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Intestinal binding of seasonal influenza A viruses to DC-SIGN$^+$ CD68$^+$ cells

Martin C. W. Chan,[a] Nelson Lee,[b,c] Paul K. S. Chan,[a,c] Ka-Fai To,[d] Rity Y. K. Wong,[b] Carmen O. K. Law,[e] Karry L. K. Ngai,[a] Joseph J. Y. Sung[e]

[a]Department of Microbiology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China. [b]Division of Infectious Diseases, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China. [c]Stanley Ho Centre for Emerging Infectious Diseases, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China. [d]Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China. [e]Institute of Digestive Disease, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China.
Correspondence: Nelson Lee, MD, 9/F Clinical Sciences Building, Division of Infectious Diseases, Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China. E-mail: leelsn@cuhk.edu.hk

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To the editors:

We have recently reported that influenza A viral RNA was frequently detected in the feces of adult patients hospitalized for severe seasonal influenza infection.[1] However, there was no correlation with gastrointestinal symptoms and clinical outcomes, and human-like influenza virus receptor was absent from the small and large intestinal epithelial surface. We also showed that seasonal influenza A (H1N1) and (H3N2) viruses did not bind to intestinal epithelial cells, but to a subset of lamina propria and submucosal intestinal CD45$^+$ (leukocyte common antigen) cells *in vitro*. In this study, we have further characterized these virus-binding cells, which may help to explain the phenomenon of seasonal influenza A virus presence in the extra-pulmonary sites.

In vitro binding of seasonal influenza A viruses to archival human small (duodenum) and large (colon) intestinal tissues was performed as we previously described.[1] Two representative formalin-inactivated human virus isolates were used: H1N1 (A/HongKong/CUHK-13003/2002) and H3N2 (A/HongKong/CUHK-22910/2004). These two virus isolates were selected for binding assay because comparison of key amino acid residues comprising the glycan-binding site on hemagglutinin showed that they were very similar to historical major circulating strains of the same virus subtype. To identify virus-binding cells, double immunofluorescence staining was performed using a virus subtype-specific monoclonal antibody against viral nucleoprotein (clone A1 for H1N1 and clone A3 for H3N2; Millipore, Billerica, MA, USA) and a panel of immune cell surface markers: T cells (CD3, clone F7.2.38; Dako, Glostrup, Denmark), natural killer cells (CD56, clone 123C3; Dako), B cells (CD19, clone LE-CD19; Dako; CD20, clone L26; Dako), plasma cells (CD138, clone MI15; Dako), dendritic cells (DC-SIGN, clone 5D7; Abcam, Cambridge, MA, USA), and macrophages (CD68, clone PG-M1; Dako). Double labeling among different combinations of cell surface markers was also performed in a similar manner where appropriate. Expression of human-like influenza virus receptor, namely terminal sialic acid α-2,6 galactose, on targeted mononuclear cells was determined by lectin histochemistry using lectin isolated from *Sambucus nigra*. All staining was performed on specimens obtained from at least two individuals. Detailed protocols are available upon request.

Our findings are summarized in the Figure 1. Seasonal influenza A viruses of subtypes H1N1 and H3N2 were shown to bind to a sub-population of mononuclear cells interspersed in lamina propria and the submucosal region, but not to the intestinal epithelial cells (Figure 1, panel A). Notably, these virus-binding cells appeared to be more abundantly located in the crypts region and in the submucosa (Figure 1, panel A). These cells were stained negative for cell surface markers for T cells, natural killer cells, B cells, and plasma cells. Instead, they were found to co-express markers for dendritic cells (DC-SIGN$^+$) and macrophages (CD68$^+$) (Figure 1, panel B, C). Further, we had confirmed the expression of human-like influenza virus receptor (sialic acid α-2,6) in nearly all such DC-SIGN$^+$ and CD68$^+$ cells (Figure 1, panel D).

Our study identified the influenza virus binding intestinal cells as DC-SIGN$^+$ CD68$^+$ dendritic cells. In the human small intestine, DC-SIGN$^+$ CD68$^+$ cells are

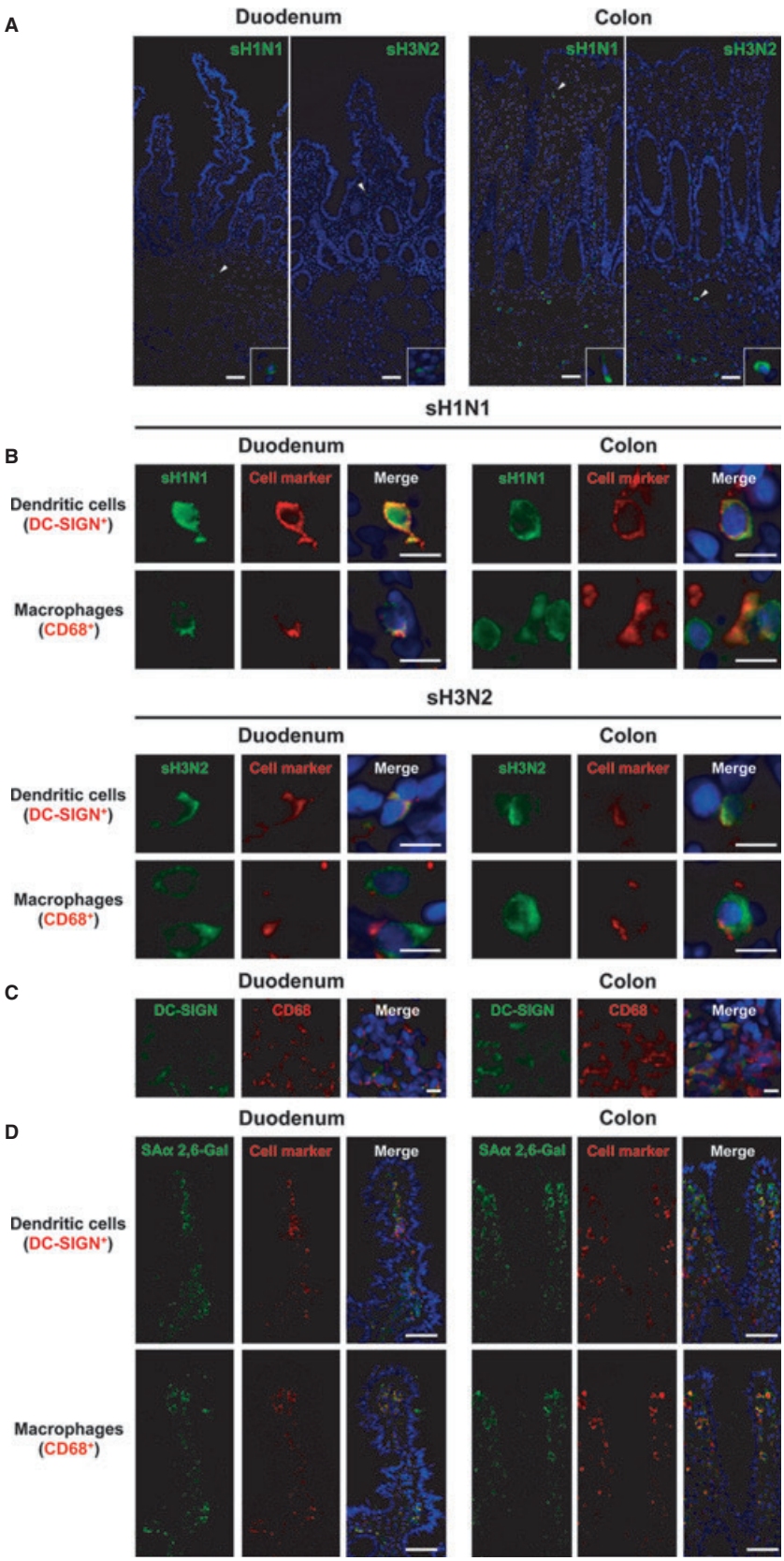

Figure 1. *In vitro* binding of inactivated seasonal influenza A (H1N1) and (H3N2) viruses to intestinal DC-SIGN$^+$ CD68$^+$ cells of human small (duodenum) and large (colon) intestinal tissues. (A) Immunofluorescence staining showing the attachment of seasonal influenza viruses (green) to cells of lamina propria and submucosa. Arrows denote representative virus-binding cells with magnified view shown in insets. (B) Double immunofluorescence staining of virus-binding cells (green) and surface markers suggestive for dendritic cells (DC-SIGN, red) and macrophages (CD68, red). (C) Double labeling of intestinal lamina propria cells co-expressing DC-SIGN (green) and CD68 (red). (D) Double immunofluorescence staining showing expression of human-like influenza virus receptor, terminal sialic acid α-2,6 galactose (green), on intestinal DC-SIGN$^+$ and CD68$^+$ cells (red). sH1N1/sH3N2, seasonal influenza A H1N1/H3N2 viruses. Scale bars, 50 μm (panels A and D) and 10 μm (panels B and C).

concentrating around the dome region of Peyer's patches, the gut-associated lymphoid tissue.[2] In the human large intestine, the distribution of DC-SIGN$^+$ CD68$^+$ cells is less well defined; but available data suggest that these cells may be abundant in colonic submucosa and rectal mucosa.[2,3] It is generally believed that these intestinal DC-SIGN$^+$ CD68$^+$ cells act as antigen-presenting cells and participate in the stimulation of immunity through T-cell activation.[4] Recent evidence indicates that antigen-presenting cells of multiple origins are susceptible to influenza virus infection of different subtypes,[5,6] and they may act as vehicles for extrapulmonary virus dissemination.[7] Notably, production of infectious seasonal influenza viruses were absent in infected circulating dendritic cells (non-productive infection).[8] Our findings thus provide further explanation to the fecal presence of seasonal influenza virus, which is likely related to the detection of viral RNA remnants in infected intestinal antigen-presenting/immune cells, rather than direct infection of intestinal epithelium.[1] In contrast, A(H1N1)pdm09 virus, which has become a widely circulating seasonal virus, binds to both human-like and avian-like virus receptors and has a characteristic glycan-binding profile different from other influenza A viruses.[9] This virus can bind to avian-like virus receptor expressed on human colon epithelial cells and replicates efficiently in intestinal cells.[10] In summary, we demonstrate intestinal binding of seasonal influenza A viruses to DC-SIGN$^+$ CD68$^+$ cells. Further characterization on the nature and role of these cells, particularly in naturally occurring infections, may shed light on the pathogenesis and host–virus control of this important human pathogen.

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Potential conflict of interest

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