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Hemagglutination inhibiting antibodies and protection against seasonal and pandemic influenza infection

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Summary *Objectives:* Hemagglutination inhibiting (HI) antibodies correlate with influenza vaccine protection but their association with protection induced by natural infection has received less attention and was studied here.
Methods: 940 people from 270 unvaccinated households participated in active ILI surveillance spanning 3 influenza seasons. At least 494 provided paired blood samples spanning each season. Influenza infection was confirmed by RT-PCR on nose/throat swabs or serum HI assay conversion.
Results: Pre-season homologous HI titer was associated with a significantly reduced risk of infection for H3N2 (OR 0.61, 95%CI 0.44–0.84) and B (0.65, 95%CI 0.54–0.80) strains, but

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not H1N1 strains, whether re-circulated (OR 0.90, 95%CI 0.71—1.15), new seasonal (OR 0.86, 95%CI 0.54—1.36) or pandemic H1N1-2009 (OR 0.77, 95%CI 0.40—1.49). The risk of seasonal and pandemic H1N1 decreased with increasing age (both $p < 0.0001$), and the risk of pandemic H1N1 decreased with prior seasonal H1N1 (OR 0.23, 95%CI 0.08—0.62) without inducing measurable A/California/04/2009-like titers.

Conclusions: While H1N1 immunity was apparent with increasing age and prior infection, the effect of pre-season HI titer was at best small, and weak for H1N1 compared to H3N2 and B. Antibodies targeting non-HI epitopes may have been more important mediators of infection-neutralizing immunity for H1N1 compared to other subtypes in this setting.

Background

Each year, seasonal influenza is responsible for three to five million severe illnesses and 250,000 to 500,000 deaths worldwide. An accurate and complete understanding of the mechanisms of immunity to influenza is critical in order to assess the risk posed by new virus variants and to optimize immunization strategies. Influenza viruses infect human cells through the binding of the viral surface hemagglutinin (HA) protein to the terminal sialic acid molecules of glycoproteins and glycolipids expressed on host cell membranes, and the subsequent fusion of viral and cell membranes.[1] Antibodies directed at targets surrounding the receptor-binding pocket of the HA can block binding, and are the best-defined correlate of influenza immunity. Serum concentrations of antibodies that block receptor binding are traditionally measured using the hemagglutination inhibition (HI) assay, and HI titers of between 18 and 40 are associated with a 50% reduction in infection risk.[2—8] However, the determinants of immunity to influenza in humans remain incompletely understood, with HI antibodies providing only a partial explanation. Indeed, in his seminal paper describing the protective effect of pre-existing HI antibodies on H3N2 and B infection, Hobson noted that people with no detectable HI antibodies may be resistant to infection,[3] and it is well recognized that immunity to infection can span major antigenic variants within a subtype.[9—13] When H1N1 re-emerged in 1977 after an absence of 20 years, resistance to infection in people aged over 20 years was not dependent on HI antibodies[6,10] and in 2009, adults in several Asian countries experienced low rates of pandemic H1N1 infection despite the virtual absence of detectable homologous HI antibodies.[12—16]

Influenza viruses have a high potential for genetic and antigenic diversity, and influenza epidemiology is characterized by regular epidemics of antigenically distinct strains.[17] Since the binding region of the HA1 protein is a key target for neutralising antibodies, it is under intense immune-mediated positive selection pressure, resulting in the acquisition and retention of amino acid substitutions that favor escape from immunity. However, the rate of antigenic evolution of the HA1 differs between subtypes, with H3N2 evolving faster than H1N1,[18,19] an observation for which there is considerable uncertainty over the mechanisms underlying this difference.[20]

We set out to re-examine the contribution of serum HI antibody to protection against natural influenza infection in an unvaccinated Vietnamese cohort followed over three consecutive influenza transmission periods, which included re-circulating strains, new antigenic variants, and the first wave of the 2009 H1N1 pandemic.

Participants

The research was approved by the institutional review board of the National Institute of Hygiene and Epidemiology, Vietnam; the Oxford Tropical Research Ethics Committee, University of Oxford, UK; and the Ethics Committee of the London School of Hygiene and Tropical Medicine, UK. All participants provided written informed consent.

The procedures for selecting the study site and for selecting and investigating individual participants are described in detail elsewhere.[21] In brief, households in Thanh Ha commune, Thanh Liem District, Ha Nam Province, Viet Nam were selected at random. This semi-rural commune is in the Red-River delta around 60 km from Hanoi. 940 members of 270 randomly selected households consented and were enrolled. The cohort is ongoing but the analysis described here covers three consecutive influenza seasons detected up until April 2010 (Table 1). Influenza seasons were detected via active surveillance for influenza-like-illness (ILI), defined as a fever > 38 °C and cough or sore throat. Study health workers examined participants with ILI and collected nose and throat swabs. Investigation was enhanced during the first wave of pandemic H1N1 transmission (September—December 2009) when all members of ILI case households were swabbed daily for up to 15 days. Blood samples were collected for serology at baseline in December 2007 and between each confirmed influenza season (Table 1).

Methods

Virology and serology

Combined nose and throat swabs were assessed by real-time reverse-transcriptase polymerase chain reaction (RT-PCR), according to WHO/US CDC protocols (CDC reference no. I-007-05, Accessed November 30, 2009, at http://www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf). Viruses were isolated from participants' swabs and propagated in MDCK cells. The HA genes of seasonal H1N1 and H3N2 isolates