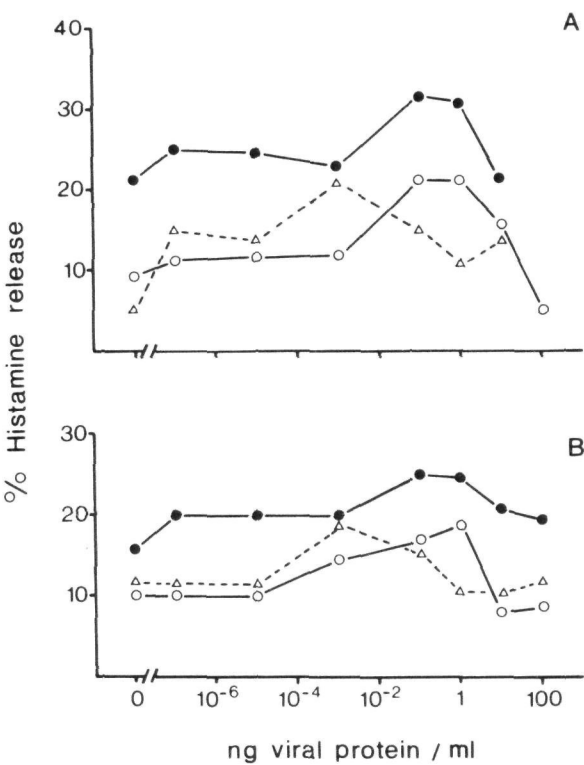


Fig. 2. Potentiation by influenza A virus of histamine release induced by specific allergens in leukocyte suspensions from 3 patients sensitized to house dust mite (A) and 3 patients sensitized to grass pollen (B). Duplicate determinations. Note that the virus *per se* caused no release of histamine.

No release and no potentiation were obtained when cells from normal individuals were challenged with the allergens, or when cells from cat dander-allergic patients were exposed to unspecific allergen, e.g. house dust mite. This was also the case when the IgE molecules were removed from the surface of the cells derived from grass or birch pollen-allergic patients prior to stimulation with the specific allergens.

To elucidate whether the enhancement of

Table 1

Potentiation by influenza A virus of histamine release induced by specific allergens in sensitized persons. The histamine release is given independent of the antigen and virus dilution in the experiments. Mean $\pm$ SEM is given

Patients allergic to	No. of subjects tested	No. of responders	Histamine release caused by allergen alone	Maximal histamine release caused by allergen + influenza A virus
House dust mite	5	4	14.4 ($\pm$ 2.7)	25.4 ($\pm$ 1.9)
Grass pollen	7	5	10.8 ($\pm$ 1.6)	18.6 ($\pm$ 1.5)
Birch pollen	2	2	9.5	19.0
Cat dander	3	2	12.0	20.3

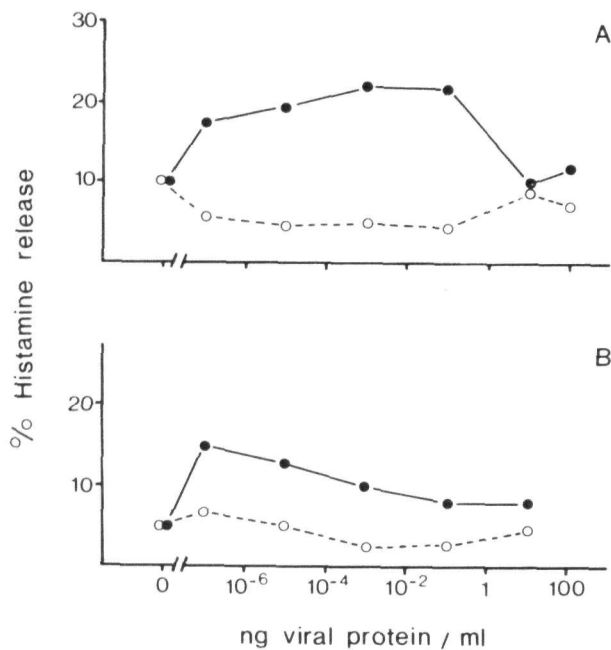


Fig. 3. Potentiation of histamine release by influenza A virus (●—●) and the complete abolishment by the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (○---○). Cells from a patient sensitized to house dust mite (A) and to grass pollen (B). Duplicate determinations.

mediator release by influenza A virus was due to viral neuraminidase, experiments were performed with virus in the presence or absence of the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid. The potentiation of histamine release was completely abolished by the neuraminidase inhibitor. Fig. 3. shows examples of a person sensitized to house dust mite (Fig. 3A) and a person sensitized to grass pollen (Fig. 3B).

To examine whether purified neuraminidase could mimic the effect of virus, influenza A virus was replaced by a preparation of *V. cholerae* neuraminidase. A potentiating effect similar to that of virus was obtained by the purified neuraminidase, and this was seen in experiments with cells from two patients allergic to house dust mite and two patients allergic to grass pollen (Fig. 4A), and furthermore with cells from two birch pollen-allergic patients (not shown). In all these experiments, the peak was obtained in final concentrations of neuraminidase corresponding to the range of 5×10^{-8} to 5×10^{-1} mU/ml. The neuraminidase did not cause histamine release *per se*. When the neu-

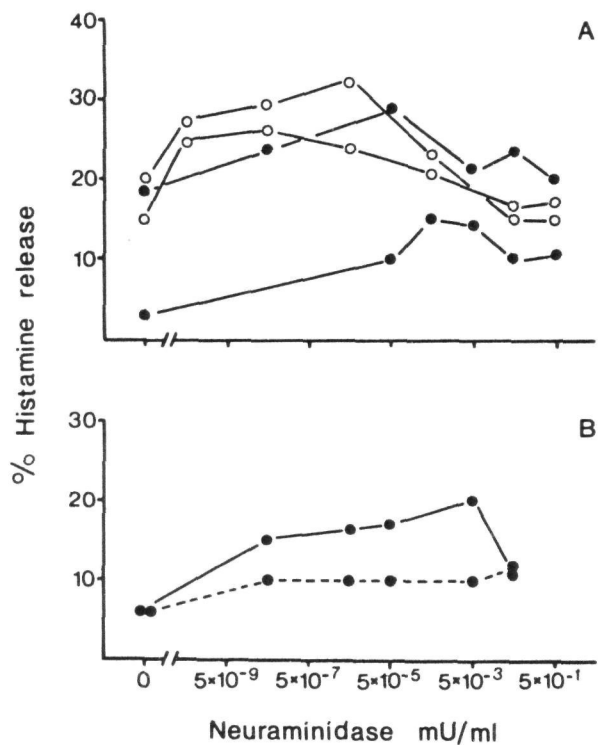


Fig. 4. Potentiation of histamine release by a purified neuraminidase (—●—) and the complete abolishment by the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid (---○---). Cells from patients sensitized to grass pollen (●) and to house dust mite (○). Duplicate determinations.

raminidase inhibitor was included in the samples, the potentiating effect of the purified neuraminidase on antigen-induced histamine release was completely abolished. Fig. 4B shows an experiment with cells from a patient allergic to grass pollen. A possible interference of the neuraminidase inhibitor with the histamine release was examined by stimulating the cells from the sensitized individuals with the specific allergens in the absence of virus and purified neuraminidase. A similar release was obtained in the presence or absence of the inhibitor, and examples are shown from two patients allergic to house dust mite (Fig. 5A) and from two patients allergic to grass pollen (Fig. 5B).

DISCUSSION

The influence of virus on histamine release from human basophil leukocytes has previously been investigated by Ida et al. (5), who found that Herpes simplex virus type 1, adenovirus

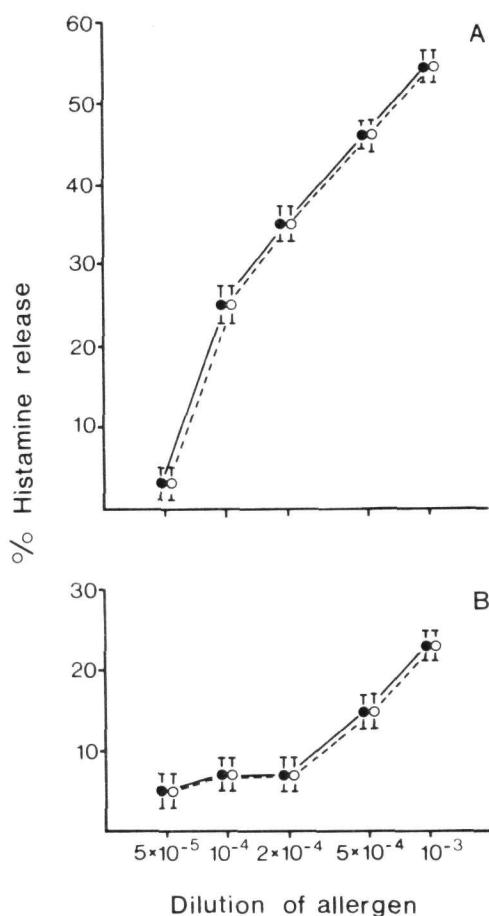


Fig. 5. No interaction of the neuraminidase inhibitor with histamine release. Cells from 2 house dust mite-allergic patients (A) and 2 grass pollen-allergic patients (B) were stimulated with specific allergen in the presence (○---○) and absence (●—●) of the inhibitor. Final dilutions of the allergen preparations are given. Six determinations, mean $\pm$ SEM.

type 1 and influenza A virus did not induce mediator release in leukocyte suspensions from normal individuals. However, preincubation of the cells with virus followed by stimulation with anti-IgE enhanced the IgE-mediated histamine release. It was necessary to preincubate the cells with virus for at least 8 h to obtain this effect, and it seemed that interferon production was responsible for the enhancement of the histamine release (5). Busse et al. (1) found a similar indirect effect of influenza A virus on the anti-IgE-induced basophil histamine release. However, virus, which causes no production of interferon, was also able to enhance the anti-IgE-induced histamine release, thus suggesting that other, unknown factors are responsible for the potentiating effect of virus (2).

In short-term experiments we examined a direct effect of influenza A virus on IgE-mediated histamine release from human basophil leukocytes. Cells from normal individuals were stimulated with anti-IgE for 40 min at 37°C to release histamine. When influenza A virus was included in the samples, the histamine release was enhanced. Also when histamine release was induced by specific allergens in cell suspensions from patients allergic to house dust mite, grass pollen, birch pollen or cat dander, inclusion of influenza A virus caused an enhanced mediator release. The potentiating effect of virus was due to synergism, since virus did not release histamine *per se*, either in cell suspensions from normal individuals or in cell suspensions from the sensitized patients.

It seems doubtful whether a production of interferon could be responsible for the potentiating effect of virus in these short-term experiments, since a virus-induced production takes more time (5). However, in these experiments the enhancement of histamine release by influenza A virus could be due to viral neuraminidase on the surface of the virus, since a similar potentiating effect was obtained when virus was replaced by a purified neuraminidase preparation obtained from *V. cholerae*. Further, the effect of both virus and neuraminidase was completely abolished when the neuraminidase inhibitor 2-deoxy-2,3-dehydro-N-acetylneuraminic acid was included in the samples. This potent neuraminidase inhibitor is known to inhibit different viral and bacterial neuraminidases (12), and it was found to be a suitable tool for investigating the potentiating effect of virus, as it did not interfere with the histamine release process induced by specific allergen. It has also previously been shown that the inhibitor did not affect histamine release induced by other stimulators, such as anti-IgE and the calcium ionophore A23187, or by endotoxin-induced complement activation (3).

All these short-term experiments indicate that the neuraminidase on the surface of influenza A virus is responsible for the potentiating effect of virus on the IgE-mediated histamine release. This theory is supported by similar findings in experiments with basophil leukocytes, where

purified neuraminidase was found to enhance histamine release induced by anti-IgE, concanavalin A and the calcium ionophore A23187 (6, 7).

On the basis of the finding that influenza A virus enhances allergic histamine release *in vitro*, it is tempting to speculate that a potentiation of IgE-mediated mediator release by virus could play a role in the conversion from latent to manifest asthma.

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