Towards identification of influenza A virus strains with pandemic potential in vitro: species-tropism and inflammatory cytokine responses

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Key words
Avian influenza virus, H5N1, tropism, innate immune system

Aim of the study
The aim of this project was to characterize how influenza viruses with different hosts specificity, pathogenicity and virulence interact with cells of the innate immune system. We hypothesized that differences observed would relate to important virus characteristics, and that such in vitro systems would permit a controlled analysis of viral factors important in determining the thread of particular influenza viruses for animal and human health.

Material and methods
In vitro infection studies of various human, porcine and chicken cell systems were preformed. These included various types of dendritic cells (DC), primary endothelial cells, macrophages as well as epithelial cell lines. The selected viruses included viruses of avian, human and swine origin of the following subtypes H1N1, H3N2, H5N1, H7N1, H7N2, H7N7. In addition, viruses generated by reverse genetics were employed to identify the role play by haemagglutinin (HA). As readout, virus infection detected by nucleoprotein expression, virus replication detected by titration, cell activation detected by cytokine and interferon release were employed.

Results and significance
We have identified two cell systems which provide information not found with epithelilal cell lines classically used for tropism studies. In plasmacytoid DC a difference between avian and mammalian isolates in terms of inducing interferon-alpha was found. Avian viruses independent of their pathogenicity and virulence were sensed more efficiently at low doses but at high doses induced cytopathogenic effects. This was partially mediated by the HA. In endothelial cells we identified a peculiar tropism of virulent H5N1 isolates. The cells were efficiently infected and activated to produce interferon and cytokines but also killed by the viruses. This effect was independent of the HA cleavage site and contrasted with human isolates which were inefficient at infecting endothelial cells. The endothelial cell tropism was completely attributed the H5 HA. These in vitro systems permit the identification of viruses with a particular tropism which appear to relate to virulence. Future studies will now enable to identify the molecular determinants of this tropism.

Publications, posters and presentations
Macchi, M. et al. “Immunobiology and Pathogenesis of Influenza Virus Infections”, 31.5-6.5.2008 Atlanta U.S.A. Differential infection and activation of porcine conventional dendritic cells by mammalian and avian influenza viruses. (Poster presentation at the conference)

Bel, M. et al. “Immunobiology and Pathogenesis of Influenza Virus Infections”, 31.5-6.5.2008 Atlanta U.S.A. Efficient sensing of avian influenza viruses by plasmacytoid dendritic cells: good news or not? (Poster presentation at the conference)

Bel, M. “Plasmacytoid dendritic cells and immune responses” 12.-13. December, 2007; Institut Curie, Paris. (Poster presentation at the Workshop)

Summerfield, A.; McCullough, KC. (2009) Dendritic Cells in Innate and Adaptive Immune Responses against Influenza Virus. Viruses 1, 1022-1034

Ocaña-Macchi, M. et al. Hemagglutinin-dependent tropism of H5N1 avian influenza virus for human endothelial cells. J Virol. 2009;83(24):12947-55.

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