New technologies for the control of influenza

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Summarizing discussion
Chapter 7

The research described in this thesis focuses on various aspects of influenza control. The rapid evolution of influenza strains established in the human population, and incidental introduction of new virus subtypes from animal reservoirs make continuous surveillance a necessity. Originally, embryonated chicken eggs were used for this purpose, but since the 1960s, cell culture, particularly of MDCK cells, has been the preferred method for isolating influenza strains from clinical swabs. This is because cultivation of influenza viruses in eggs can lead to the selection of antigenic variants [1-6], and is sometimes impossible altogether. In recent years however, growth of clinically representative influenza isolates has become increasingly difficult due to changes in their receptor binding properties that have occurred during their continued circulation in the human host [7]. In chapter 2 we have assessed whether overexpression of the human CMP-N-acetylneuraminic beta-galactosamide α-2,6-sialyltranferase (ST6Gal1) in PER.C6 cells results in increased α2,6-sialylation of glycoconjugates on the cell surface and increased replication of, and susceptibility for, recent human influenza viruses. We found that although viral titres were not increased, increased α2,6-sialylation did result in an increased recovery rate of influenza A viruses from limiting dilutions of virus inoculum. Furthermore, virus was isolated from a greater proportion of PCR positive clinical specimens. Particularly the finding that a virus was isolated only in PER.C6-a2,6ST cells indicate that these cells may be an important adjunct in influenza surveillance.

The same reasons that complicate the use of eggs for influenza surveillance; the inability of some human strains to replicate in this substrate, and the introduction of egg adapting mutations, complicate the use of eggs for vaccine production. Furthermore, a production system requiring large numbers of embryonated chicken eggs is inherently inflexible, prone to microbial contamination, and cannot enable surge capacity to be met in the event of a vaccine shortage or a pandemic, particularly if chickens themselves are susceptible to the circulating virus. Large-scale cell culture-derived vaccine manufacturing circumvents all these problems and licensure has been obtained for trivalent seasonal influenza vaccines produced on MDCK cells, and a monovalent prepandemic H5N1 vaccine produced on Vero cells [8-10].

The applicability of cell culture is furthermore enhanced by the development of the reverse genetics technology. However, the requirement of technically suitable and at the same time fully tested and licensed cell banks forms a barrier to using reverse genetics in vaccine production and Vero cells are presumed to be the best available option [10]. In chapter 3, we have shown that PER.C6 cells are highly suitable for the generation of influenza vaccine seed strains by reverse genetics. Due to the fact that they are both easily transfectable and highly susceptible to influenza infection, PER.C6 cells allow for the generation of vaccine seed strains and subsequent production from a single cell bank (figure 1). Moreover, the establishment of reverse genetics on suspension PER.C6 cells makes it possible to do so in a completely animal component free procedure. These two aspects greatly simplify the procedure from both technical and regulatory perspectives.
Although reverse genetics has become the method of choice for the generation of vaccine seed viruses for vaccines against highly pathogenic avian influenza viruses, high-growth reassortants of H3N2 and H1N1 viruses for the trivalent seasonal influenza vaccines are currently still generated using the traditional method of co-infection of eggs. The requirement for an isolate capable of egg growth introduces a bias in the strains that are considered for inclusion in the vaccine. By generating a vaccine seed strain that grows well in both eggs and cell culture with the antigenic properties of a strain that itself did not grow in eggs, we have shown in chapter 4 that such a bias can be avoided by using reverse genetics. However, before the reverse genetics technology can be used for the production of seasonal vaccines, intellectual property issues will need to be addressed. The rapid evolution of influenza viruses does not only complicate prevention of disease by means of vaccination, but also leads to resistance against currently available antivirals for treatment and short-term (post-exposure) prophylaxis. Recently discovered human mAbs with heterosubtypic neutralizing activity hold promise for future mAb-based immunotherapy against influenza. In chapter 5, we have compared the prophylactic and therapeutic efficacies of CR6261, representative of these broadly neutralizing mAbs, with those of neuraminidase inhibitor oseltamivir, the leading antiviral drug. We have shown that a single administration of 15 mg/kg CR6261 outperforms a five day treatment with 10 mg/kg/day of oseltamivir in both prophylaxis and treatment of mice challenged with lethal doses of H1N1 and H5N1 viruses. The ferret is generally considered a good model for influenza in humans as the course of infection and symptoms in influenza infected ferrets resemble those in humans. In chapter 6, we have evaluated the prophylactic and therapeutic efficacy of the CR6261 against lethal challenge with the highly pathogenic avian H5N1 virus in ferrets. Prophylactic administration of 30 and 10 mg/kg CR6261 prior to viral challenge completely prevented mortality, weight loss and reduced the
amount of infectious virus in the lungs by more than 99.9%, abolished shedding of virus in pharyngeal secretions and largely prevented H5N1-induced lung pathology. When administered therapeutically 1 day after challenge, 30 mg/kg CR6261 prevented death in all animals and blunted disease, as evidenced by decreased weight loss and temperature rise, reduced lung viral loads and shedding, and less lung damage. Together, these results justify further (pre-)clinical evaluation of mAb CR6261 for the prevention and treatment of disease caused by influenza.

References

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