



Title	Localized antibody responses in influenza virus-infected mice
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by other IAVs, we compared seasonal with avian virus strains. The proportion of both infected and ISG15-positive cells was 3-4-fold higher after infection with low-pathogenic avian IAV in comparison with seasonal IAV, suggesting that these non-human viruses are debilitated in inhibiting ISG15-expression. The proportion of double-positive cells increased up to 6 times upon infection with a mutant virus lacking the NS1 gene, indicating a role for NS1 in the suppression of ISG15 in infected cells. Along this line, transfection-based experiments demonstrated that the presence of an active viral polymerase was sufficient to induce ISG15, which was inhibited by co-expressed NS1 protein. In a similar transfection assay, NS1 proteins of seasonal IAV significantly reduced subsequent IFN-stimulated ISG15 induction, whereas this was not observed for NS1 proteins of low-pathogenic avian IAV. Notably, the NS1 protein of the newly emerged H7N9 virus also did not inhibit ISG15 induction. Finally, knock down of ISG15 had no effect on replication of seasonal IAV but led to 2-6-fold higher virus titers of low-pathogenic avian IAV at different time points. Conclusions: Our study demonstrates on a single-cell level that NS1 proteins of seasonal IAV strains are able to suppress ISG15 induction in infected or NS1-transfected cells. On the contrary, NS1 proteins expressed by low-pathogenic avian IAV or the novel H7N9 virus are unable to inhibit IFN-induced ISG15 expression. Preliminary data suggests that the suppression of ISG15 by NS1 is most likely due to a mechanism on the post-transcriptional level, which is currently being investigated. In conclusion, we propose that the ISG15 system contributes to limiting the IAV host range.

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Background: Traditionally, vaccine-mediated protective responses were quantified by measuring the level of increase of influenza virus-specific antibodies circulating in blood. However, virus-specific antibodies in serum do not necessarily correlate with protection in vaccinees receiving intranasally administered live attenuated influenza vaccines (LAIVs). Local mucosal and cellular immune responses are believed to be the protective mechanism induced by LAIVs. Recently, antibody secreting cell (ASC) responses derived from peripheral blood mononuclear cells (Cherukuri A, et al. *Vaccine*. 2012;356:685-696) of ferrets and antibody obtained via human nasal washes, but not the systemic serum (Barria MI, et al. *J Infect Dis*. 2013;207:115-124), were found to better correlate with B-cell responses induced by LAIV. ASCs are found in the upper and lower respiratory tract in influenza infections and play an important role in combating influenza infections. Analyses of antigen-specific B-cell receptors on these ASCs were limited by cell-based assays such as ELISPOT or FACS probe by hapten or B-cell tetramers. Although the localized mucosal and systematic ASC responses of Influenza A virus-infected mice are different (Joo HM, et al. *Vaccine*. 2010;28:2186-2194), direct comparisons of antibodies secreted by ASCs at these locations are lacking. Here, we isolate antibodies secreted by ACSs at multiple anatomical sites and characterize the epitope specificity and other properties of these antibodies systematically. Materials and Methods: Mice intranasally infected with influenza virus (A/HK/68) were used as a model. Lymphocytes from different nodes (eg, cervical lymph nodes [CLNs], which drain the upper respiratory tract, and mediastinal lymph nodes [MLNs], which drain the lower respiratory tract) and from the spleen of infected mice were harvested for cell cultures at days 3, 7, and 28 post-infection. In addition, supernatants of nasal washes, bronchoalveolar lavage fluid, and serum of the mice were harvested. Antibodies secreted by the cultured cells and antibody presented in the harvested body fluids were characterized by influenza A-specific isotyping ELISA, micro-neutralization assay, as well as fine epitope mapping assay using a yeast surface display library for H3 hemagglutinin. Results: Antibody in lymphocyte supernatants (ALS) from cultured cells of MLNs and spleen and antibody in serum were found to be positive for influenza virus-specific IgM at day 3 post-infection. Nasal washes, bronchoalveolar lavage fluid, and ALS from CLNs and MLNs were found to be IgA-positive at day 7 post-infection. High IgG1 and IgG2a responses were detected in ALS from MLNs at day 7 post-infection. The control ALS from cells derived from iliac lymph nodes, which drain the mouse tail but not the respiratory tract, was negative for influenza-specific IgA, IgG1, IgG2a, and IgM throughout the study. Bronchoalveolar lavage fluid collected at day 28 post-infection and ALS from MLNs collected at day 7 post-infection demonstrated

a weak neutralization property in a standard micro-neutralization assay. Antibody repertoire mapping of ALS from MLNs collected at day 7 and of serum collected at day 28 post-infection was also performed. The antibody repertoire mappings were comparable and both identified a major immunodominant antigenic site in HA1 and a weaker antigenic site located in HA2. However, two additional antigenic sites were identified in the mapping of ALS from MLNs collected at day 7 when compared with the mapping of the serum obtained at day 28 post-infection. Conclusions: This study illustrated the feasibility of recovering ASC specificity at different localizations after influenza A challenge. With the use of cell-free supernatant, the properties of the ASC-secreted antibodies can be further characterized by various methods traditionally used for serum. The method described will provide information about influenza A-induced antibody responses early post-infection, ie, at the time when the virus was cleared.

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A comparison of human influenza A H1N2 nucleoprotein T-cell epitopes with circulating influenza A viruses from 1918 to 2003

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Background: In 2000, an H1N2 influenza virus arose in the human population and was reported as a sporadic cause of illness across the globe for approximately 3 years. Its greatest impact was in the UK, where it accounted for 54% of influenza A viruses isolated during the 2001-2002 season. The greatest number of infections occurred in the 5-14-year age group (39%) followed by the 14-44-year age group (32%). This H1N2 virus did not contain novel surface antigens or genes because it was a reassortant of the H1N1 and H3N2 viruses that were circulating in the population at the time. Natural influenza infection induces production of neutralising antibodies and stimulates long-lived CD8+ memory T-cell immunity. T cells are targeted directly at the conserved internal proteins of the influenza virus, thereby providing a level of cross-protective immunity against type A influenza viruses. This study examined the T-cell epitopes for all HLA types found in the H1N2 nucleoprotein (NP) for evidence of drift or novel epitopes that might explain its emergence as the dominant circulating strain in the UK over a season. **Materials and Methods:** H1N2 NP was used as the reference data set and consisted of 46 H1N2 NP sequences originating worldwide, with 10 originating from the UK. The NP epitopes were compiled using Immune Epitope Database and Analysis Resource. Epitopes were identified in the data sets using the Identify Short Peptides feature available in the Influenza Research Database. Data sets of H1N2, H2N2, H1N1 and H3N2 viruses isolated from 1918 to 2003 were sourced from GISAID EpiFlu™. The full spectrum of reference epitopes available for each site in combined H1N1, H2N2 and H3N2 NP data sets was identified and characterised as either a perfect match or otherwise with the reference epitope data set. The percentage of strains achieving a perfect match for each epitope sequence was recorded in 5-year blocks to allow assessment of the potential for exposure of population cohorts over time to viruses antigenically related to the emergent H1N2. **Results:** The reference H1N2 data set had a high degree of similarity in all 55 epitopes expressed in the NP, except for 3 viruses—from Asia (2) and North America (1)—with sporadic point mutations expressing novel epitopes not seen elsewhere. These were not considered further, restricting the reference data set to 43 strains. The extent of H1N2 epitope match with the aggregated historical virus data set was as follows: 100% match (20% of epitopes), 75%-99% match (20%), 50%-74% match (49), 25%-49% match (4%) and 15%-24% match (7%). Four epitopes circulated from 1918 until the mid 1960s, re-emerging again in the late 1970s, and epitope NP₁₇₋₂₅, which had circulated before 1950 and re-emerged in the late 1990s; 14% of epitopes found in the H1N2 virus emerged as novel epitopes in the early 1970s, and a further 16% of epitopes also emerged for the first time in the mid 1990s. A further two epitopes that had been predominant in the