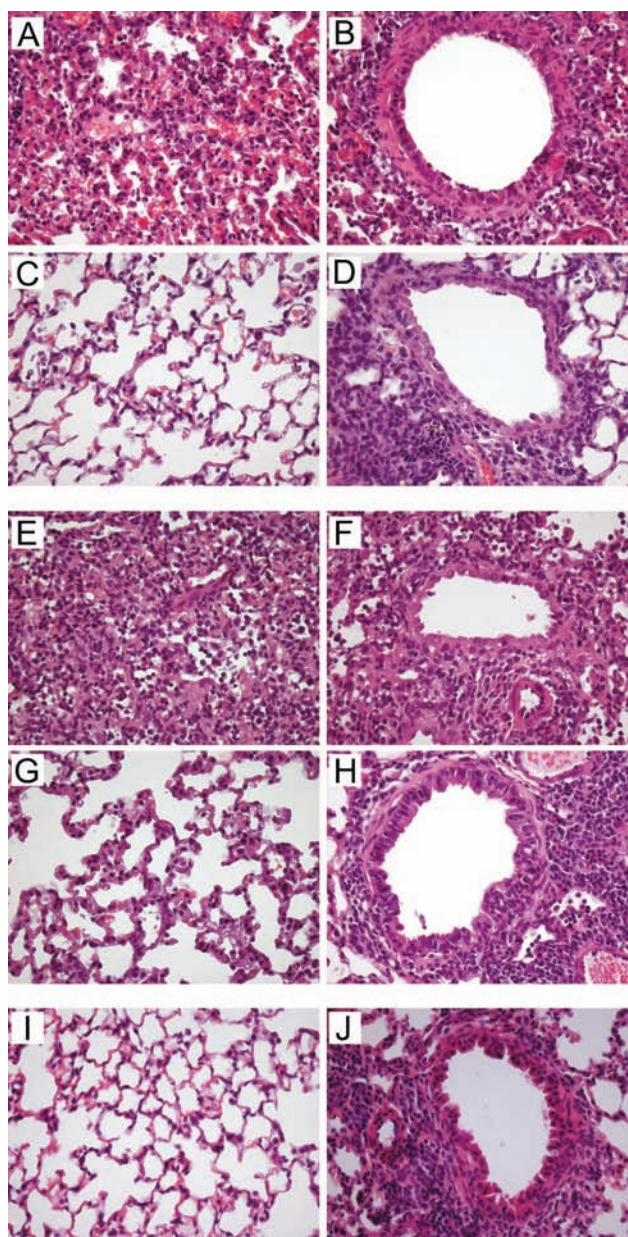
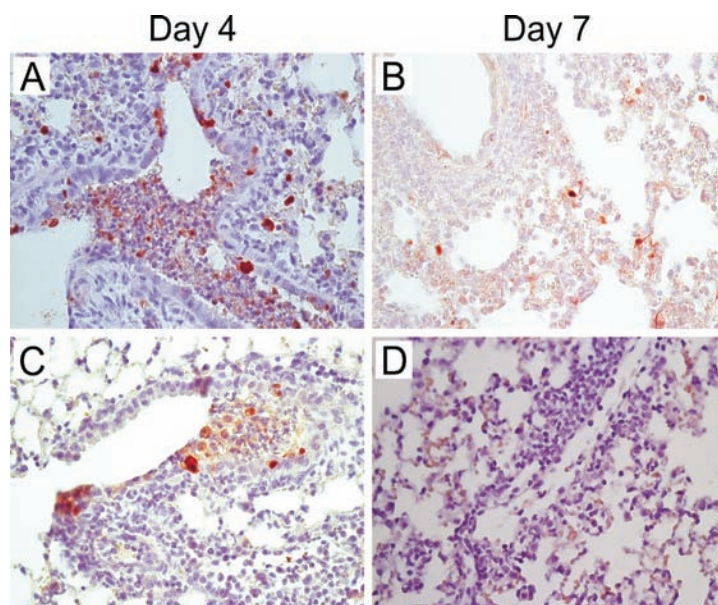


APPENDIX I



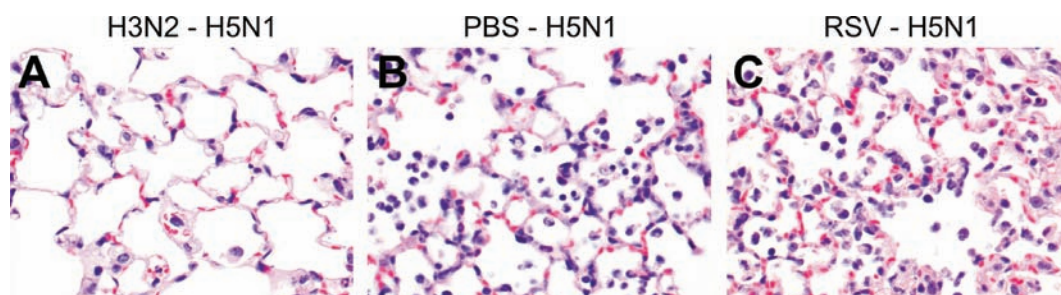
Chapter 2 - Figure 5 Histology of the lungs of mice infected with either influenza virus X-31, influenza virus A/PR/8/34 or both sequentially. A+B: lung of a naive mouse 4 days after PR/8 infection with flooding of the alveoli (A) and peri-broncheolar and -vascular lymphoid infiltrate (B). (C+D) lung of an X-31 experienced mouse 4 days after PR/8 challenge lacking flooding of the alveoli (C) and stronger peri-broncheolar and -vascular lymphoid infiltrate (D). (E+F) lung of a naive mouse 7 days after PR/8 infection, The alveoli are filled with cell debris and fluid, and type II pneumocyte hyperplasia is seen in the alveolar walls (E) combined with strong peri-broncheolar and -vascular lymphoid infiltrate (F). (G+H) lung of an X-31 experienced mouse 7 days after PR/8 challenge, with mild type II pneumocyte hyperplasia (G) and marked peri-broncheolar and -vascular lymphoid infiltrate (H). (I+J) lung of an X-31 experienced mouse on day 28 after PR/8 challenge with normal looking alveoli (I) and a peri-broncheolar and -vascular lymphoid cuff (J).

APPENDIX II

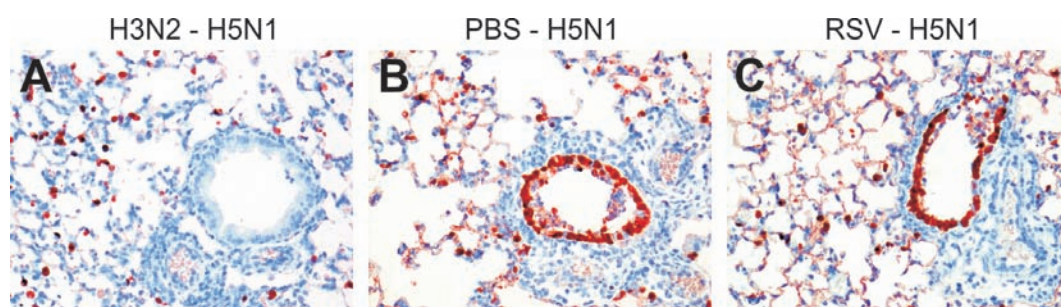


Chapter 2 - Figure 6 Mouse lung sections stained for influenza virus A NP. Cytoplasm of influenza A virus infected cells stains red, the nuclei stain deep red (A) naive mouse on day 4 after infection with influenza A virus A/PR/8/34 with virus antigen positive epithelial cells alining the bronchiole, and positive cells in the alveoli (B) naïve mouse on day 7 p.i. with virus antigen positive cells in the alveoli (C) X-31 experienced mouse on day 4 after infection with Influenza virus A/PR/8/34 with virus antigen positive cells around the bronchioles (D) X-31 experienced mouse on day 7 p.i. lacking virus antigen positive cells.

APPENDIX III

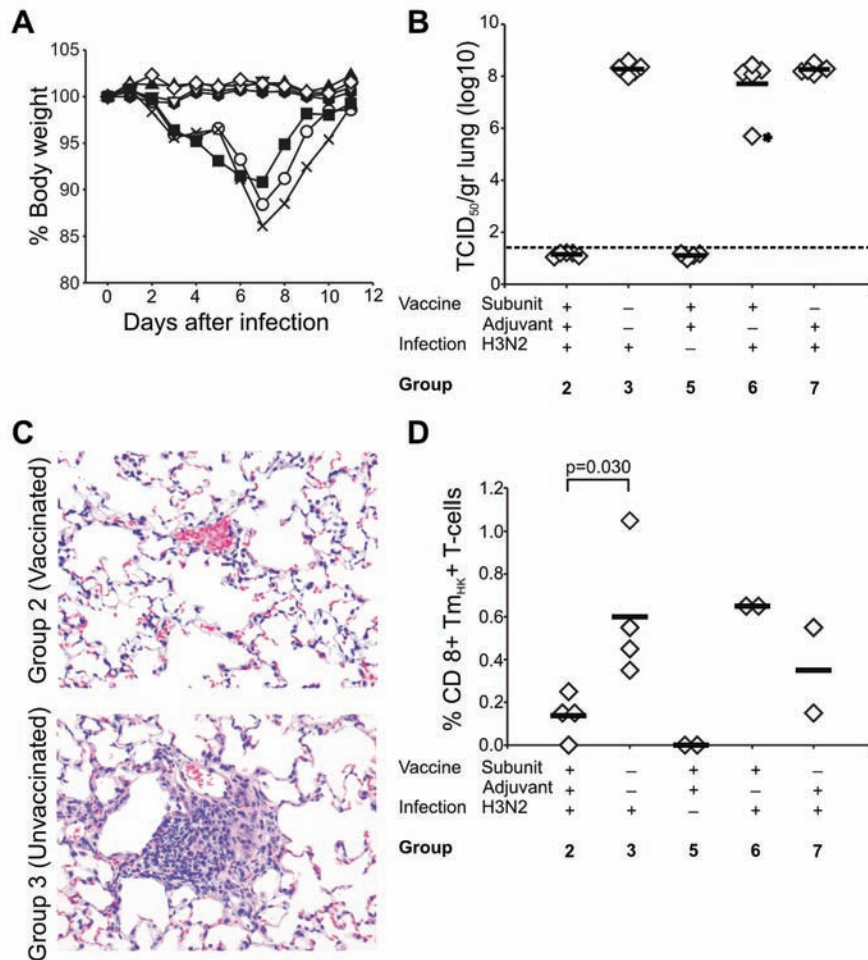


Chapter 3 - Figure 3 Histopathology was examined in the lungs of mice after infection with influenza virus A/IND/5/05. Four days after infection the lungs of H₃N₂-primed animals showed a multifocal mild broncho-interstitial pneumonia with mild inflammatory infiltrates consisting of predominantly lymphocytes and neutrophils (A). The mock-infected control mice (B) and RSV-primed mice (C) displayed a multifocal moderate necrotizing broncho-interstitial pneumonia with marked infiltration, consisting of inflammatory cells, mainly neutrophils and lymphocytes, in the alveoli.



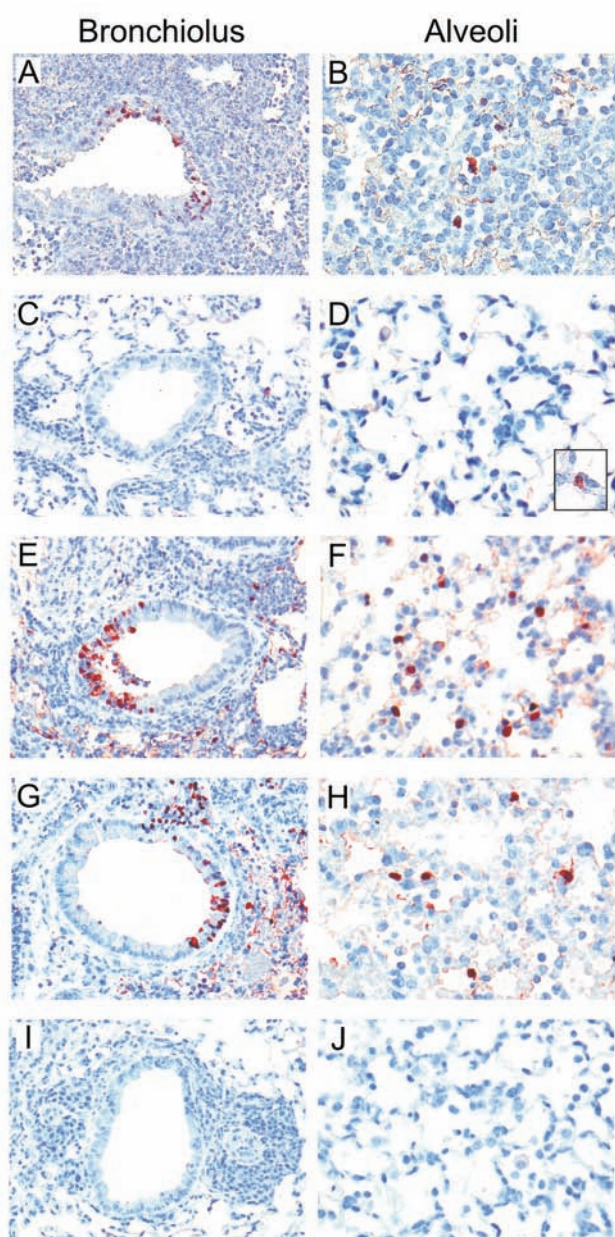
Chapter 3 - Figure 4 Detection of virus-infected cells by immunohistochemistry in the lungs of influenza virus A/IND/5/05 (H₅N₁)-infected mice on day 4 p.i. in mice primed by infection with influenza virus A/HK/2/68 (H₃N₂) (A), mock-infected mice (B), or RSV-primed mice (C).

APPENDIX IV



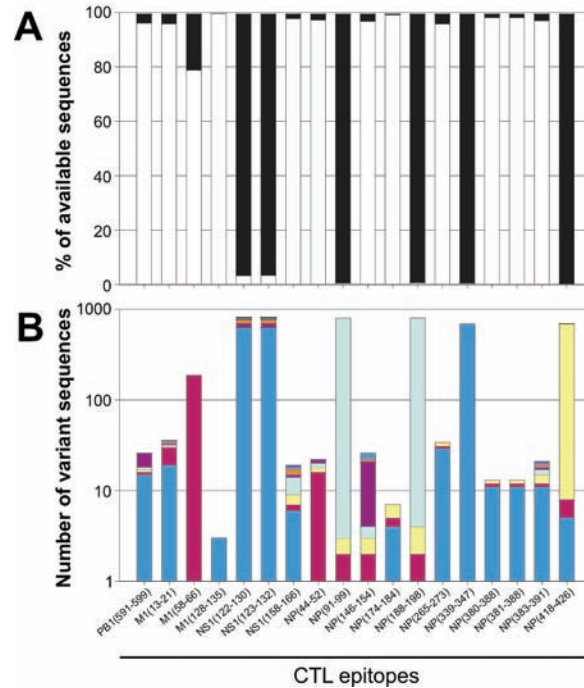
Chapter 4 - Figure 2 Outcome of infection with IAV HK/68 (H₃N₂). Mice were inoculated with IAV HK/68 (groups 2 (▲), 3 (○), 6 (■) and 7 (×)) or PBS (groups 1 (●), 4 (▽) and 5 (◇)). **(A)** Body weight after infection was determined daily and expressed as the percentage of the original body weight before infection. **(B)** Lung virus titers measured on day 4 p.i. in mice from the indicated experimental groups. Horizontal bars represent the average titers of five mice. The dotted line represents the cut-off value for obtaining a positive result. *This mouse from group 6 had before infection an HI antibody titer of 40. **(C)** Vaccination prevented the induction of iBALT after infection. Twenty-eight days post infection with IAV HK/68 iBALT was detected in mice from group 3, but not in mice from group 2. Lung tissue sections were stained with HE. **(D)** Virus-specific CD8+ T cell responses detected 28 days post infection. Splenocytes of mice from the indicated experimental groups were tested for the presence of CD8+ T cells that bound the H2-Db NP_{HK} Tetramer. Horizontal bars represent the average of 2-4 mice. The difference in %CD8+ Tm+ T cells between groups 2 and 3 was statistically significant ($P=0.030$).

APPENDIX V



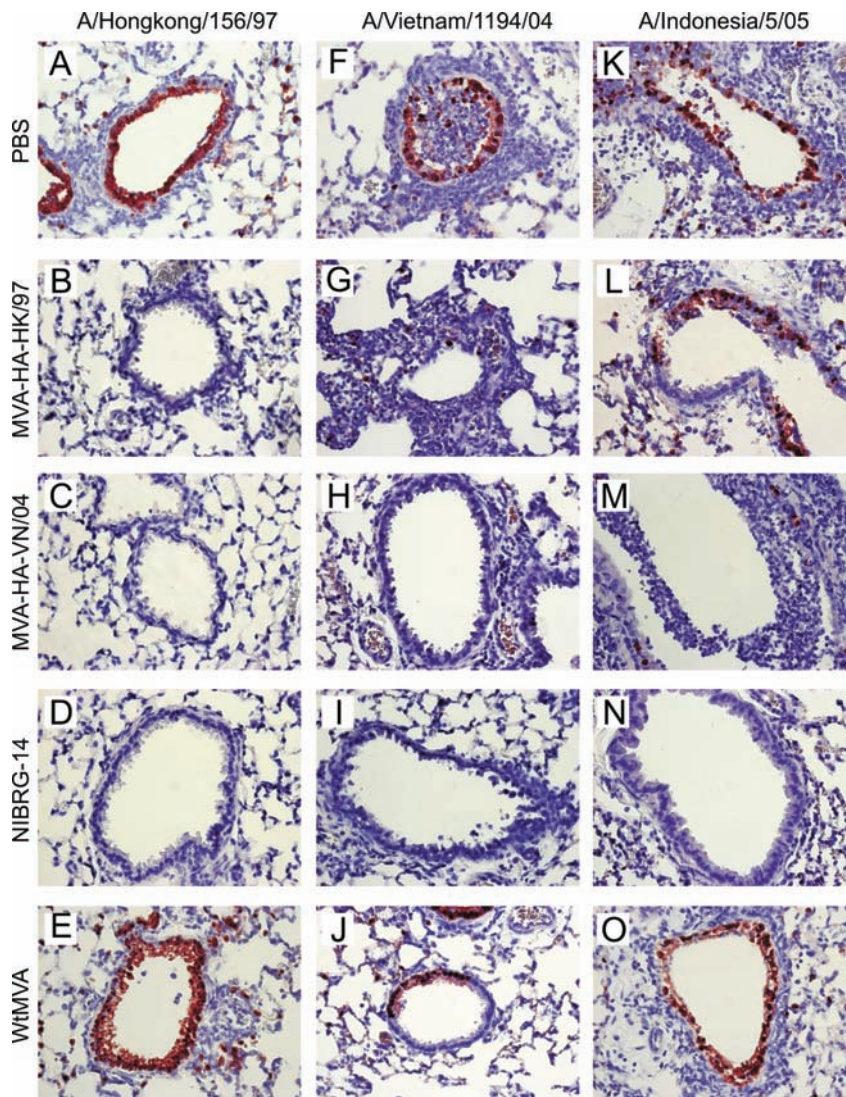
Chapter 4 - Figure 4 Histopathological analysis and immunohistochemistry of the lungs of mice infected with IAV IND/o5. Mouse lung sections were stained for influenza A virus nucleoprotein. Cytoplasm of infected cells stain red, the nuclei of infected cells stain deep red. In the groups without a history of productive A/ H3N2 infection, including group 2 (**A,B**), infection with IAV IND/o5 led to severe histopathological changes and to viral antigen expression in cells of the bronchiolar walls and in the alveoli (group 4: **E,F** and group 5: **G,H**). In mice of groups 3 (**C,D**) and 7 (**I,J**) that had experienced a productive infection with IAV HK/68 only moderate histopathological changes were observed and virus infected cells were detected sporadically (see insert in panel D). For more information please see text.

APPENDIX VI



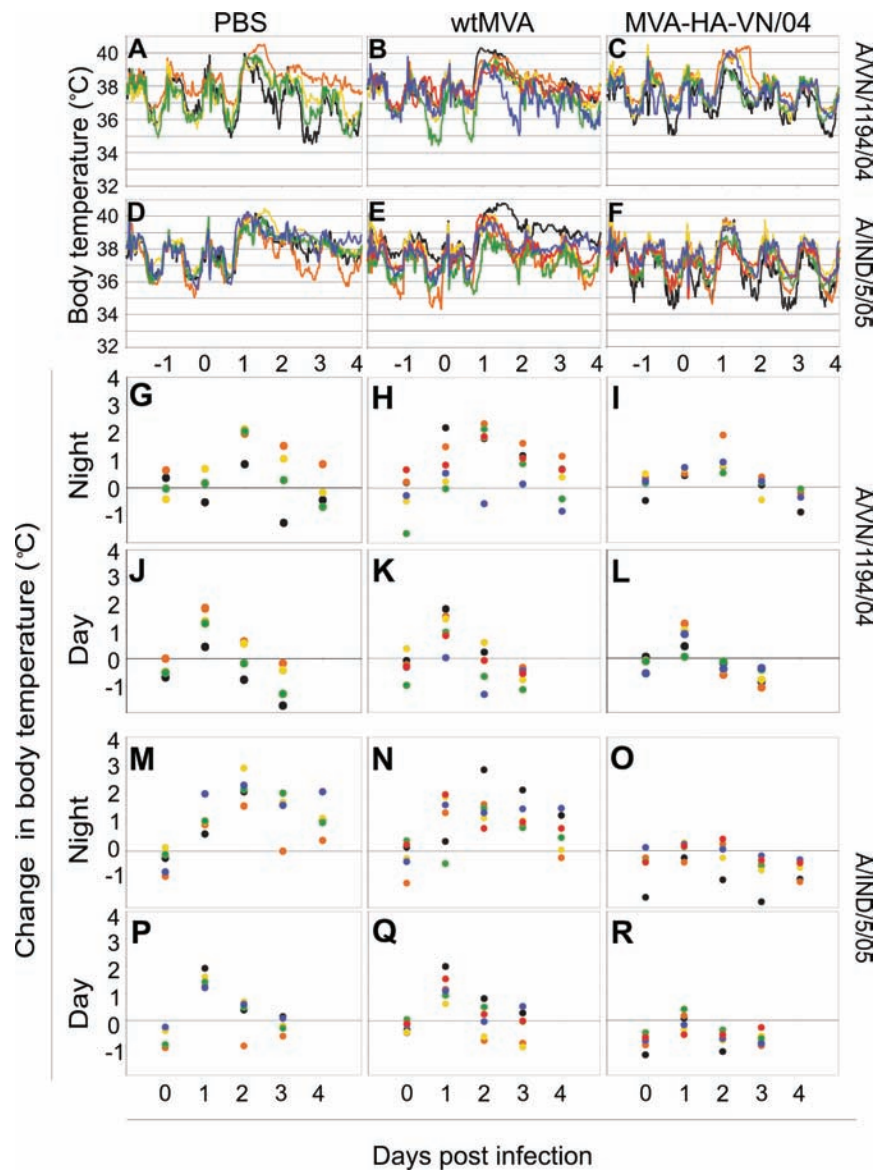
Chapter 5 - Figure 1 The presence of known CTL epitopes in H5N1 strains. The percentage of H5N1 viruses with an epitope sequence identical to human influenza viruses (white bars) is shown in Figure 1. The black bars indicate the percentage of H5N1 viruses with one or more amino acid substitutions in the epitope sequence. The absolute numbers of each variant of an epitope are shown in Figure 1B, each color represents a single variant (sequences can be found in table 1). For this analysis almost 900 H5N1 viruses were analyzed for which sequence information was available in the influenza sequence database [290].

APPENDIX VII



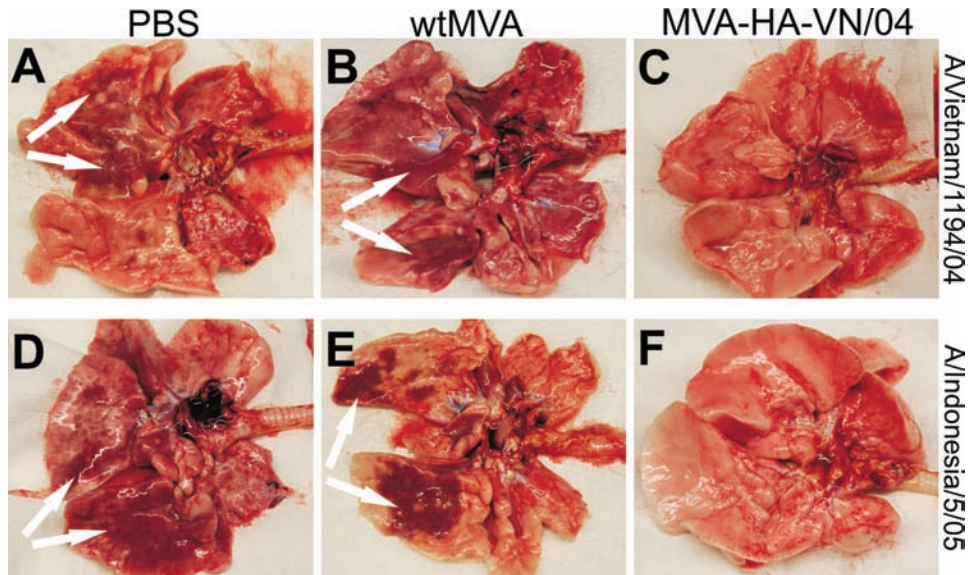
Chapter 6 - Figure 4 Histopathology and immunohistochemistry of the bronchioles and alveoli in lungs of mice infected with either influenza virus A/HK/157/97, A/VN/1194/04 or A/IND/5/05 as indicated. Influenza virus A/HK/156/97 infection led to viral antigen expression in cells of the bronchiolar wall of PBS (A) and wtMVA immunized mice (E), combined with mild peribronchiolar inflammatory infiltrate, while in the lungs of MVA-HA-HK/97 (B), MVA-HA-VN/04 (C) and Stimune®-adjuvanted NIBRG-14 (D) immunized mice no viral antigen was detected. Infection with influenza virus A/VN/1194/04 resulted in expression of viral antigen in cells of the bronchiolar walls of PBS (F), MVA-HA-HK/97 (G) and wtMVA (J) immunized mice, also combined with moderate peribronchiolar infiltrate (except for the wtMVA immunized mice). No viral antigen expression or morphological changes were detected in MVA-HA-VN/04 (H) and Stimune®-adjuvanted NIBRG-14 immunized mice (I). Infection with influenza virus A/IND/5/05 resulted in abundant viral antigen expression in the bronchioles of PBS (K), MVA-HA-HK/97 (L) and wtMVA (O) immunized mice, combined with moderate peribronchiolar infiltrate. Only minimal viral antigen expression was detected in the bronchiolar wall of MVA-HA-VN/04 (M) immunized mice, combined with moderate inflammatory infiltrate. No viral antigen was detected in the lungs of Stimune®-adjuvanted NIBRG-14 (N) immunized mice after infection with influenza virus A/Indonesia/5/05.

APPENDIX VIII

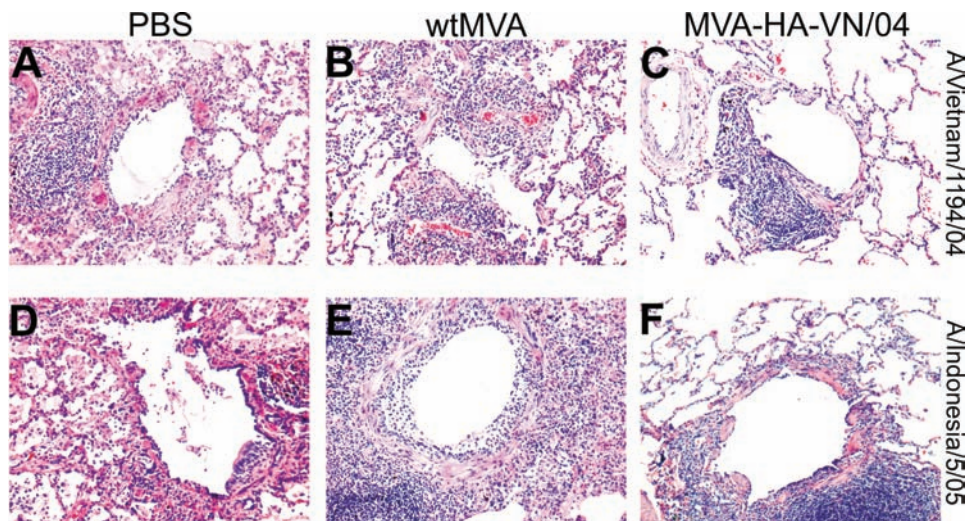


Chapter 7 - Figure 2 Body temperature recorded before and after infection with influenza virus A/Vietnam/1194/04 (A-C) or A/Indonesia/5/05 (D-F). The animals were immunized with PBS, wtMVA or MVA-HA-VN/04 as indicated. Changes in body temperature of individual animals after infection with influenza virus A/Vietnam/1194/04 (G-L) or A/Indonesia/5/05 (M-R) were calculated for each individual animal. Each dot represents an individual animal. Line colors in Figure 2A-C correspond with dot colors in figure 2G-L. Line colors in Figure 2D-F correspond with dot colors in figure 2M-R.

APPENDIX IX

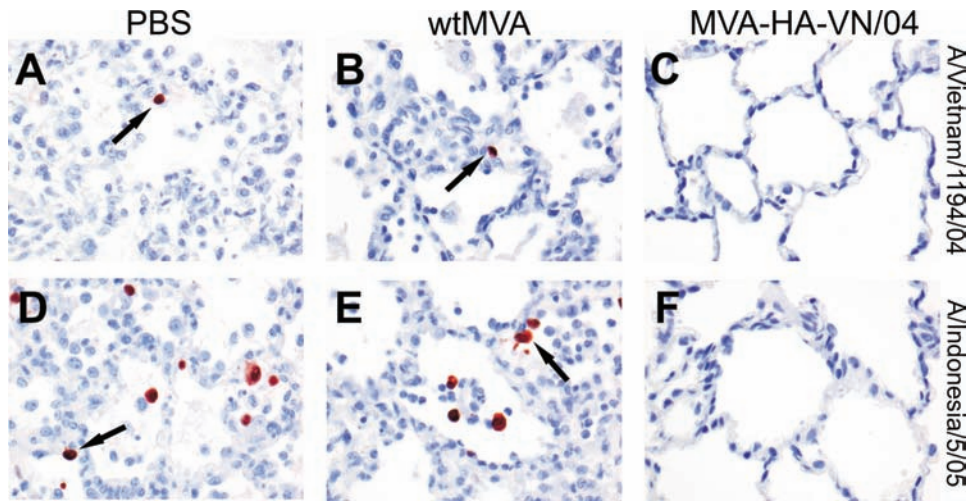


Chapter 7 - Figure 5 Macroscopic lesions of the lungs after infection with H₅N₁ influenza virus. The lungs of animals immunized with PBS (A, D), wtMVA (B, E) or MVA-HA-VN/04 (C, F) were fixed in formalin on day 4 after infection with influenza virus A/Vietnam/1194/04 (A-C) or A/Indonesia/5/05 (D-F). The arrows indicate consolidated areas present in the lungs of PBS and wtMVA immunized animals after infection (A, B, D, E). Lungs from the MVA-HA-VN/04 immunized animals had no macroscopical lesions (C, F).



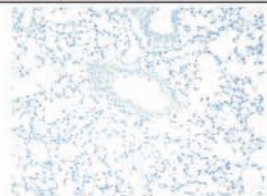
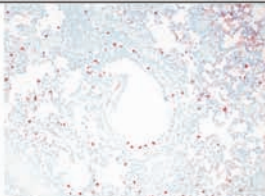
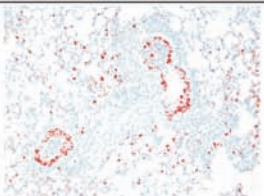
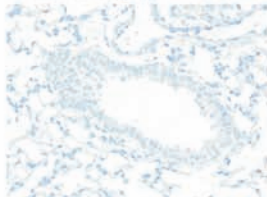
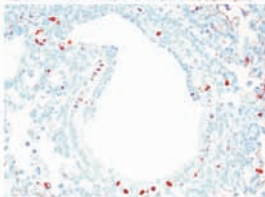
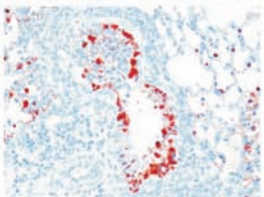
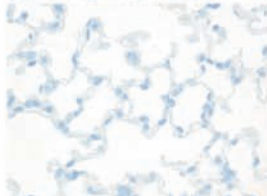
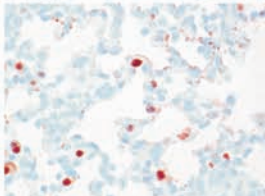
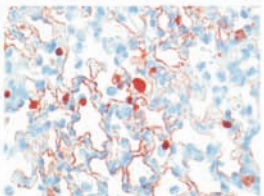
Chapter 7 - Figure 6 Histopathologic analysis of the lungs on day 4 after infection with influenza virus A/Vietnam/1194/04 (A, B, C) or A/Indonesia/5/05 (D, E, F). Histopathological changes were comparable in PBS and wtMVA inoculated animals with extensive lesions in the lungs of these animals. There was mild necrosis, edema, hypertrophy and hyperplasia of type II pneumocytes combined with peribronchiolar and -vascular infiltration. The epithelium of some bronchioles is denuded due to necrosis of the epithelial cells. (A, B, D, E). In the lungs of the MVA-HA-VN/04 immunized animals no histopathological changes were observed (C, F).

APPENDIX X



Chapter 7 - Figure 7 Detection of virus-infected cells in the lungs four days post infection with H5N1 influenza viruses. Immunohistochemistry was used to stain cells that are positive for the presence of viral antigen showing a deep red staining in the nucleus. Influenza viruses A/Vietnam/1194/04 (arrows indicate single infected cells) or A/Indonesia/5/05 antigen expression was seen in alveolar epithelial cells and some alveolar macrophages of PBS (A, D) and wtMVA (B, E) inoculated animals. No viral antigen was observed in the lungs of MVA-HA-VN/04 immunized animals (C, F).

APPENDIX XI

Classification	None		Moderate		Severe		
	normal alveoli normal bronchioles		Alveolar and bronchiolar cells expressing viral antigen		Alveoli and necrotic bronchioles display viral antigen expression and proteinaceous fluid		
Overview							
							
							
	None		Moderate		Severe		
	Challenge virus	Vaccine	Dose	Vaccine	Dose	Dose	
One immunization	VN/04	MVA-HA-VN/04	10^8	MVA-HA-VN/04	10^4 10^5 10^6	PBS wtMVA MVA-HA-VN/04	n.a. 10^8 10^3
	IND/05	MVA-HA-VN/04	10^8	MVA-HA-VN/04	10^4 10^5 10^6	PBS wtMVA MVA-HA-VN/04	n.a. 10^8 10^3
	VN/04	MVA-HA-VN/04	10^5 10^6	MVA-HA-VN/04	10^4	PBS wtMVA MVA-HA-VN/04	n.a. 10^6 10^3
Two immunizations	IND/05	MVA-HA-VN/04	10^5 10^6	MVA-HA-VN/04	10^4	PBS wtMVA MVA-HA-VN/04	n.a 10^6 10^3

Chapter 8 - Figure 3 Histopathological changes and immunohistochemistry of the lungs after infection with influenza A/H5N1 virus. Representative pictures were selected for the different classifications. Magnification: overview (10x), bronchiole (20x), alveoli (40x).

