HA-specific CD4+ T cells respond to influenza virus infection with a Th1 phenotype. Naïve WT HA-specific CD4+ T cells were adoptively transferred into WT mice with $2.5 \times 10^3$ PFU of influenza virus infection as described in text. HA-specific CD4+ T cells were retrieved from the lungs for analysis on stated days post infection. (a) WT HA-specific CD4+ T cells responded with the production of Th1 effector cytokines and IL-10. (b) Many IFN-γ producing cells secreted IL-10 as well, as shown by the percentage of double positive cells with and without re-stimulation of cognate HA peptide. (c) T-bet expression of HA-specific 6.5 CD4+ T cells. Values are mean ± s.d. of at least 3 experiments.
More desialylation, LAP binding, and active TGF-β in 6.5 HA-specific CD4+ T cells in the absence of IL-10 during the early phase of influenza virus infection.

Naïve WT or IL-10KO HA-specific CD4+ T cells were adoptively transferred into WT or IL-10KO mice with 2.5 X 10^3 PFU of influenza virus infection, as described in text. Lungs and mediastinal lymph nodes (MLN) were collected for single cell suspension analysis on day 4- post infection. Recipient mice without infection served as control. (a) PNA lectin binding, the binding to desialylation sites, on 6.5 HA specific CD4+ T cells. (b) Anti- LAP monoclonal antibody binding on 6.5 HA-specific CD4+ T cells. (c) Active TGF-β in 6.5 HA-specific CD4+ T cells as revealed by intracellular staining.
Supplementary Figure 3

Absence of IL-10 leads to more active TGF-β production in the lung during the early phase of infection. Naïve WT or IL-10KO HA-specific CD4+ T cells were adoptively transferred into WT or IL-10KO mice with 2.5 X 10³ PFU of influenza virus infection, as described in text. Lungs were collected and crushed in PBS on day 4- post infection. Recipient mice without infection served as control. (Left panel) All cells retrieved from homogenized lung were analyzed for active TGF-β by intracellular staining. (Right panel) The lung lysates were analyzed for active TGF-β by ELISA. Values are mean ± s.d.
IL-10KO mice suffer from ameliorated disease severity compared to WT mice, although the kinetics of viral clearance is not changed. IL-10KO and WT mice were infected with influenza virus of different inoculum sizes. (a) Body weight and survival after infection. (b) Viral titers in the lungs at various time points after infection. Values are mean ± s.d. (n = 6; ***=p<0.0001; **=p<0.001; NS=non-significant, p>0.05).
Supplementary Figure 5

Basal level IL-10 production by 6.5 HA-specific CD4+ T cells upon stimulation with cognate HA peptide. Splenocytes from naïve WT 6.5 mice were stimulated in vitro overnight with cognate HA peptide. Non-stimulated splenocytes served as control.
Supplementary Figure 6

**Splenocytes cultured in vitro with influenza virus**

**A:** Total splenocytes

![Graph showing membrane desialylation](image)

**B:** Splenocytes and individual population of cells of splenocytes

![Bar charts showing membrane desialylation](image)

**Higher levels of membrane desialylation by influenza virus in the absence of IL-10.** Naïve splenocytes of WT or IL-10KO 6.5 mice were cultured in vitro with influenza virus for overnight or for 48 hours. Membrane desialylation was defined by PNA binding. (a) Membrane desialylation was higher on IL-10KO 6.5 compared to the WT splenocytes after overnight co-culture (left panel). The desialylation waned at 48-hours (right panel). Splenocytes from naïve WT or IL-10KO 6.5 mice cultured without virus served as control (grey filled area). (b) Membrane desialylation on individual populations of cells of IL-10KO or WT 6.5 splenocytes after an overnight co-culture with virus.
TGF-β mediates the suppressed IFN-γ production in IL-10KO 6.5 HA-specific CD4+ T cells in vitro. Splenocytes from naïve WT or IL-10KO 6.5 mice were cultured in vitro with influenza virus. IL-10KO cells produced less IFN-γ as revealed by intracellular staining after an overnight culture. While both were added at the onset of culture, anti-TGF-β (10 mg ml⁻¹) reversed and TGF-β (10 ng ml⁻¹) aggravated the suppressed IFN-γ production. Data are mean ± s.d.