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

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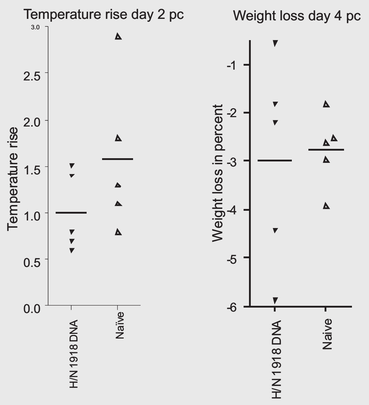
DNA VACCINE FERRETS

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 administrated 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

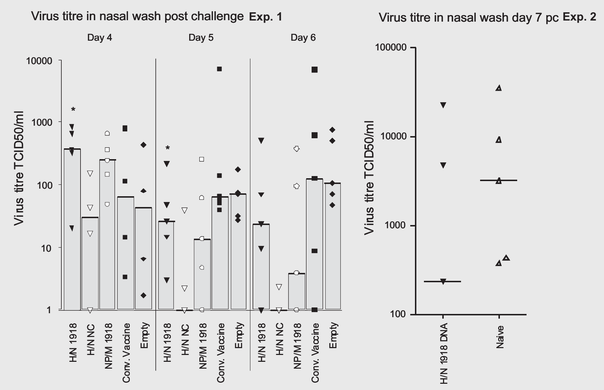
We wanted to assess the induced immune response and level of cross-protection by ferrets vaccinated with a H1N1 DNA vaccine based on the pandemic "Spanih flu" virus from 1918 and challenged with a contemporary H1N1 virus A/New Caledonia/20/99 (NC)



RESULTS

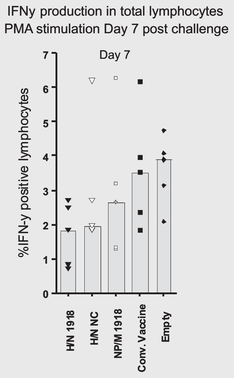
Clinical symptoms

- Unvaccinated ferrets had a higher rise in body temperature at day two (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



Reduction in virus load

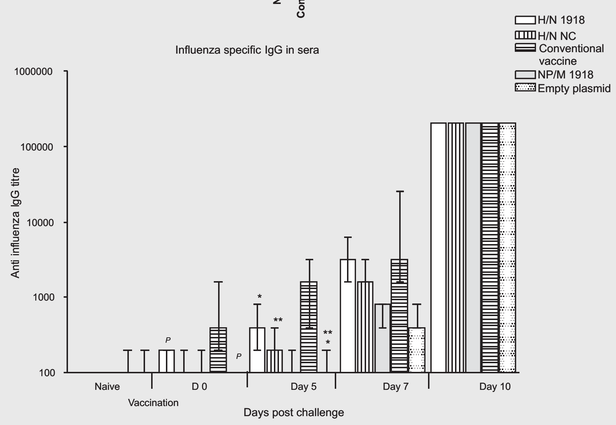
- DNA vaccinated groups had a reduction in virus titre during infection not seen in the conventional vaccine or the empty plasmid group.
- The H/N 1918 DNA vaccinated group had a significant (P<0.05) reduction in virus titre from day four to day five (Figure 1).
- The H/N NC DNA vaccine was the most 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 naïve group.
- Notably, also the ferrets vaccinated with NP and M DNA had a better virus clearance than the control groups.



Reduction in IFNy-production

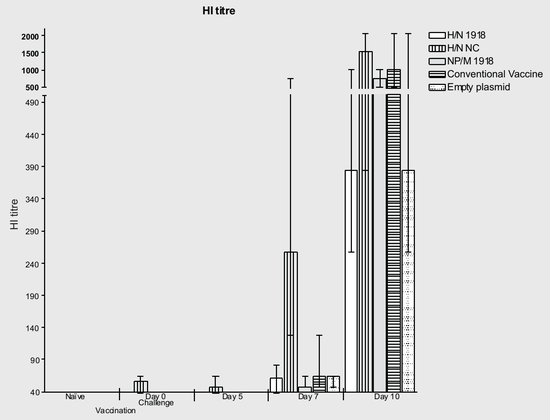
IFN-y positive lymphocytes were estimated by flow cytometry as a measure of virus infection.

- A higher percentage of the total lymphocytes produce IFN-y 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 are significantly less IFN-y produced day seven in the H/N 1918 H1N1 DNA vaccine group compared to the negative control group (P<0.05)



Influenza specific antibody response after DNA vaccination

- A significant higher influenza A IgG titer (P<0.05) compared to the negative control group was observed at the day of challenge for the H/N 1918 DNA vaccinated ferrets, indicating induction of influenza specific antibodies after DNA vaccination.
- At day five post infection, both H/N DNA vaccinated animals and conventional vaccine vaccinated animals showed vaccine induced influenza specific antibodies.
- The H/N 1918 DNA and the conventional vaccine vaccinated animals had comparable recall antibody titre at day seven post infection



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 MGT was accomplished after vaccination measured the day of challenge.

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-y positive lymphocytes upon unspecific antigen stimulation. Animals in the conventional vaccine group and negative control group, with an ongoing infection, still had active IFN-y 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

The 1918 pandemic H1N1 genes were designed from nucleotide sequences of A/South Carolina/1/18 and A/Brevig Mission/1/18 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 25 ferrets (Mustela Putorius Furo), approximately seven months old, were divided in five animals per group by a chip-tag for cats (E-vet, pet-id, Haderslev, 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)(Influvac, Solvay Pharmaceuticals), (5) empty plasmid vaccinated (negative vaccine control).

The ferrets were gene gun (Helios, Bio-Rad, Hercules, CA) inoculated (400 psi compressed helium) on shaved abdominal skin, using 2 µg plasmid DNA-coated gold particles (1.6 µm-sized particles), 80-95% coating efficiency each shot. Each ferret received two shots, three times, two weeks apart. Ferrets were challenged ten days after third immunisation by 5.5x10E5 50% tissue culture infectious dose (TCID50) of A/New Caledonia/20/99(H1N1) administrated into the nostrils with a syringe.

Quantitative real time RT-PCR assay for influenza A.

Nostrils of each ferret were flushed with 1 ml PBS and the flushings were frozen down immediately for real-time RT-PCR analysis.

Serum antibody determined by ELISA

ELISA plates (96 wells) were coated with 100 µl, split influenza vaccine 2006/07 (Including A/New Caledonia/20/99(H1N1))(Vaxigrip, Sanofi Pasteur, Belgium) diluted 1:100 in 35 mM $NaHCO_3$ pH 9.6 and 15 mM Na_2CO_3 over night at 4°C.

Intracellular staining for FACS analyses of gamma-interferon levels

Blood was drawn in heparinised tubes. The Peripheral Blood Leucocytes were stimulated with phorbol-12-myristate-13-acetate (PMA, Sigma) to a final concentration of 20 ng/ml together for 4 hours with Brefeldin A (Sigma; St. Louis, USA) to a final concentration of 10 µg/ml culture and Ionomycin (Sigma) to a final concentration of 1 µg/ml. The primary antibody was 500 ng of a cross-reactive mouse monoclonal antibody to bovine gamma-interferon (Serotec). Incubation was one hour in 4°C. 0.5 µl FITC-conjugated rabbit anti-mouse was applied as secondary antibody (Dako)and the cells were incubated again for one hour at 4°C.

Haemagglutination inhibition (HI)

Performed after WHO recommendations with A/New Caledonia/20/99(H1N1) virus standardised to 8 HAU.

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