Identification of H1N1 influenza viral proteins and peptide pools that stimulate interferon-gamma secretion in peripheral blood mononuclear cells and lung mononuclear cells from post infected ferrets

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Identification of H1N1 influenza viral proteins and peptide pools that stimulate interferon-gamma secretion in peripheral blood mononuclear Cells and lung mononuclear cells from post infected ferrets.

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Aims
To identify the commonly immunogenic H1N1 A/California/04/09 virus proteins and peptides that are capable of stimulating an immune response, assessed by interferon gamma (IFN-γ) ELISpot for uninfected, H1N1 and H3N2 ferret groups.

Introduction

- One billion people became infected with influenza A virus (IAV) every year with 3-5 million cases showing severe disease and approximately 500, 000 deaths.

- Seasonal IAV vaccines are designed on mutations in either the haemagglutinin or neuraminidase viral surface proteins.

- IAV vaccines are based on strain prediction that is recommended by the WHO using epidemiology data from the winter season in the opposite hemisphere.

- There is high interest in developing a universal vaccine based on the capability of the conserved IAV proteins to provide immunogenic protection to influenza A infection.

- The ferret is the ‘gold standard’ small animal model to study IAV infection and is the model of choice to assess seasonal vaccine efficacy and safety testing as they display similar clinical symptoms and transmission traits to humans.

- The significance of T-cell responses in the role of both protection from natural infection and vaccination is a relatively new field of study within the influenza community as the importance of T-cells was only identified during the 2009 H1N1 pandemic.

- IFN-γ secretion can be measured as a biomarker for a T-cell mediated immune response.

- Ferret peripheral blood mononuclear cells (PBMCs) and lung mononuclear cells (Lung MNCs) were resuscitated and stimlated using different influenza proteins using an influenza A (H1N1) peptide array. Analysis of the magnitude and frequency of the immune response was performed for IFN-γ ELISpot.

- To develop new immunological tests and identify new biomarkers of IAV disease and assess their role in IAV infection.

- Identification of H1N1 specific IFN-γ secretion was higher in lung MNCs compared to the PBMCs. For lung MNCs, the conserved influenza A proteins NP, M1 and NS1 had both common and highest response for both low dose H1N1 and H3N2 groups.

- The data reported from this research is new and expanded information for the ferret model of IAV infection and indicates that the site of infection and disease may play a key role in immunity, which is also a novel area for exploration.

- Lung MNCs gave the highest and most frequent IFN-γ secretory response compared to PBMCs for both H1N1 and H3N2 groups, suggesting that Lung MNCs are important when studying IAV infection. Similar evidence has also been reported in ferrets and humans.

- Common IFN-γ responses were detected between H1N1 and H3N2 test groups. The mega pools of nucleoprotein, matrix protein 1 and non-structural protein 1. Refined IAV specific cellular immune responses to specific peptide sub-pools from each protein was observed. This confirms that conserved proteins of IAV can initiate a heterosubtypic cellular immune response between different IAV subtypes.

- The use of the ferret model for studying H1N1 specific IFN-γ secretion for using peptide arrays for each IAV protein allowed the opportunity to understand which protein were significant in cellular immunity.

- The data reported supports and strengthens the use of the ferret as the ‘gold standard’ small animal for study IAV disease.

Results

- An Influenza A H1N1 peptide array was obtained from the BEI resources repository, which consists of the ten major virus proteins from sub-type A/California/04/09: HA, NA, NP, M1, M2, NS-1, N2, NS2, PB-1, PB-2, and PA.  Peptides (15 mers overlap) were made into sub-pools consisting of 11-15 peptides (150µg/mL per peptide).

- A mega pool containing all the peptides from each influenza protein was also made and tested (Agpm, per peptide).

- Three main test groups were studied: Naive, Low dose H1N1 infected and Low dose H3N2 infected ferrets.

- All data were derived from cryopreserved cells as a means of refinement and reduction in line with the three RRR's for animal welfare.

- Cryopreserved ferret PBMCs and Lung MNCs were resuscitated and counted using a Nucleocounter-200 to obtain an accurate cell count and to check % cell viability for ELISpot.

- Peptide pools were used to stimulate PBMCs or Lung MNCs (30° C, 5% CO2, overnight) on pre-coated IFN-γ ELISpot plates using a ferret IFN-γ ELISpot kit (Mabtech, L2312-4APW-10).

- Plates were scanned, counted, and QC checked using a CTL scanner and Immunospot® 5.1.14. ELISpot results were calculated in Microsoft Excel to subtract background and to express the results as spot forming units per million cells (SFU x 10). Test group data were analysed for IFN-γ secretion using GraphPad Prism® version 7.3.

- An empirical cut-off was defined by setting response limits using the mean results from the naïve group plus 2x standard deviations for each peptide pool tested.

- Cut-off values were subtracted from the low dose H1N1 and H3N2 ELISpot data.

Discussion

- To study peptide sequences and map epitope in greater depth by studying cell-mediated immunity and characterization of the adaptive immune response to the antigens identified from this research.

- To design, characterize and develop MVA constructs of the most immunogenic proteins NP, M1 and NS-1.

- To deliver MVA constructs expressing immunogenic IAV proteins to study cellular immunity in the lower respiratory tract (the lung) using the ferret model.

- To develop new immunological tests and identify new biomarkers of IAV disease and assess their role in IAV infection.

References


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