Homosubtypic and heterosubtypic antibodies against highly pathogenic avian influenza H5N1 recombinant proteins in the H5N1 and non-H5N1 subjects

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Abstract:
TK- cells infected with recombinant vaccinia virus constructs containing HA, NA, NP, M or NS gene inserts derived from A/Thailand/1(KAN-1)/2004(H5N1) were used as the antigens for detection of specific and cross reactive antibodies in human sera by indirect immunofluorescence assay (IFA). Expression and localization of these proteins was confirmed by Western blot (WB) assay and confocal microscopy, respectively. The test sera comprised sequential serum samples from 4 H5N1 survivors, paired blood samples from 5 patients infected with influenza A (H3N2) virus and 5 patients infected with 2009 pandemic influenza A (H1N1) (H1N1pdm), and single blood sample collected from 18 healthy subjects. The result showed that sera from all 4 survivors contained antibodies to H5N1 virus which lasted for longer than 4 years as determined by hemagglutination inhibition (HI) assay, microneutralization (microNT) assay; and these sera also contained antibodies specific to the 5 recombinant proteins as determined by IFA. On the other hand, no neutralizing antibody was detected in non-H5N1 subjects as determined by microNT assay; but all of them had antibodies that could cross react with all 5 recombinant proteins as detected by IFA. Nevertheless, antibody titers to HA and NA were markedly higher in the survivors, while antibodies to NP, M and NS in both groups of subjects were of comparable titers. Interestingly, some H1N1pdm patients had a 4-fold rise in antibody titer to HA, NA and/or NP antigen. The geometric mean titer (GMT) against HA and NA proteins in convalescent sera of the H1N1pdm patients were significantly higher than that of the acute blood samples (Wilcoxon Signed Ranks test, p<0.05); while no significant different was found with the GMT to NP, M and NS proteins. Only one H3N2 patients had a 4-fold rise antibody titer to NA and M proteins. However, there was no statistically significant difference in GMT
to all 5 recombinant proteins in their paired blood. Most of healthy individuals contained antibodies to all of the 5 recombinant proteins with comparable GMT to the H1N1pdm or H3N2 patients. Our findings suggested that these cross-reactive non-neutralizing antibodies against conserved epitopes of the H5N1 viral proteins could be induced in the non-H5N1 subjects, presumably as a result of the previous infection by seasonal influenza viruses or annual influenza vaccination against seasonal influenza vaccine. In terms of vaccine development for preparedness against pandemic influenza, it is difficult to predict which subtype of vaccine should be prepared in advance. The robust antibody response to conserved epitopes of a universal vaccine might exert the anti-viral activities against various influenza subtypes as has been reported by previous investigators.