1918 pandemic H1N1 DNA vaccine protects ferrets against 2007 H1N1 virus infection

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Influenza vaccines with the ability to induce immune responses cross-reacting with drifted virus variants would be of great advantage for vaccine development against seasonal and emerging new strains. We demonstrate that gene gun administered DNA vaccine encoding HA and NA and/or NP and M proteins of the H1N1 pandemic virus from 1918 induce protection in ferrets against infection with a H1N1 (A/New Caledonia/20/99(H1N1)) virus which was included in the conventional vaccine for the 2006-2007 season. The viruses are separated by a time interval of 89 years and differ by 21.2% in the HA1 protein. These results suggest not only a unique ability of the DNA vaccines, but perhaps also natural infection, to induce cross-protective responses against even extremely drifted virus variants.

**INTRODUCTION**

DNA vaccines induce an immune response which is comparable to the response acquired by natural virus infection by activating both humoral and cell-mediated immunity. We wanted to assess the induced immune response and level of cross-protection by ferrets vaccinated with a H1N1 DNA vaccine based on the pandemic "Spanish flu" virus from 1918 and challenged with a contemporary H1N1 virus A/New Caledonia/20/99 (NC)

**RESULTS**

- Clinical symptoms
  - Unvaccinated ferrets had a higher rise in body temperature (the day of maximum temperature rise) after infection with A/New Caledonia/20/99(H1N1) than did the HA/NA 1918 DNA vaccinated ferrets (P=0.2).
  - No difference in body weight at day four (the day for maximal body weight loss) between the two groups was observed.

- In the 1918 DNA vaccinated group had a significant (P=0.05) reduction in virus titre from day four to day five (Figure 1).

- The H/N NC DNA vaccine was the most effective vaccine in preventing infection and virus clearance.

- Only three of five ferrets in the 1918 DNA vaccinated group had detectable virus load at day seven compared to all animals in the naive group.

- Notably, also the ferrets vaccinated with NP and M DNA had a better virus clearance than the control groups.

- Reduction in IFN-γ production
  - IFN-γ positive lymphocytes were estimated by flow cytometry as a measure of virus infection.
  - A higher percentage of the total lymphocytes produce IFN-γ at day seven after infection with A/New Caledonia/20/99(H1N1) in the negative control group and the conventional vaccine group compared to the DNA vaccinated groups, indicating an ongoing or recent infection.
  - There were significantly less IFN-γ produced day seven in the H/N 1918 H1N1 DNA vaccine group compared to the negative control group (P=0.05).

**CONCLUSIONS**

- HA-NA DNA vaccine based on the pandemic 1918 H1N1 virus induce specific IgG antibodies against A/New Caledonia/20/99(H1N1) (ELISA), comparable to titers induced by the conventional vaccine. However, only H+N NC DNA vaccine induced HA inhibitory antibodies (HI assay).

- The HA-NA H1N1 1918 DNA vaccinated ferrets cleared the virus infection better than the conventional vaccine.

- Ferrets, vaccinated with DNA vaccine, which cleared their infection early, also had a low percentage of IFN-γ positive lymphocytes upon unspecified antigen stimulation. Animals in the conventional vaccine group and negative control group, with an ongoing infection, still had active IFN-γ positive inflammatory cells at day seven, as a sign of infection.

DNA vaccines might be better candidates for influenza prophylaxis than annual conventional protein based vaccines which frequently need to be updated to match the circulating influenza virus. DNA vaccination induces broader cross-reactivity against drifted strains and longer memory responses.

**METHODS**

- Construction of the DNA vaccine
  - A/New Caledonia/20/99 (H1N1) genes were designed from nucleotide sequences of A/South Carolina/1/18 and A/Ardenburg/67 (H1N1) published in GenBank. A/New Caledonia/20/99 virus was sequenced in-house. The genes were codon optimised and made synthetically by GeneArt.

- Immunisations
  - A total of 23 ferrets (Maelera Patinsa Parc), approximately seven months old, were divided in five animals per group by a chip-top for care (Z:het, post:oil, Radek, Denmark).

- Five groups of five animals were vaccinated as follows: (1) HA and NA, 1918 H1N1 DNA vaccinated, (2) HA and NA, A/New Caledonia/20/99(H1N1) DNA vaccinated, (3) NP and M 1918 H1N1 DNA vaccinated, (4) conventional trivalent protein vaccine 2006-07 (incl. A/New Caledonia/20/99(H1N1)), Sanofi Pasteur (60 μg), (5) empty plasmid vaccinated (negative vaccine control).

- The ferrets were gene gun (Biolon, Bio-Rad, Hercules, CA) inoculated (400 μl compressed helium on shaved abdominal skin, using 2 μg plasmid DNA-coated gold particles (4.0 μm sized particles), 40-80% coating efficiency each shot. Each ferret received two shots, three times in two weeks apart. Ferrets were challenged ten days after third immunisation by 5x10^5 50% tissue culture infective dose (TCID50) of A/New Caledonia/20/99(H1N1) administrated into the nostrils as a spray.

- Quantitative real-time RT-PCR assay for influenza A.

- Serum of each ferret was sampled 1 week PFS and the screenings were frozen down immediately for real-time RT-PCR analysis.

**Induction of HA inhibiting antibodies after DNA vaccination**

- Ferrets vaccinated with the H/N NC H1N1 DNA vaccine had significant HI titre against the A/New Caledonia/20/99(H1N1) virus after DNA vaccination at the day of challenge.

- The H/N NC DNA vaccine gave a better recall response of inhibitory antibodies than the conventional trivalent protein vaccine.

- At day five after infection 60% of the H/N NC H1N1 DNA vaccinated ferrets had seroconverted (HI<40), compared to 40% of the ferrets in the conventional vaccine group. Also a >2.5 fold increase in HI GMT was accomplished after vaccination measured the day of challenge.