Influenza-derived peptides cross-react with allergens and provide asthma protection

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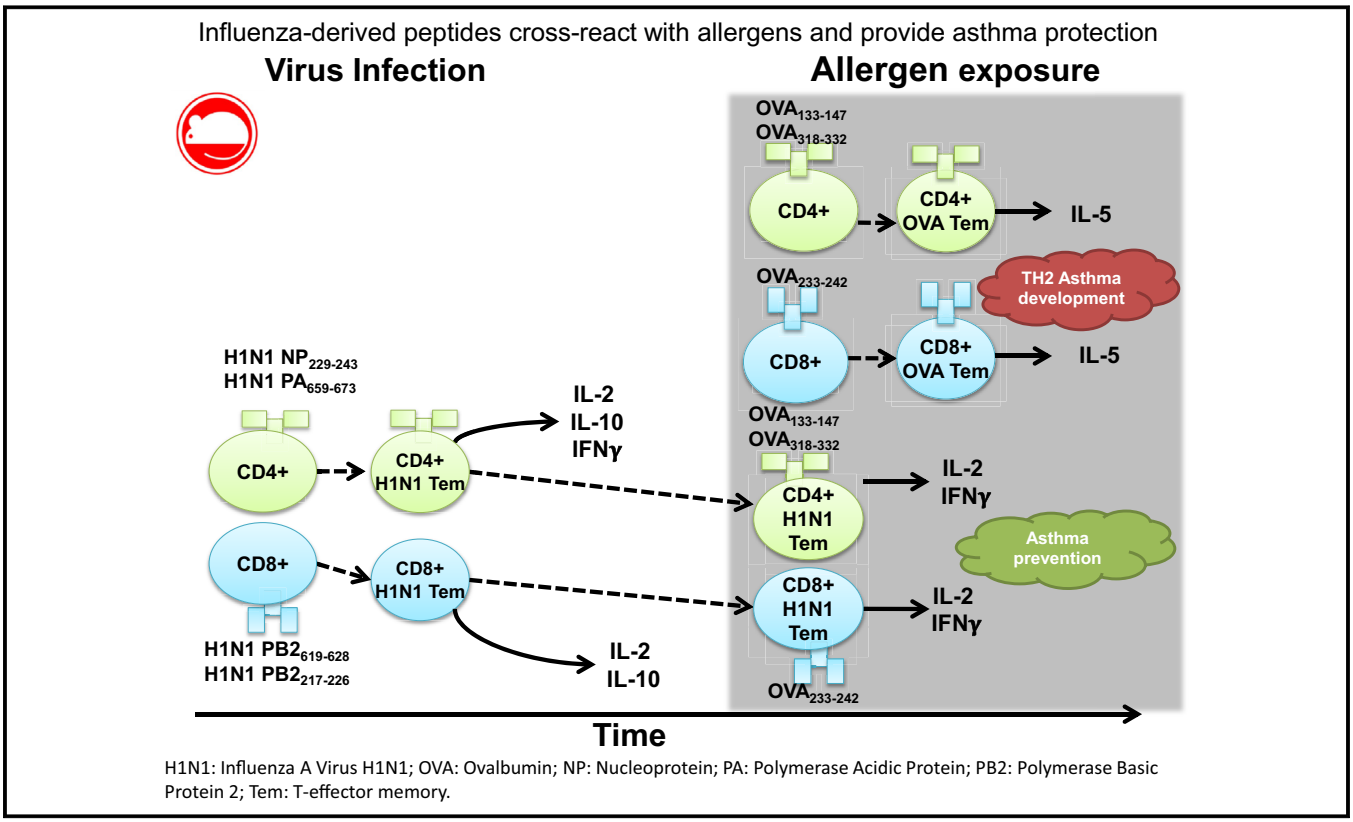
GRAPHICAL ABSTRACT



H1N1: Influenza A Virus H1N1; OVA: Ovalbumin; NP: Nucleoprotein; PA: Polymerase Acidic Protein; PB2: Polymerase Basic Protein 2; Tem: T-effector memory.

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Background: The hygiene hypothesis is the leading concept to explain the current asthma epidemic, which is built on the observation that a lack of bacterial contact early in life induces allergic T_H2 immune responses.

Objective: Because little is known about the contribution of respiratory tract viruses in this context, we evaluated the effect of prior influenza infection on the development of allergic asthma.

Methods: Mice were infected with influenza and, once recovered, subjected to an ovalbumin- or house dust mite–induced experimental asthma protocol. Influenza-polarized effector memory T (Tem) cells were transferred adoptively to allergen-sensitized animals before allergen challenge. A comprehensive *in silico* analysis assessed homologies between virus- and allergen-derived proteins. Influenza-polarized Tem cells were stimulated *ex vivo* with candidate peptides. Mice were immunized with a pool of virus-derived T-cell epitopes.

Results: In 2 murine models we found a long-lasting preventive effect against experimental asthma features. Protection could be attributed about equally to $CD4^+$ and $CD8^+$ Tem cells from

influenza-infected mice. An *in silico* bioinformatic analysis identified 4 influenza- and 3 allergen-derived MHC class I and MHC class II candidate T-cell epitopes with potential antigen-specific cross-reactivity between influenza and allergens. Lymphocytes from influenza-infected mice produced IFN-γ and IL-2 but not IL-5 on stimulation with the aforementioned peptides. Immunization with a mixture of the influenza peptides conferred asthma protection, and peptide-immunized mice transferred protection through $CD4^+$ and $CD8^+$ Tem cells. Conclusion: For the first time, our results illustrate heterologous immunity of virus-infected animals toward allergens. This finding extends the original hygiene hypothesis. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Influenza virus, asthma, hygiene hypothesis, effector memory cells, heterologous immunity*

Abbreviations used
BAL: Bronchoalveolar lavage
BLAST: Basic Local Alignment Search Tool
CD62L: CD62 ligand
dpi: Days postinfection
HBV: Hepatitis B virus
HDM: House dust mite
H1N1: Hemagglutinin 1 neuraminidase 1
IEDB: Immune Epitope Database
IFV: Influenza virus
NP: Nucleoprotein
OVA: Ovalbumin
PA: Polymerase acidic protein
PAS: Periodic acid–Schiff
PB: Polymerase basic protein
PBST: PBS/0.01% Tween-80
TCR: T-cell receptor
Tem: Effector memory T

More than one billion persons worldwide have an allergic disorder, and the prevalence is expected to reach up to 4 billion in the 2050s.[1] Asthma, a disease characterized by airway hyperreactivity and chronic inflammation, constitutes a major part of the epidemic, with approximately 300 million afflicted subjects of all ages. The ongoing increase in its prevalence has been associated with profound changes in lifestyle and environmental conditions, including sanitation, thus leading to the hygiene hypothesis.[2] Indeed, several investigators[3,4] observed an inverse relationship between the incidence of infectious diseases and asthma, particularly during the second half of the last century. Although most studies have focused on the protective role of bacteria[5] and their products, exposure to nonrespiratory[6] and respiratory[7] tract viruses has also been inversely associated with atopy and asthma development later in life. To date, functional studies[8] extending the concept of the hygiene hypothesis to respiratory tract virus contribution are still lacking. Moreover, severe virus-mediated respiratory wheezing illnesses during early life have been linked to the induction of asthma development later in childhood.[9]

A potentially protective role of respiratory tract viruses from allergic patients might have important clinical implications; furthermore, influenza virus (IFV) is a leading airway pathogen contributing significantly to pulmonary disease burden. Thus we aimed to investigate the effects of a preceding IFV infection on the development of allergic airway inflammation. Cellular immunity is largely responsible for heterologous responses to IFV,[10-12] and virus-specific $CD4^+$ effector T cells were shown to directly correlate to disease protection among experimentally infected patients with IFV.[13] Therefore we hypothesized that T-cell cross-reactivity between IFV and certain environmental allergens might be responsible for any effects over allergic airway inflammation.

METHODS

Mice

Adult 4- to 6-week-old inbred female BALB/c mice were purchased from Harlan-Winkelmann GmbH (Borchen, Germany).

Viral propagation and titration

The swine-origin pandemic influenza virus A/Hamburg/5/2009 (hemagglutinin 1 neuraminidase 1 [H1N1]) was propagated in Madin-Darby canine kidney (ATCC CCL-34) cells (ovalbumin [OVA]–free approach).[14] Virus-containing supernatants were collected 2 to 3 days postinfection (dpi) after visible cytopathic effect. The virus was clarified from cellular debris by means of low-speed centrifugation, and infectivity was determined by using a plaque assay in Madin-Darby canine kidney cells, as described previously,[15] and expressed in plaque-forming units per milliliter. Aliquots of viral suspension were stored at −80°C until use. For unspecific *ex vivo* stimulation and immunosorbent assay, viral stocks were inactivated by addition of $NaHCO_3$ (to 0.15%) and β-propiolactone (1:2000; Sigma, St Louis, Mo) for 72 hours at 4°C, followed by storage at −80°C.

Murine influenza infection model

Mice were lightly anesthetized by means of intraperitoneal injection of Ketamin (Inresa Arzneimittel GmbH, Freiburg, Germany)–Rompun (Bayer Health Care, Leverkusen, Germany), as described above, and infected intranasally with 200,000 plaque-forming units of H1N1 in 40 μL of sterile PBS. Weight loss and survival of infected mice were monitored over a 12-day period (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Animals showing more than 20% body weight loss were killed and documented as dead. Twelve days after infection, respective mice were subjected to the OVA- or house dust mite (HDM)–induced acute asthma model, as described below. Additionally, an extended H1N1 infection model was assessed with onset of the OVA-induced asthma model at 33 dpi.

Murine OVA-induced acute asthma model

For more information on the murine OVA-induced acute asthma model, see Fig E3, *A*, in this article's Online Repository at www.jacionline.org. Mice were sensitized to OVA by means of subcutaneous injection of 10 μg of OVA (grade VI; Sigma) without adjuvant in a total volume of 200 μL of PBS on days 0, 7, and 14. Subcutaneous injection was performed at the scruff of the neck and verified by the observation of a fluid bubble forming under the skin.[16] The "sensitization" phase was followed by 20 minutes of 1% OVA or PBS aerosol treatments on days 24, 25, and 26 ("challenge" phase).

Forty-eight hours after the last aerosol challenge, mice were deeply anesthetized by means of intraperitoneal injection of 300 mg/kg ketamine hydrochloride (Ketamin) plus 30 mg/kg xylazine (Rompun) before exsanguination by cutting the abdominal aorta.

Murine peptide immunization model

Peptides were synthesized by ProImmune (Oxford, United Kingdom), and purity was greater than 90%, as determined by using HPLC. Animals were immunized intraperitoneally with a pool of predicted H1N1-derived MHC class I and MHC class II T-cell epitopes (polymerase basic protein