Virus Receptors and Entry

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Differences in receptor-binding specificity of highly pathogenic avian influenza viruses and their low pathogenic precursors

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Avian influenza viruses (AIVs) are subdivided into two groups of high or low pathogenicity. Only low pathogenic AIVs (LPAIVs) of subtypes H5 and H7 can evolve into highly pathogenic AIVs (HPAIVs). The two surface viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA, play an important role in determining pathogenic properties of the virus. To characterize potential changes in receptor-binding specificity of HA and substrate specificity of NA during the emergence of HPAIVs we compared a number of LPAIVs and HPAIVs for their HA-mediated binding and NA-mediated desialylation of a set of synthetic receptor analogs, α2-3 sialylated oligosaccharides with β1-4- or β1-3-linkage in the carbohydrate core and fucosylated or non-fucosylated glucosamine moiety.

Receptor-binding specificity of HA was investigated in a direct binding assay. In brief, 96-well plates were coated with purified virus and biotinylated sialyloligosaccharides were added. Following the addition of conjugate and substrate solutions, optical density at 405 nm was determined. The affinity constants were determined from slopes of Scatchard plots. The substrate specificity of NA was studied in the fluorescent assay. The method based on the a quantitative separation of neutral fluorescent-labeled product from negatively charged fluorescent-labeled uncleaved substrate using anion exchanger microcartridges. Fluorescence was measured at 485/535 nm. Virus NA specificity for each sialoside was calculated as the slope of the linear region of the V_0 vs. S_0 kinetic curve (V_0 - initial rate of the desialylation, S_0 - initial substrate concentration).

Results obtained demonstrated that NA substrate specificity correlated with structural groups of NAs and did not correlate with pathogenic potential of the virus. When substrate specificity was plotted as 3'SLN/SiaLe^x or SiaLe^c/SiaLe^a ratios, all viruses were divided into two groups. For AIVs (both HPAIVs and LPAIVs) with N1, N5 and N8 NAs (structural group I) the ratio was between 1.5 and 2 while for those bearing N2, N7 and N9 NAs (group II) the ratio varied between 3 and 7. In contrast, all HPAIVs differed from LPAIVs by a higher HA receptor-binding affinity towards trisaccharides 3'SLN and SiaLe^c and by their ability to discriminate between non-fucosylated and fucosylated sialyloligosaccharides, 3'SLN and SiaLe^x. All HPAIVs bound to 3'SLN from 3 to 6 times stronger than they bound to SiaLe^x whereas for LPAIVs this ratio was about 1.5.

Our results suggest that alteration of the receptor-binding specificity is required for the emergence of the HPAIV from their low pathogenic precursors.

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The impact of natural taurocholate co-transporting polypeptide (NTCP) polymorphisms on HBV infection

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A hepatocyte-specific receptor for HBV and HDV has been identified as human sodium taurocholate co-transporting polypeptide (hNTCP/SLC10A1). The entry process mediated by hNTCP involves a high affinity binding with a myristoylated N-terminal preS-domain of the HBV L-protein (represented by the peptidic HBV entry inhibitor Myrcludex B). The interacting site of NTCP with Myrcludex B was pinpointed to a region near amino acid 157/158, which is differently located from sodium binding site and bile acid binding site of NTCP. However, the NTCP binding with Myrcludex B and its substrates compete with each other, suggesting a close relation between the receptor function and transporter activity of NTCP.

We investigated naturally occurring single nucleotide polymorphisms (SNPs) of hNTCP with respect to its receptor function and the bile salt transporter activity. HuH7 cells were used to express SNPs of hNTCP leading to amino acid change within sodium binding site, Myrcludex B binding site and transportation determining site. These polymorphic variants of human NTCP were characterized regarding the binding activity with Myrcludex B, taurocholate transportation, and the susceptibility to HBV/HDV infection. One SNP, G157S, does not interfere with transportation but leads to deficient Myrcludex B binding and infection. More strikingly, another SNP (S267F) leads to a drastic abrogation of bile acid transport, as well as Myrcludex B binding and HBV/HDV-receptor activity. Since the S267F SNP is predominantly found in Asian populations (9.2 % Vietnamese and 7.1% Chinese) it raises the question whether homozygous or heterozygous S267F are protected from HBV/HDV infection. To answer that, we titrated S267F with wild-type hNTCP and found that the expression level of S267F correlate in linear with the reduction of taurocholate activity and infection. In summary, this data show the NTCP polymorphsim has impacts on the two functions of NTCP. Homozygous but not heterozygous S267F carrier might protect from HBV infection.