The role of cytokine stimulation in MCMV reactivation was tested in MCMV-infected and sham treated mice. Four months after primary infection, when an active infection could be excluded, the animals were treated (i.p.) either with 10µg TNF-
post-infection (p.i.) mice were sacrificed and lungs were flushed twice with 1ml of ice-cold, sterile PBS before samples were transported to the University Hospital, Medical Research Center (ZMF), Department of Anesthesiology and Intensive Care Medicine, Tübingen, Germany. The expression of immediate early (IE) genes IE1, IE3 and early gene (gB) were determined in selected parts of tissue. The expression of immediate early (IE) genes IE1 and early gene (gB) were determined in selected parts of lung tissue. Eighteen months after primary infection, the i.p. injection of TNF-

Methods:
Healthy, 7-8 week-old BALB/c mice, purchased from Harlan Winkelmann (Germany), were housed in accordance with guidelines for animal welfare under sterile conditions. Mice were infected intranasally (i.n.) with 2x10³ PFU of purified murine cytomegalovirus (MCMV Smith strain) at PBS (sham). One, 3, 5, 15, 22, 29, 36, 43 and 50 days post-infection (p.i.) mice were sacrificed and lungs were flushed twice with 1ml of ice-cold, sterile PBS before samples were transported to the University Hospital, Medical Research Center (ZMF), Department of Anesthesiology and Intensive Care Medicine, Tübingen, Germany.

Results:
After primary infection IE1 and IE3 gene expression was detected frequently between day 1 and 36 p.i. (Fig.4). A strong IE1 gene expression was only detected between day 1 and 7 p.i. in lung tissue.

Activation of NF-κB was detected five days p.i. by EMSA. Super Shift analysis indicate the nuclear translocation of the Rel subunits p50 and p65 (Fig.5). However, the activation only occurred in Rel subunits that are not synthesized in Rel subunits of the Balb/c mice. At day 14 p.i., the production of the NF-κB dependent mediators MIP-1β and IL-16 were higher in BALs from MCMV-infected animals, whereas the concentration of CCL3, TNF-α and IL-1β were lower in virus-infected animals compared to sham animals (Fig.6). At day 5 p.i., concentrations of most mediators were higher in BAL samples collected from sham-infected animals except for IL-1β and CCL3 compared to MCMV infected mice.

Four months after primary infection, the p. injection of TNF-α or IL-1β increased the expression of IE1 and IE3 in different time courses (Fig 5). However, MCMV gene expression was also induced in single PBS treated animals between day 3 and day 21. In MCMV / TNF-α and MCMV / IL-1β treated mice a constant expression of the IE1 gene was visible between day 1 and 14. In MCMV / TNF-α treated animals strong IE3 RNA expression was seen at day 3. In MCMV / IL-1β treated animals IE1 gene expression was visible between day 1 and 14. However, faint bands representing IE3 expression were also detected between day 3 and day 10 in single long pieces collected from MCMV infected PBS treated animals.

Activation of NF-κB was seen at day 7 p.i. after TNF-α treatment (Fig6). At day 7 p.i. activation of NF-κB was mostly detected in IL-1β treated animals. Translocation of the p50 Rel subunit could be observed at day 10 in MCMV / PBS and MCMV / IL-1β. IL-1β mice and 14 days after TNF-α treatment in MCMV / TNF-α treated littermates.

In our preliminary data no specific difference in the pattern of IE gene expression was found (Ref.3). The coincidence of IE1 transcription and NF-κB activation in lung tissue were investigated by RT-PCR. Furthermore NF-κB activation in lung tissue was determined in EMSAs and EMSA-Supershift Assays for the Relprotein p50. In supernatant of the BAL samples chemokine concentration were measured by specific ELISAs.

Conclusion:
Immunogenic MCMV infection of healthy mice results in IE1, IE3 and gB expression up to day 36 p.i. During primary infection NF-κB seems to play a minor role. Indeed NF-κB regulated gene products, like MIP-2 and IL-6 were lower in BAL samples of MCMV-infected mice.

After recovery of primary infection, treatment with TNF-α or IL-1β resulted in reactivation of MCMV IE genes, but not of the gB gene in lung tissue. Therefore there is no complete reproductive reactivation cycle in TNF-α or IL-1β treated mice. We hypothesize that additional stimuli are necessary to induce a complete reactivation of MCMV in lung tissue.

In single MCMV infected mice, treated with PBS or IFN-γ (3µg) at day 1, 7 and 14 may point to an indirect role of NF-κB during MCMV gene transcription. However other transcription factors may be involved. Indeed production of NF-κB dependent genes, like IL-6 and MIP-2 were higher in sham mice than in MCMV infected animals (Fig 7).

References: