**Enhanced immunogenicity of novel influenza vaccines using ImmunoBody™ technology**

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**Introduction**

Influenza is a respiratory tract infection causing a wide range of illnesses in humans and is a major cause of hospitalisation and death in the elderly. Current methods of vaccine development are generally effective at preventing illness in children and adults though less effective in the elderly. Current vaccines attempt to induce strong antibody responses against the haemagglutinin (HA) and to a lesser extent the neuraminidase as the antibody responses against these antigens correlate with protection against influenza either by neutralising the virus or blocking viral entry into cells. Antibody mediated protection is effective against homologous influenza strains but relatively ineffective against heterologous strains. As influenza A strains can mutate their coat proteins rapidly, the failure to anticipate a major antigenic drift/shift or the emergence of a pandemic strain would lead to greatly reduced protective efficacy of the vaccine. T cells can mediate heterologous protection by targeting other viral proteins as the structure of these antigens is conserved within the type of influenza and cytokine T lymphocytes (CTL) have a role in clearing influenza virus from the lungs. Current subset vaccines are effective at inducing neutralising antibody responses but less effective at inducing cellular immunity. Antigen/antibody complexes allow the efficient internalisation of the complex via the Fc receptor. When internalised through the FcγRIIa the antigen can be processed and presented in both the MHC class I and class II pathways allowing a broader immune response to the antigen. Using the approach of HA fused to an Fc tail (HA-Fc) which promotes binding to Fc receptors on the cell surface, ImmunoBiology Ltd has developed an approach that potentially enables HA to enter both the MHC class I and class II pathways resulting in broader humoral and cellular immune responses. Here we show that using HA-Fc in vitro to restimulate human PBMC, we are able to generate antigen-specific IFNγ and antibody cells to the Fc & these cells are also able to recognize heterologous HA proteins. Using a murine in vivo immunogenicity model, we have found that HA-Fc generates a better anti-HA IgG & IFNγ responses than those with a commercially available influenza vaccine containing the same HA. These data suggest that an influenza vaccine (Flubiovax), based on HA-Fc will generate an effective immune response as seen by the targeting of antigen-presenting cells via the Fc receptor & this immune response has the potential to protect against subsequent infection with influenza.

**Methods & Results**

**Recognition of heterologous influenza strains**

Briefly, human PBMC were prepared from 3 buffy coats supplied from the NHS Blood & Transplant service. PBMC were then stimulated for 9 days with either Flubiovax (HA-Fc) or no antigen as a negative control in RPMI-1640 + 10% FBS. On days 3 & 7, the cells were cultured in fresh medium without antigen or IL-2 & rested overnight. ELISPOT plates were prepared & the ELISPOT performed according to the manufacturers protocols. The number of IFNγ producing cells was detected using ELISPOT using a kit from R&D Systems.

PBMC from 3 donors stimulated with HA-Fc in vitro & restimulated with the same antigen had IFNγ-producing cells above background levels. A similar result was obtained when the PBMC were restimulated with A5/Myery (H5N1). When stimulated with either A/Verona/1904/H1N1 (H1N1), A/Solomon Islands/306/2006 (H1N1) (all NSBIC) or A/H3N2-Panama-FluBiovax™ & performed in duplicate. Following the development of the assay the plates were read on a BIOSYS Bioreader 5000.

PBMC from young, unvaccinated & vaccinated donors responded to stimulation with both H1N2. The vaccine & HA-Fc with higher IFNγ responses at all antigen concentrations used being seen in those donors that had previously been vaccinated within the last 5 years. In the elderly donors, PBMC from vaccinated donors responded to stimulation with the H1N2 vaccine whereas there were only low IFNγ responses in the unvaccinated donors to stimulation with this vaccine in vitro. Good IFNγ responses were seen in the elderly vaccinated donors in response to the HA-Fc vaccine & in contrast to the H1N2 vaccine, good IFNγ responses were seen in the elderly unvaccinated donors suggesting that HA-Fc influenza vaccine may be able to stimulate IFNγ responses in a high risk population that is poorly served by current influenza vaccine strategies.

**Immune responses in the elderly**

PBMC were prepared from samples taken from 6 healthy elderly donors. The donors were divided into young (age 22-33) or elderly (age 60-81) & further subdivided into vaccinated (within the last 5 years) or unvaccinated (no history of vaccination or disease within the last 5 years). The recruitment & mode of blood taking were approved by the Innsbruck ethics committee.

PBMC were cultured in 96-well Multiscreen filtration plates (Millipore) at 1 x 10^5 cells/well. Cells were cultured for 24 hours in the presence of the following inactivated antigenic antigens, A/Flubiovax, A/Verona/1904/H1N1 (H1N1), A/Solomon Islands/306/2006 (H1N1) (all NSBIC) or A/H3N2-Panama FluBiovax™ & performed in duplicate. Following the development of the assay the plates were read on a Zeiss KS ELISPOT imaging system.

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**Conclusions**

Following infection, influenza is cleared from the lungs by CTL that are recruited to the lungs by a Th1 response that is specifically due to the production of IFNγ (1.2). It has been shown that a shift from Th1 to Th2 cytokines occurs with ageing & is associated with reduced CTL activity & protection against infection with influenza (3-6). The data presented here show the potential of the ImmunoBody™ technology to be used for an effective vaccine against influenza particularly in the elderly.

- Flubiovax (HA-Fc) is able to generate IFNγ secreting cells against influenza HA
- HA-Fc is more effective at generating IFNγ responses in elderly unvaccinated donors than a commercially available monovalent vaccine
- HA-Fc is as effective at generating IFNγ responses in young unvaccinated donors as a commercially available monovalent vaccine
- The immune response generated by HA-Fc also recognises the HA from heterologous strains of influenza
- HA-Fc generates a stronger IgG1 & IgG2a antibody response in mice than a commercially available monovalent vaccine

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**ImmunoBody™ technology**

**Vaccine Characteristics**

- Adjuvant pathways where key target antigens (s) identified
- Rapid vaccine development when new antigens identified as important in protection or influenza or heterosubtypic
- Potential to incorporate multiple antigens
- Exact and predictable prophylaxis
- Modulating development risk, time & cost
- Multiple antigen targeting for vaccine prime and/or booster, expansion system providing fast, high factor production & low cost of goods

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**ImmunoBody™ is a registered trademark of ImmunoBody Ltd**

References: