

## **A pandemic vaccine is available...in the library!**

**The following is a summary of a presentation by Dr Ulf Schröder at the WHO meeting on pandemic influenza preparedness on May 4-5, 2006.**

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A number of studies, both in humans and animals, have demonstrated that nasal vaccination gives rise to cross-protection against drifted strains. As can be seen in Table 1, it was reported from human studies as early as in 1970 that nasal immunization with formalin-killed whole virus gave rise to cross-protection exceeding that achieved after parenteral immunization<sup>1</sup>. The authors concluded “ ..it could make vaccine development against the multitudes of respiratory viruses much more feasible”. A few years later (1977), Shvartsman et al.<sup>2</sup>, described that secretory antibodies had a wider spectrum of activity than circulating antibodies.

When a pandemic vaccine is considered, results demonstrating that nasal immunization of mice with H3N2, provided 100 % protection to lethal challenge with H5N1 should be of particular interest<sup>3,4</sup>. Using live attenuated influenza vaccine (FluMist) in humans, Belsche et al. showed that good protection was achieved even though the epidemic influenza strain was not contained in the vaccine<sup>5</sup>. In a number of studies in animal models, similar data have been obtained also with other agents. Cross-clade immunity against HIV was described after nasal DNA-prime followed by nasal peptide boost<sup>6,7</sup>. The vaccine contained epitopes of clade B, and high, long-lasting serum IgG and IgA titers against the neutralizing gp41 ELDKWAS epitopes from clades A, B, C and D were observed.

Today, it is time that the scientific society and the public health authorities would allow people to benefit from these findings. However, in order to obtain good cross protection, either formalin killed whole virus particles, split or subunit vaccines with powerful adjuvants (e.g. cholera toxin, CT) or live vaccines have to be used. For inactivated whole virus it was demonstrated that only formalin-killed virus, and not the ether-killed counterpart, was sufficiently immunogenic for nasal vaccination<sup>4</sup>. Other successful experiments were based on live attenuated virus<sup>8</sup>. A nasal vaccine comprising a split/subunit antigen without a powerful adjuvant does not seem to be sufficiently immunogenic.

The mucosal adjuvant most frequently used in studies of nasal influenza vaccination has been the cholera toxin (CT), or its "relative", heat labile toxin (LT) originating from *Escherichia coli*. Unfortunately, when LT was used as a mucosal adjuvant in a human nasal influenza vaccine, the vaccine had to be withdrawn from the market due to reports of a possible immunization-associated Bell's Palsy among the vaccinees<sup>9</sup>. In mice CT has been shown to induce retrograde transport of antigen into the brain, as well as morphological changes of the olfactory nerve<sup>10</sup>, indicating that the toxic adjuvant rather than the antigen contributed to the palsies in humans.

### **Correlation between animal tests and human**

It has been suggested that animal data from mucosal immunisation in general, and from nasal immunogenicity studies in particular, correlate well with results in humans and considerably better than results obtained from studies on parenterally administered vaccines. Thus initial results from nasal vaccination of mice often predict success in humans<sup>11</sup>. A new, and most likely successful approach to combating pandemic influenza vaccines is therefore a rapid development of nasal vaccine formulations.

### **What knowledge and understanding of the mucosal immune-system could explain these results?**

Antibodies produced by the systemic immune system are predominantly of the IgG class, while antibodies in external secretions are primarily constituted by secretory IgA (SIgA) derived from local plasma cells (PCs) via receptor-mediated IgA export through mucosal epithelia<sup>12</sup>. PCs of the systemic immune system originate mainly from B cells generated by germinal-centre (GC) reactions in peripheral lymph nodes. Mucosal B cells, on the other

hand, are stimulated primarily in lymphoid structures with direct access of antigens and mitogens from epithelial surfaces. Such mucosa-associated lymphoid tissue (MALT) includes the tonsils and other nasopharynx-associated lymphoid tissue (NALT), as well as intestinal Peyer's patches<sup>13</sup>. The latter are usually referred to as gut-associated lymphoid tissue (GALT), which also includes numerous isolated lymphoid follicles and the appendix<sup>13</sup>.

In humans, MALT shows GCs shortly after birth as a sign of immune activation<sup>13</sup>. The GC reaction is of vital importance for T cell-dependent generation of memory B cells, affinity maturation of the B-cell receptor (BCR), and Ig-isotype switching<sup>14</sup>. It has been shown that naïve B cells are first stimulated in the T-cell zone just outside the primary lymphoid follicle by cognate interactions with activated CD4<sup>+</sup> T cells<sup>15</sup>. The initially stimulated B cells produce un-mutated IgM (and some IgG) antibody which binds antigen with low affinity, and the resulting immune complexes are deposited on follicular dendritic cells (FDCs) in the GCs. There antigen is retained to induce prolonged B-cell memory<sup>16,17</sup>. Among the cell surface molecules, the complement receptors CR1/CR2 (CD35/CD21) are considered to play a crucial role in the GC reaction. CD21 is expressed abundantly on both FDCs and B cells, and may function by localizing antigen to the FDC network.

In addition to complement, also other components of the innate immune system are involved in MALT GC formation, as reportedly shown in experimental animals. Normally, the GC reaction is driven by competition for a limited amount of antigen, but two recent publications suggested that if there is also a BCR-independent drive, B cells may survive with a restricted repertoire and rather low affinity. The first study showed – in the germ-free appendix model in rabbits – that selected commensal bacteria are efficient in promoting GALT development, and that this ability depends on certain stress responses in the same bacteria, suggesting a non-specific (superantigen?) impact on the B cells<sup>18</sup>. Another study showed independency of BCR engagement for GALT development in mice where the Epstein-Barr virus protein LMP2A was transgenically introduced as a constitutive BCR surrogate providing a weak signalling pathway<sup>19</sup>. The authors concluded that commensal bacteria can promote the MALT GC reaction independently of BCR-mediated antigen recognition by interacting with innate immune receptors. These reports constitute useful information to explain the enormous IgA drive provided by the commensal microbiota in the absence of high-affinity BCR development, thereby leading to the production of large amounts of IgA with a restricted

repertoire and a capacity to bind with low affinity to redundant epitopes of commensal bacteria<sup>20</sup>.

Mouse experiments have likewise demonstrated the importance of commensal bacteria for the development of NALT<sup>21-23</sup>, and the cervical lymph nodes are clearly connected to these regional immune-inductive MALT structures in the same manner as the mesenteric lymph nodes are associated with GALT.<sup>13</sup> Recent characterization of homing molecules on human NALT-derived B cells has indeed documented an integration of the regional mucosal immune system, and the systemic immune system<sup>24</sup>, which offers an explanation for the results obtained by vaccination via the nasal route. For adequate protection against most pathogens, immunization should induce both mucosal and systemic immunity.

As GCs are principally designed to generate B-effector cells for the production of antibodies against T cell-dependent antigens, MALT structures can mount adaptive pathogen-directed responses of high affinity and specificity over time. On the other hand, the GC reaction induced by the constant background challenge provided by the commensal flora may to some extent rely on BCR-independent polyclonal stimuli via innate mechanisms<sup>19,20</sup>, and thereby continuously generate a response of a restricted IgA repertoire a broad reactivity. Such a dual model for antimicrobial SIgA responses may be adequate for the host's coexistence with the indigenous microbiota, as well as providing immediate protection against pathogens by cross-reactivity before the high-affinity response is elicited.

Considerable levels of polyreactive SIgA antibodies directed against self as well as against microbial antigens also occur in human external secretions<sup>25</sup>. As in mice, the reason for this may be microbial polyclonal activation of MALT independently of BCR-mediated antigen recognition. This is an important distinction between systemic and mucosal immunity, which can be exploited by targeting NALT with adjuvanted nasal vaccines, which at the same time will also stimulate the systemic immune system<sup>26</sup>.

### **Where to go from here?**

The current knowledge concerning the mucosal immune system and cross-protection after nasal vaccination, in combination with the imminent threat of an H5 pandemic, should constitute incitements to start an immediate development of a nasally delivered pandemic

influenza vaccine. Such a vaccine could also be used inter-pandemically if, in addition to an H5 strain, it would also contain “average” strains circulating during the last decade. The vaccine could consist of formalin-killed whole virus alone, whole-virus or subunits combined with an acceptable mucosal adjuvant, or live attenuated viruses. Provided that the results published until now are valid, such a vaccine should for instance prevent initial spread of a pandemic strain outside the area of origin. In the best possible scenario, the vaccine could totally prevent influenza infection. Even if not fully protective, such a vaccine would at least lessen the severity of H5 infections in humans and thereby reduce mortality.

A “crash-vaccine” like the one suggested above may not be the final solution to the pandemic influenza vaccine once the strain is known. However, in our opinion it would be better to achieve a relatively good protection than none at all when the next pandemic is already a fact. Several governments have recently set aside a substantial financing for the production of a parenteral, pandemic vaccine despite existing knowledge that if the strains does not match, the vaccine will have only very limited protection.

In addition to the pandemic use, the highly relevant issue of the yearly “flu shot” should be considered. Provided that the data listed in Table 1 are all relevant to humans, the influenza vaccine manufacturers may totally change their production if a nasal vaccine is introduced. There would be no need to make a “new” vaccine every year and the influenza vaccine could be produced in the same manner as all other vaccines, which would certainly benefit the vaccine industry. A direct result of such a change could be that the constant shortage of influenza vaccine would be history. Another important issue is that it has been suggested that there is a need for at least two immunizations of a pandemic vaccine because there is no pre-existing immunity in the individual. Switching to annual nasal vaccination with a vaccine containing the strains as suggested above for the pandemic vaccine, would solve also this problem.

To achieve these goals, the industry and governmental bodies will have to change gears and direction, leaving the path of well-established parenteral immunization and focus their efforts on the development of an efficient nasal vaccine for human use. If this approach is agreed upon, the present worry concerning a pandemic vaccine would most likely be obsolete.

**Table 1.****Published studies on cross-protection after intranasal vaccine immunisation.**

<b>Vaccine strain</b>	<b>Adjuvant or formulation</b>	<b>*Protection **Crossreactivity</b>	<b>Species</b>	<b>Efficacy</b>	<b>Year</b>
A2/Taiwan/1/64	Formalin-killed virus	**A2/HongKong/8/68 or A/PR8 A <sub>0</sub>	Human	Cross nasally, none in serum	1970 <sup>1</sup>
A/HongKong/1/68 (H3N2)	Formalin-killed virus	**A/Singapore/1/57 (H2N2)	Human	Broader cross nasally compared to serum	1977 <sup>2</sup>
H3N2/Fukuoka or H3N2/Sichuan	CTB <sup>a</sup>	*H3N2/Guizhou	Mice	P<0,001	1992 <sup>27</sup>
H3N2/Sichuan	CTB <sup>a</sup>	*H3N2/Guizhou	Mice	P<0,001	1992 <sup>28</sup>
H1N1/Kumamoto/79	CTB/CT	*H1N1/Yamagata/86	Mice	P<0,001	1994 <sup>29</sup>
Prime: H1N1/Kumamoto Boost: H1N1/Bangkok	CTB/CT	*H1N1/Yamagata	Mice	P<0,001	1995 <sup>30</sup>
H1N1/Shenzhen and H3N2/Wuhan	Live - attenuated	*H3N2/Sydney	Human	See comments	2000 <sup>5</sup>
H3N2	Formalin-killed virus and LT	*H5N1	Mice	100% (lethal challenge)	2001 <sup>3</sup>
H1N1 A/Yamagata or H1N1 A/Beijing	CTB/CT	*A/PR8	Mice	100%	2002 <sup>31</sup>
H1N1 + H3N2	Live virus	*H1N2	Swine	"robust protection"	2003 <sup>8</sup>
H1N1(PR8)/H3N2	Formalin-killed virus	*H5N1	Mice	100%	2003 <sup>4</sup>
HIV clade B, DNA/prime + peptide/boost	N3 and L3	**Clade A, C and D	Mice	See comments	2004 <sup>6,7</sup> 2005 <sup>7</sup>

a) the CTB in these studies most likely contains small amounts of CT.

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