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LETTER TO THE EDITOR

Dear Editor

Aberrant Cytokine Responses to Influenza A Virus in a Child with Severe Influenza A Infections

Influenza is a common respiratory virus infection. Although it causes significant morbidity, clinical courses of the infection are self-limited in most cases. However, the influenza viruses sometimes cause severe and lethal diseases such as acute respiratory distress syndrome, encephalopathy, and myocarditis. These severe infections usually occur in patients who had been apparently healthy and immunocompetent before infection, and underlying mechanisms for severity are often obscure. We describe a case of 9 year old boy with repeated severe influenza A infections in whom aberrant lymphocyte responses to influenza antigens *in vitro* were observed.

He had been healthy except for egg allergy outgrown by age of 2 with no history of severe infections before the episodes of severe influenza. At age of 6, he was infected with influenza A/H3N2 resulted in acute respiratory distress syndrome and brain edema within 24 hours of onset. With methylprednisolone pulse therapy he recovered without sequelae. He had second severe influenza A/H1N1 infection leading to lobar pneumonia with severe hypoxemia at age of 9. Again, he could recover with an intensive care. At the time of discharge, eosinophilia (34%) was noted without symptoms.

He had been vaccinated with diphtheria, pertussis, tetanus (DPT), BCG, polio, Japanese encephalitis, rubella, and measles without complications. He had natural varicella infection without complication. However, he had large topical vesicles at injected region following first vaccination against influenza at 5 years of age. This vaccine was trivalent inactivated influenza HA vaccine. Since then he stopped to receive influenza vaccinations. He had no previous history of influenza infection.

At 6 months after his second influenza virus infection episode, we tested for general immune competence and found no significant abnormalities, including peripheral blood leukocytes, peripheral blood mononuclear cell subpopulations, phytohaemagglutinin-stimulated proliferative responses of peripheral blood mononuclear cells, NK cell function, serum immunoglobulins, and antibody titers to viruses, except for high serum IgE (3430 IU/ml), sensitization to house dust mite, and eosinophilia (11%). Furthermore, we examined viral immune responses of the patient *in vitro*. We found high proliferative responses to influenza virus A antigens compared to age-matched controls. The supernatants of mononuclear cells cultured for 48 hours with the 2 strains of influenza virus A antigen were assayed for a panel of cytokines and chemokines by a beads array system (Luminex). As shown in Figure 1, production of interferon (IFN)-γ, interleukin (IL)-17, IL-6 and MIP-1α appeared low and that of IL-2, IL-4 and IL-5 and IL-13 by the influenza virus A antigens was high compared to age-matched healthy (n = 1) and mild allergic children (n = 3) who had influenza infection in the same season. In addition, production of IL-1β, IL-7, G-CSF, GM-CSF, IL-6, IL-8, IL-17, MIG, MIP-1α, MIP-1β, RANTES, and eotaxin also appeared low compared with the controls. Proliferative responses and cytokine/chemokines productions to measles, rubella, and varicella were similar between the patient and controls. Specific IgE levels to the 2 influenza virus A strains by ELISA were also high in the patient (Table 1). No significant mutations in Toll-like receptor 3, 7, 8, and RIG-I genes were identified.

Hypercytokinemia has been reported to correlate with severe illness in patients with influenza virus infections.[1,2] Conversely, inadequate adaptive immune responses, or defective cytokine production, that lead to robust viral replication and apoptotic crisis are also suggested in severe diseases.[3] It is still unclear the role of cytokines and why some of the patients with influenza virus infection became severe. We describe here a case of severe influenza, which may be due to inadequate Th1 and Th17 cytokine responses and aberrantly high Th2 cytokine responses. Although the source of Th2 cytokines is not known, it could be Th2 cells or basophils stimulated by IgE cross-linking since the patient had specific IgE to influenza.[4] The presence of specific IgE may also explain the fact that the patient had severe disease only with influenza infection and immunocompetent to other pathogens since IgE antibody may suppress protective immune responses. Our case presents a new type of severe infection, which may hint the unknown pathogenesis. The study was approved by the Ethics Committee of Mie National Hospital. Written informed consent was obtained from the subjects.

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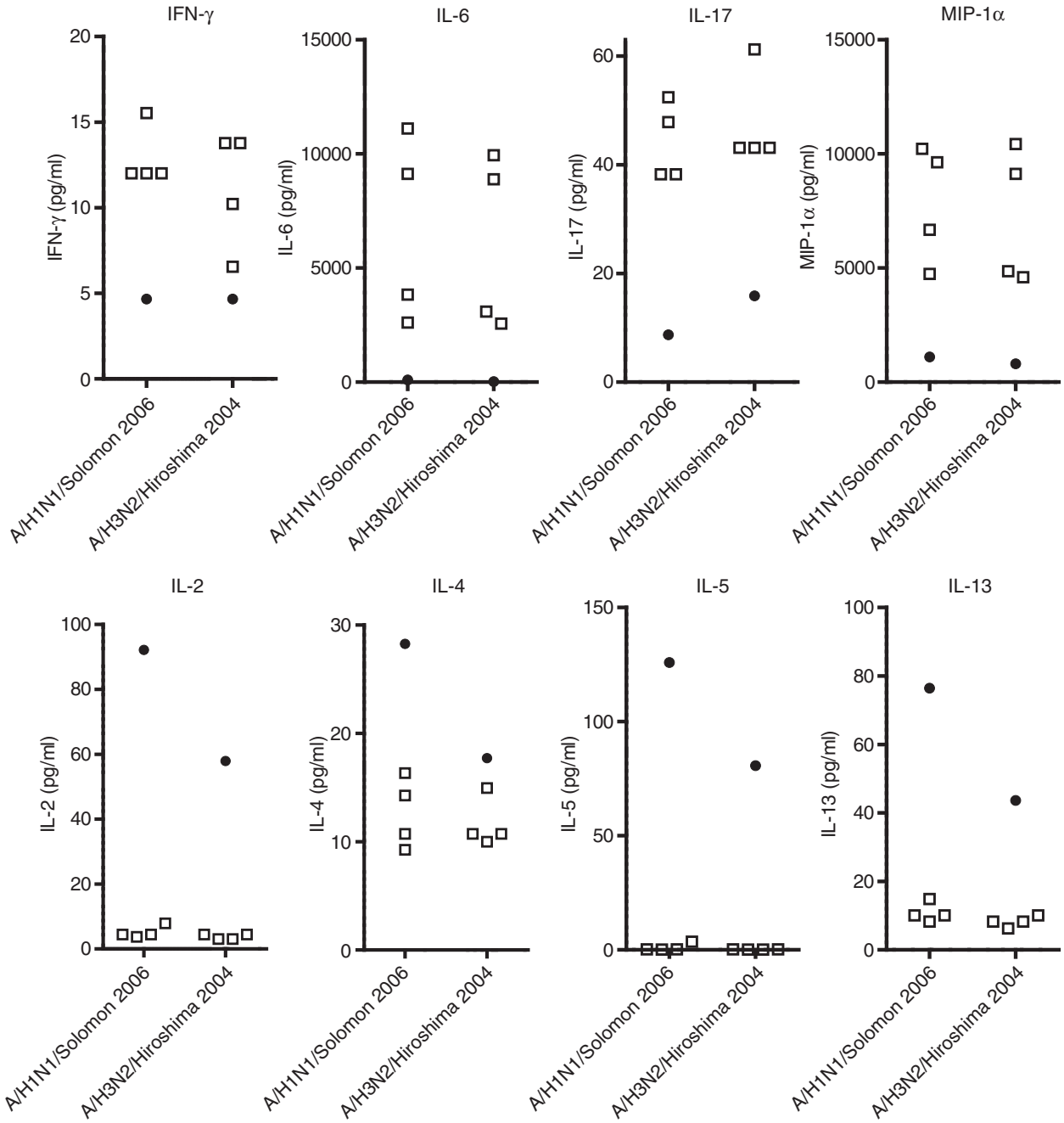

Fig. 1 Production of cytokine in response to the influenza antigens. Isolated PBMCs and 1 : 190 diluted inactivated influenza HA vaccine components, namely, A/Solomon islands/2006 (H1N1) at 689 μgHA/ml and A/Hiroshima/2005 (H3N2) at 1200 μgHA/ml, kindly provided by the Kanonji Institute of the Research Foundation for Microbial Diseases of Osaka University, were incubated in 24 well microplate at 37°C in 5% CO2 for 2 days. The supernatants after 48 hours of incubation were tested for 20 cytokines, namely, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IFN-γ, TNF-α and GM-CSF, eotaxin, GRO-α, IP-10, MCP-1, RANTES, MCP-2, MCP-3, MIP-1α, MIP-1β, MIG with a beads array system (Luminex Corp., Austin, TX, USA) using a multiplex cytokine kit (Invitrogen, Carsbad, CA, USA).

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Table 1 IgE antibody titers to influenza antigens by ELISA

Subjects	History		IgE antibody titer (OD)		
	Allergy	Influenza	A/Solomon 2006 (H1N1)	A/Hiroshima 2004 (H3N2)	B/Malaysia 2006
Patient	FA	+	2.92	3.00	3.03
Control child 1	AR	+	2.61	1.52	2.31
Control child 2	none	+	0.36	0.21	0.19
Control child 3	AR	+	0.22	0.25	0.08
Control child 4	BA	+	0.40	0.24	0.64
Control child 5	none	+	0.27	0.25	0.41

Influenza-specific IgE antibodies were measured with ELISA according to a method reported elsewhere.[5]
FA, food allergy; AR, allergic rhinitis; BA, bronchial asthma.

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