A UNIVERSAL VACCINE AGAINST INFLUENZA

by

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"Twenty per cent of the world's population falls ill. One in every hundred of those ill is hospitalized (if enough beds are available). Seven million deaths occur in a few months and 28 million are hospitalized. This is how the next influenza pandemic might look, according to optimistic estimates. Estimates from other models are far more frightening, but even this best–case scenario is cause for considerable concern" (Stöhr and Esveld , WHO, Science, 306, 2195-96, 2004).

Nowadays, the media report almost daily on the avian influenza strain H5N1, mainly because there is the possibility that the virus may acquire the ability for human to human transmission, resulting in a pandemic. We, however, have been concerned with this problem since quite a long time, in fact since the late seventies.

New influenza epidemics arise almost every year, usually coinciding with the winter months. Two out of three epidemics are caused by influenza A, the others by influenza B. As quite often the same virus strain has already been involved in an epidemic outbreak in another part of the world, it is usually possible on the basis of such an early isolate to prepare a conventional vaccine before the epidemic spreads wildly. Occasionally the guess as to the identity of the forthcoming epidemic is wrong, which means that the commercial vaccine does not protect. WHO estimates that on average the world death toll due to influenza amounts to 500,000 to 1 million people per year. But much more worrisome is the occasional occurrence of a pandemic, such as the Spanish Flu in 1917-1918 which caused in the order of 50 million deaths (Johnson & Mueller, 2002). As a pandemic strain is totally novel in terms of antigenic profile, it is hardly possible to anticipate and prepare a specific vaccine in time. Indeed, all conventional vaccines so far are based on growth of viruses, and the subsequent isolation of the antigenic hemagglutinin (HA) and neuraminidase (NA) glycoproteins.

Viruses, and especially RNA-containing viruses, are well known to have an unstable genome. The result is that almost every year new strains arise which evade the immunity resident in the population at large. The viruses achieve this by accumulation of a number of mutations, mainly in the HA, but also in the NA, a phenomenon known as "drift". As a result of this drift, a new strain emerges which is not hindered by the immunity in the population, and therefore has a selective advantage, propagates actively and causes a new epidemic. Occasionally, however, a human-type HA-gene, possibly also an NA-gene, is replaced by a homologous gene derived from the much larger reservoir of avian influenza viruses. This phenomenon, known as "shift", leads to the emergence of a totally new, human-adapted strain, which will now spread over the whole world as no human has specific antibodies to it. This means a new pandemic!

We were the first to clone an HA-gene from a human influenza virus and to report its total sequence (Min Jou *et al.*, 1980), to elucidate on a molecular basis the phenomena of "drift" and "shift" (Verhoeyen *et al.*, 1980 ; Fang *et al.*, 1981), and to report protection against influenza disease by vaccination with a recombinant vaccine. The latter achievement means that one is no longer dependent on the tedious production of viruses as a starting material for vaccine preparation, that one avoids the handling of sometimes dangerous virus strains, and that one is no longer limited by the supply of chicken eggs for vaccine production. Our first recombinant vaccine was directed against the viral HA (Vanlandschoot *et al.*, 1993), and a later vaccine against NA (Deroo *et al.*, 1996).

But a major breakthrough was undoubtedly the development of a "Universal Influenza vaccine", based on the viral M2-protein. The external domain, referred to as M2e, is under normal conditions in the field not, or almost not, immunogenic. Therefore there is no M2e-based immunity in the population, and hence no selection pressure on the virus to escape from prevalent immunity by mutation. The net result is that unlike HA and NA, the human M2e-sequence remains remarkably stable, independent of epidemics or pandemics (Neirynck *et al.*,1999 ; Fiers *et al.*, 2004). M2e is a peptide sequence of only 23 amino acids. The M2-protein is a proton pump, required for virus entry and for virus production by the infected cell. Although few M2-protein molecules are present in the virus, it is fairly abundant on virus-infected cells. As mentioned above, natural M2e is almost not immunogenic. But by fusing one, two or three copies of M2e to each subunit of Hepatitis B virus core, we converted this non-immunogenic peptide into a highly immunogenic one (Neirynck *et al.*,1999 ; De Filette *et al.*, 2005). High titers were achieved in vaccinated mice, which became fully protected from a lethal influenza infection. By using a mucosal adjuvant, we could also obtain similar high titers in the systemic circulation following intranasal administration (De Filette *et al.*, 2006). We know that the protection is mediated by antibodies, and the evidence so far obtained indicates that the mechanism involved antibody dependent NK-cell mediated killing of virus-infected cells.

Considering that the target of the vaccine-induced antibodies is M2e on infected cells, and that M2e has a conserved sequence in all human virus strains so far isolated, we can conclude that this vaccine provides "universal" protection against human influenza disease. On top of this "universality", it may be noted that the M2e-HBc vaccine is very efficiently expressed in E. coli cells, and hence can be economically produced on a mass scale.

Last year, the VIB licensed the "universal vaccine" to Acambis PLC, a British-American vaccine company. In collaboration, experiments in ferrets are now ongoing, and Phase I clinical trials may start at the end of 2006 or Q1 2007. Also preclinical research with H5N1 variants of the vaccine are in progress.

Conclusions

- **Vaccination with M2e-VLPs (Virus Like Particles) protects completely against a lethal influenza virus challenge, also when using different virus subtypes and strains**
- **Immunogenicity of M2e-VLPs has been enhanced, and anti-carrier (HBc) responses reduced, via the fusion of tandem repeats of M2e**
- **Intranasal (mucosal) immunization with CTA1-DD adjuvanted M2e-VLPs protects against a lethal influenza challenge and reduces morbidity**
- **Protection is mediated by antibodies and involves Antibody dependent NK-cell killing of virus-infected cells**
- **Serum from M2e-HBc vaccinated mice reacts with a broad panel of M2e peptides**

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