B-1 cell response and its regulation during influenza virus infection

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KEY MESSAGES

1. Pleural cavity B-1a cells rapidly infiltrate lungs during influenza infection.
2. Pulmonary B-1a cells produce natural antibodies as first-line protection against influenza lung infection.
3. IL-17A deficiency impairs natural antibody production by B-1a cells.
4. IL-17A promotes B-1a cell differentiation into high-rate IgM producing cells in lung tissue.

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Introduction

Outbreaks of influenza infection are a threat to public health in Hong Kong. Influenza infection is generally localised in the respiratory tract where virus-binding antibodies provided by B cells are essential for antiviral immune response against influenza infections by opsonisation of pathogens and activation of complement receptor-mediated phagocytosis. During influenza infection, virus-binding antibodies are produced by two sources, B-1 cells and conventional B-2 cells. Owing to low frequency of viral antigen-specific B-2 cells at the onset of infection, early induction of natural antibody response by B-1 cells becomes critical for immune protection against influenza infection. Although natural IgM antibodies produced by B-1 cells have been recognised to provide the first-line protection by directly neutralising influenza virus,[1-5] it remains unclear what molecular mechanisms regulate this process.

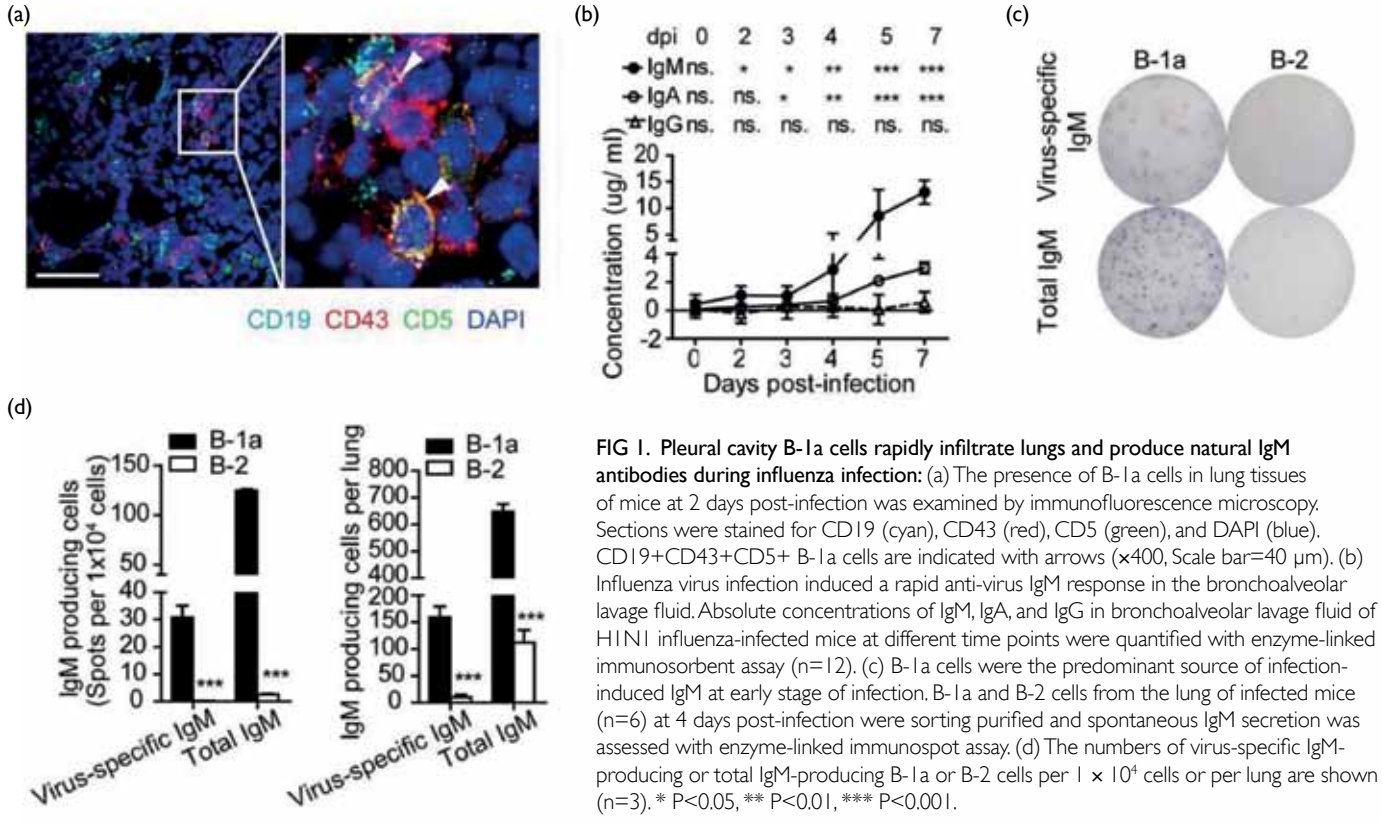

FIG 1. Pleural cavity B-1a cells rapidly infiltrate lungs and produce natural IgM antibodies during influenza infection: (a) The presence of B-1a cells in lung tissues of mice at 2 days post-infection was examined by immunofluorescence microscopy. Sections were stained for CD19 (cyan), CD43 (red), CD5 (green), and DAPI (blue). CD19+CD43+CD5+ B-1a cells are indicated with arrows (×400, Scale bar=40 μm). (b) Influenza virus infection induced a rapid anti-virus IgM response in the bronchoalveolar lavage fluid. Absolute concentrations of IgM, IgA, and IgG in bronchoalveolar lavage fluid of H1N1 influenza-infected mice at different time points were quantified with enzyme-linked immunosorbent assay (n=12). (c) B-1a cells were the predominant source of infection-induced IgM at early stage of infection. B-1a and B-2 cells from the lung of infected mice (n=6) at 4 days post-infection were sorting purified and spontaneous IgM secretion was assessed with enzyme-linked immunospot assay. (d) The numbers of virus-specific IgM-producing or total IgM-producing B-1a or B-2 cells per 1×10^4 cells or per lung are shown (n=3). * P<0.05, ** P<0.01, *** P<0.001.

Results and discussion

We discovered that airway exposure to influenza caused migration of B-1a cells, a subset of B-1 cells, to the lung tissue in infected mice. Lung-infiltrating B-1a cells underwent further differentiation into plasma cells with enhanced production of protective natural IgM antibodies (Fig 1). As an important cytokine locally induced by influenza virus infection, IL-17A critically regulated this process by driving B-1a cell differentiation into high-rate IgM producing plasma cells in the lung tissue during influenza infection. Notably, deficiency of IL-17A led to reduced production of virus-binding natural antibodies by B-1 cells. Furthermore, we elucidated the molecular mechanisms by which IL-17A activates Blimp-1 gene expression and promotes B-1 cell differentiation into plasma cells for natural antibody production (Fig 2). Together, these results

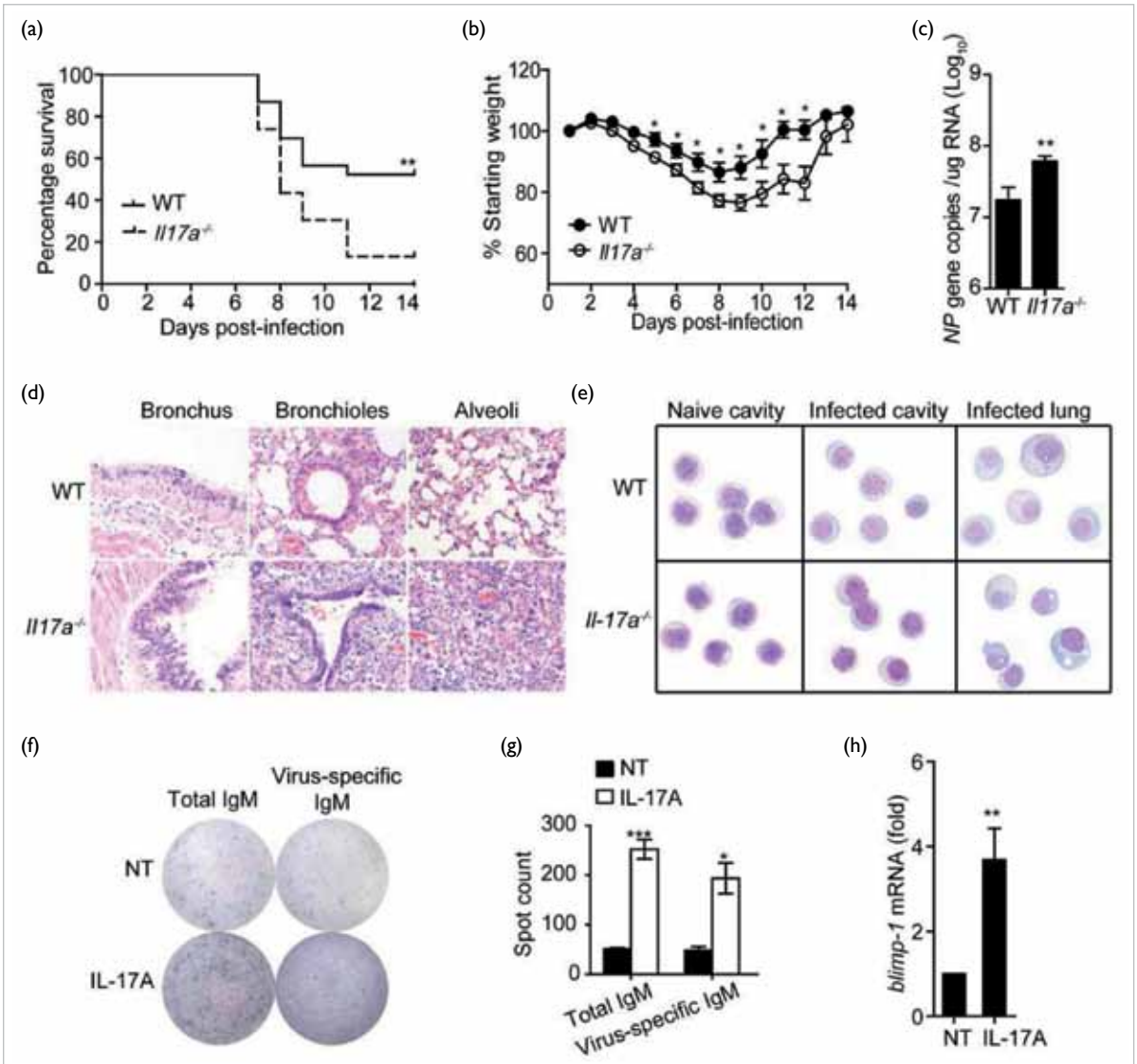

FIG 2. IL-17A promotes B-1a cell differentiation into high-rate IgM producing cells by activating *blimp-1* gene expression: (a, b) H1N1 influenza virus-infected IL-17A deficient (*Il17a*$^{-/-}$) mice exhibited significantly reduced survival rate and body weight compared with wild-type control mice (n=23). (c) The copy number of influenza virus NP gene in the lung tissues at 5 days post-infection was measured by quantitative real-time PCR. The *Il17a*$^{-/-}$ mice exhibited a much higher viral burden than wild-type controls (n=6). (d) Histological analysis revealed substantially increased severity of lung damage in *Il17a*$^{-/-}$ mice, characterised by pronounced inflammatory destruction and leukocyte infiltration at 5 days post-infection following challenge with influenza virus (×200). (e) Morphology of B-1a cells from the pleural cavity of naïve mice or pleural cavity and lung tissues of H1N1-infected wild-type and *Il17a*$^{-/-}$ mice at 5 days post-infection was examined by cytospin preparation and Wright's staining. B-1a cells from lung tissue of infected *Il17a*$^{-/-}$ mice showed morphologically reduced differentiation level. (f, g) B-1a cells were sorting-purified from pleural cavity of wild-type mice and cultured with or without recombinant murine IL-17A (rmIL-17A) (20 ng/ mL) for 5 days. Production of total IgM and virus-specific IgM in supernatants of cultured B-1a cells was examined with enzyme-linked immunospot assay. (h) Sorting purified B-1a cells from pleural cavity of wild-type mice were cultured with or without rmIL-17A (20 ng/ mL) for 24 hours. Real-time PCR analysis showed that IL-17A promoted blimp-1 gene expression in B-1a cells. * P<0.05, ** P<0.01, *** P<0.001.

have demonstrated that IL-17A is a key factor that modulates natural antibody production by B-1 cells in the lung during influenza infection.

These findings provide new insights in understanding how natural antibody production by B-1 cells is regulated by IL-17A, an important process in early immune response against influenza infection. This study will facilitate further investigations to validate IL-17A as an immune stimulator in designing effective vaccines for preventing influenza infection in human. We will further study the functional modulation of B-1 cell response and develop a new strategy by targeting B-1 cells for the effective treatment of influenza infection.

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References

1. Hayakawa K, Hardy RR, Honda M, Herzenberg LA, Steinberg AD, Herzenberg LA. Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies. Proc Natl Acad Sci U S A 1984;81:2494-8.
2. Ha SA, Tsuji M, Suzuki K, et al. Regulation of B1 cell migration by signals through Toll-like receptors. J Exp Med 2006;203:2541-50.
3. Berberich S, Forster R, Pabst O. The peritoneal micromilieu commits B cells to home to body cavities and the small intestine. Blood 2007;109:4627-34.
4. Baumgarth N, Herman OC, Jager GC, Brown L, Herzenberg LA, Herzenberg LA. Innate and acquired humoral immunities to influenza virus are mediated by distinct arms of the immune system. Proc Natl Acad Sci U S A 1999;96:2250-5.
5. Choi YS, Baumgarth N. Dual role for B-1a cells in immunity to influenza virus infection. J Exp Med 2008;205:3053-64.