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## IgE cross-linking impairs monocyte antiviral responses and inhibits influenza-driven T<sub>H</sub>1 differentiation

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### To the Editor

Mechanisms underlying exacerbations of allergic asthma, although poorly defined, are clearly linked with viral infections.<sup>1</sup> Rhinoviruses are the most common viruses associated with asthma exacerbations.<sup>E1–E3</sup> Influenza virus infections have a similar association,<sup>E4–E6</sup> resulting in increased disease severity in this population<sup>E7,E8</sup> (see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Defects in innate cell antiviral responses represent one potential mechanism underlying the link between viral infections and atopic disease exacerbations. For example, high-affinity IgE receptor (FcεRI) stimulation on plasmacytoid dendritic cells inhibits influenza-induced IFN-α secretion<sup>2</sup> and suppresses Toll-like receptor 7<sup>2</sup> and Toll-like receptor 9 expression.<sup>3</sup> Decreasing serum IgE with omalizumab in allergic patients with asthma restores *ex vivo* antiviral IFN-α responses, and correlates with improved clinical outcomes.<sup>4</sup> Monocytes are also recruited to respiratory sites during viral infections<sup>E17</sup> and allergen exposure,<sup>5, E18</sup> and implicated in food allergy development.<sup>6</sup> In addition, allergic stimulation via IgE cross-linking impacts monocyte functions including apoptosis,<sup>E19</sup> cytokine secretion, and phagocytosis.<sup>7</sup> Defining IgE-mediated effects on monocyte antiviral responses could reveal additional pathways involved in atopic disease pathogenesis.

Antigen presentation, which drives T-cell activation and differentiation, represents a critical monocyte antiviral function. Influenza exposure increased monocyte-driven T<sub>H</sub>1 priming of naive CD4<sup>+</sup> lymphocytes in primary human monocyte-T-cell cocultures, but did not significantly affect T<sub>H</sub>2 differentiation (Fig 1, *A*; see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Influenza-driven T<sub>H</sub>1 differentiation inversely correlated with monocyte surface FcεRI expression (Fig 1, *B*), a surrogate for serum IgE,<sup>E20</sup> suggesting that IgE-mediated stimulation may impact monocyte-directed T<sub>H</sub>1 priming during viral infection. Confirming this concept, IgE cross-linking of influenza-exposed monocytes significantly disrupted T<sub>H</sub>1 differentiation (Fig 1, *C*, and Fig E1), a finding consistent across all donors (Fig 1, *D*). Similarly, IgE cross-linking decreased IFN-γ

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secretion (Fig 1, *E*) and T-cell proliferation (Fig 1, *F*, and Fig E1), but had no effect on T<sub>H</sub>2 development (see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The decrease in T<sub>H</sub>1 cell stimulation was not due to IgE-mediated inhibition of influenza virus infection of monocytes because IgE cross-linking did not affect percentages of monocytes expressing the influenza proteins hemagglutinin and nucleoprotein (Fig 1, *G* and *H*). These data suggest that allergen-mediated IgE cross-linking on monocytes during concomitant viral infection could result in impaired development of virus-specific T<sub>H</sub>1 cells in sensitized individuals *in vivo*.

We evaluated potential roles for multiple cytokines in IgE-mediated inhibition of monocyte CD4<sup>+</sup> T lymphocyte development, including IL-12, a critical factor in T<sub>H</sub>1 differentiation (see Figs E3 and E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Our results indicate that IL-12, TNF- $\alpha$ , IL-6, and IL-10 do not regulate IgE-mediated suppression of monocyte-driven antiviral T<sub>H</sub>1 priming.

Upregulation of MHC and costimulatory molecules is required for engagement and activation of the T-cell receptor complex. These signals direct CD4<sup>+</sup> T-cell maturation; stronger antigen signal strength favors T<sub>H</sub>1 differentiation, whereas weaker signals promote T<sub>H</sub>2 or regulatory phenotypes.<sup>E21</sup> We evaluated the effect of IgE cross-linking on influenza-induced monocyte maturation. Influenza exposure significantly upregulated surface CD80, CD86, and MHC class I (HLA-ABC) and II (HLA-DR) expression, with levels significantly diminished by IgE-mediated stimulation (Fig 2, *A–C*). We hypothesized that IgE-mediated downregulation of HLA-DR and CD86 may disrupt monocyte-T-cell interactions. Visualization and quantitation of monocyte-T-cell interactions revealed that monocyte-T-cell clusters were significantly increased by influenza exposure, but impaired by IgE cross-linking (Fig 2, *D* and *E*). Because cell proliferation directly reflects T-cell activation, the IgE-mediated decrease in cell division (Fig 1, *F*) provides additional evidence that IgE cross-linking prevents monocytes from forming high-affinity interactions needed to activate multiple T cells simultaneously. These data support the model that allergic stimulation significantly impairs upregulation of proteins essential for antigen presentation, resulting in weaker T lymphocyte stimulation and diminished antiviral T<sub>H</sub>1 responses (see Fig E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

IgE-mediated inhibition of virus-induced T<sub>H</sub>1 priming was remarkably conserved across all donors, and may reflect an evolutionarily maintained mechanism. Parasite-produced proteins have effects on antigen-presenting cells similar to IgE cross-linking on monocytes—downregulation of antigen presenting molecules, altered CD4 T-cell activation, and decreased T<sub>H</sub>1 responses.<sup>E22,E23</sup> Parasite infection can even alter viral immune responses in coinfection models.<sup>E24–E27</sup> Parasite-specific IgE is protective during parasitic infections<sup>E28,E29</sup>; diminished T<sub>H</sub>1 responses induced by IgE cross-linking could thus be immunomodulatory in the setting of either parasitic or atopic diseases. Along these lines, many epidemiological studies reveal that children with parasitic infections have decreased atopy.<sup>E30–E32</sup> Parasitic infections are characterized by T<sub>H</sub>2-mediated immunity. Activation of IgE-mediated pathways by parasites, or allergen-specific IgE, could thus negatively regulate T<sub>H</sub>1 development, creating a T<sub>H</sub>2-predominant, more optimal antiparasitic response. Decreased T<sub>H</sub>1 differentiation could then dampen proinflammatory responses

during parasitic (or even viral) infections, resulting in decreased inflammation and tissue damage,<sup>E33–E36</sup> and providing potential benefit in allergic airway disease.

Alternatively, IgE-mediated disruption of antiviral T<sub>H</sub>1 responses could promote more severe viral disease via dysregulation of normal T<sub>H</sub>1/T<sub>H</sub>2 balance and contribute to exacerbations of allergic disease. The samples in our study were predominantly from unknown donors obtained via a local blood bank; no information regarding atopic status or serum IgE levels was available. We can thus only extrapolate our findings to those with defined atopic disease. However, the range of surface FcεRI expression suggests a spectrum of IgE levels in our study participants, and the inverse correlation with influenza-induced T<sub>H</sub>1 differentiation supports the concept that elevated IgE may contribute to defective monocyte-directed antiviral T<sub>H</sub>1 responses *in vivo*. Consistent with our findings, others have reported blunted T<sub>H</sub>1 responses to viral infections associated with atopy. Following rhinovirus infection of patients with asthma, blood and airway CD4 T cells secreted decreased IFN-γ.<sup>8</sup> Similarly, patients with atopic dermatitis had decreased virus-specific T<sub>H</sub>1 cells after transcutaneous live virus vaccination, which inversely correlated with serum IgE levels.<sup>9</sup> In future studies, it will be important to evaluate effects of IgE cross-linking on T-cell priming by monocytes exposed to other viruses, including rhinoviruses, and compare responses in atopic (high IgE) versus nonatopic individuals.

To our knowledge, this is the first study demonstrating IgE-mediated impairment of human monocyte antiviral responses, via suppression of virus-induced monocyte maturation and T<sub>H</sub>1 differentiation. This extends our understanding of how allergen-mediated pathways alter host antiviral responses, highlighting a potential role for monocytes in virus-associated exacerbations of allergic disease. Further studies investigating the mechanisms underlying IgE-mediated suppression of virus-induced monocyte responses will enhance our comprehension of allergic disease pathogenesis and potentially uncover novel targets for therapeutic development.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

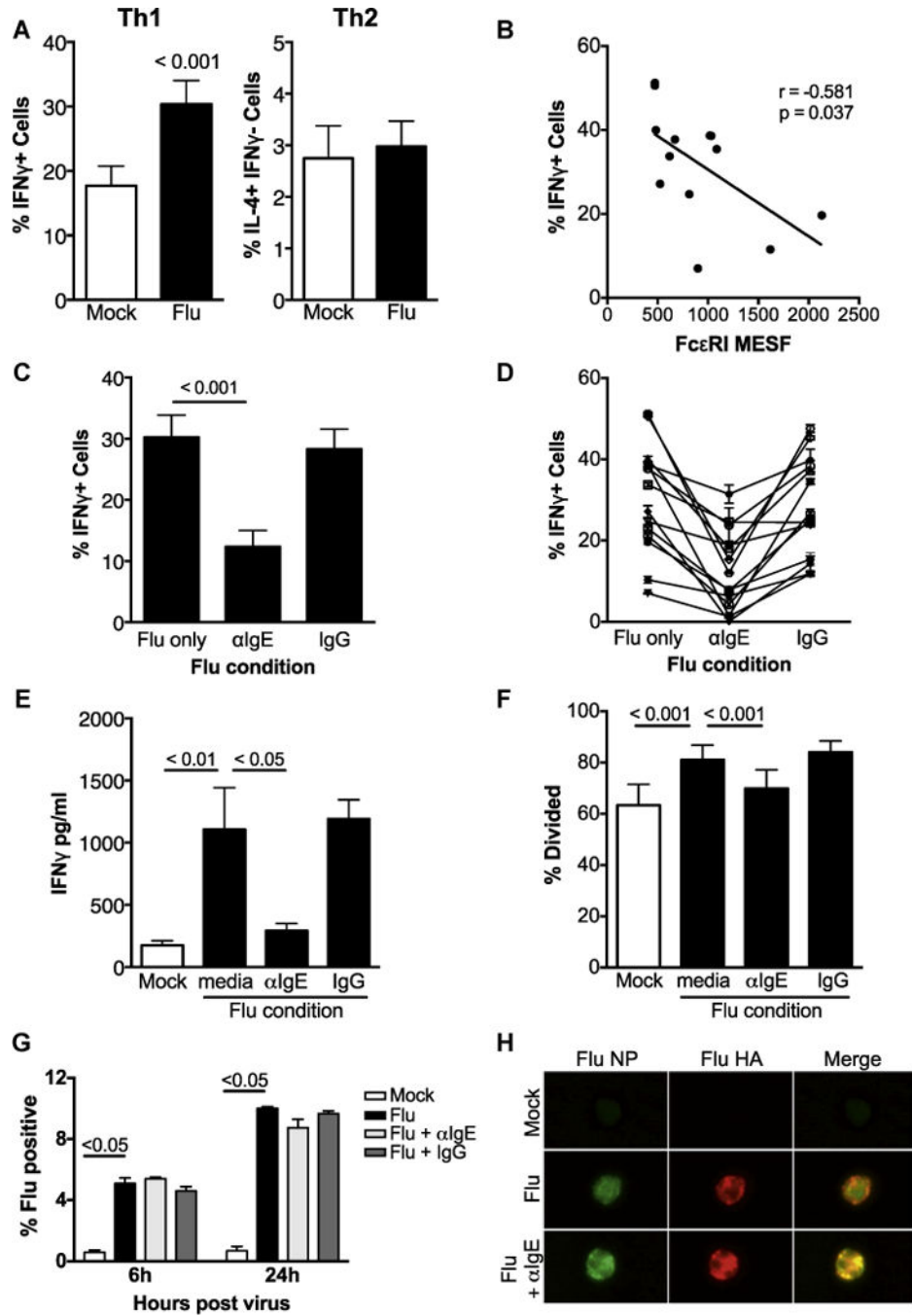
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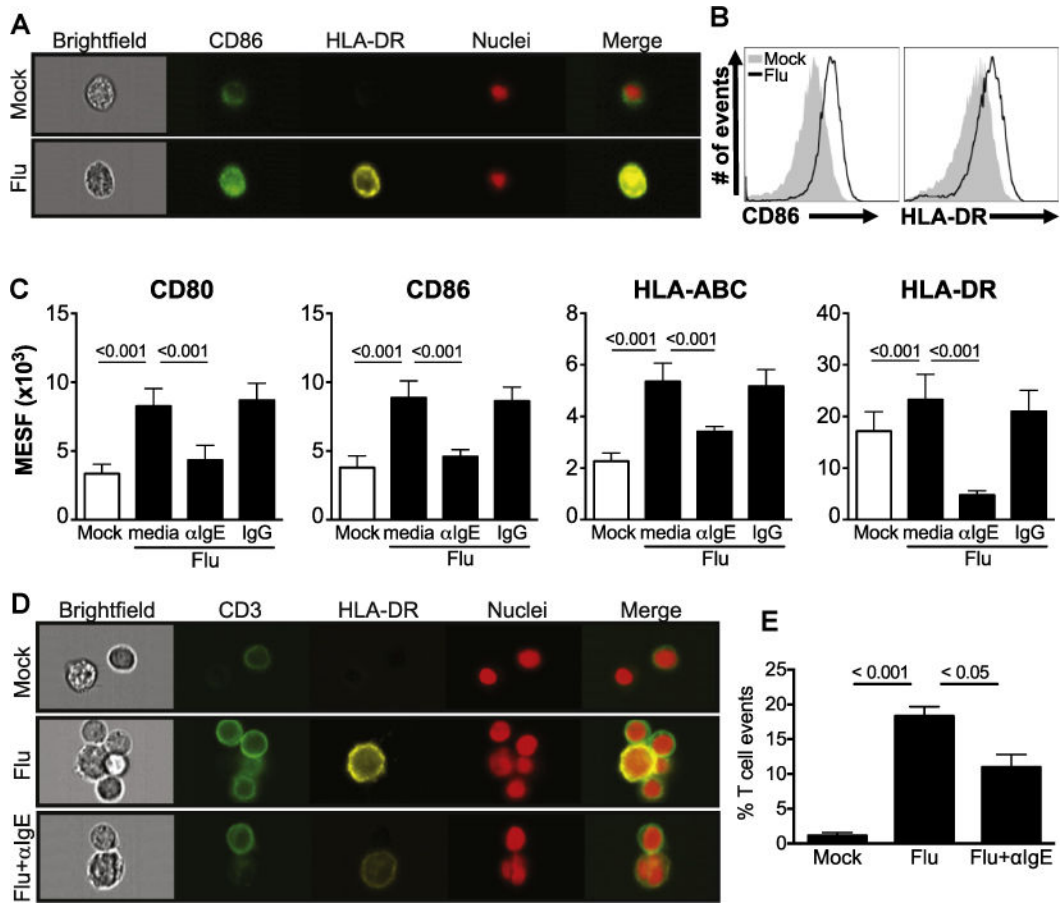
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**FIG 1.**

IgE cross-linking inhibits virus-driven monocyte priming of T<sub>H</sub>1 cells. **A**, Naive CD4 T cells polarized with monocytes exposed to no virus (mock) or influenza A virus (Flu). **B**, Linear regression analysis was used to determine correlation between baseline monocyte Fc $\epsilon$ RI expression (x-axis) and T<sub>H</sub>1 priming capacity (%IFN- $\gamma$ + cells, y-axis) of influenza-exposed monocytes with  $P$  values and  $r^2$  values noted.  $N = 15$ . **C** and **D**, % IFN- $\gamma$ + T cells in monocyte-T-cell cocultures under IgE cross-linking conditions: (Fig 1, **C**) mean and (Fig 1, **D**) individual donor pairs.  $N = 15$ . **E**, Mean IFN- $\gamma$  concentration in T-cell supernatants ( $N =$

5). **F**, IgE cross-linking diminishes T-cell proliferation. Naive CD4 T cells labeled with VPD450 cellular dye before coculture. % divided defined as cells with lower VPD450 fluorescence compared with unstimulated T-cell controls, N = 3. Error bars represent the SEM. Pertinent *P* values are shown and were obtained with 2-way ANOVA analysis. **G** and **H**, Influenza A virus protein expression in monocytes at 6 and 24 hours postinfection by (Fig 1, *G*) flow cytometry of Flu-positive cells (NP/HA double positive) and (Fig 1, *H*) flow cytometry imaging at 6 hours postinfection (NP, green; HA, red). Shown is representative data from N = 3 experiments. *HA*, Influenza hemagglutinin; *MESF*, mean equivalent soluble fluorochrome; *NP*, influenza nucleoprotein. Error bars represent SEM, and *P* values represent results of 1-way ANOVA. All Material and Methods can be found in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).



**FIG 2.**

IgE cross-linking inhibits virus-induced monocyte maturation and monocyte-T-cell interactions. **A–C**, Monocytes treated with media alone (mock) or influenza A virus (Flu) ± IgE cross-linking or IgG control antibody and stained for various cell surface markers. Representative flow cytometry (Fig 2, *A*) imaging analysis and (Fig 2, *B*) histograms for CD86 and HLA-DR staining are shown for mock and Flu only (Fig 2, *C*). Mean cell surface marker expression by flow cytometry analysis of the mean equivalent soluble fluorochrome (MESF, expressed as  $\times 10^3$  units) value was determined under the various conditions,  $N = 3$ . **D**, Flow cytometry imaging analysis of monocyte-T-cell interactions. Cell clusters from monocyte-T-cell cocultures stained with anti-CD3 (green), anti-HLA-DR (yellow), and nuclei (red) were visualized, 60 $\times$  magnification on the Amnis ImageStream flow cytometer (Amnis, Seattle, Wash). **E**, Quantitation of total monocyte-T-cell clusters was performed using the IDEAS Software (Amnis) ( $N = 3$ ). Error bars represent SEM, and  $P$  values represent results of 1-way ANOVA. All Material and Methods can be found in this article’s Online Repository at [www.jacionline.org](http://www.jacionline.org).