Supplemental Material

Supplemental results

Reduced CTL activity in rLCMV/INDG infection

To assess potential differences in CTL activity elicited by LCMV-ARM versus rLCMV/INDG we infected C57BL/6 mice with $2x10^4$ pfu of either virus i.v. At the peak of the CTL response in rLCMV/INDG infected mice (day 6, not shown) we measured CTL activity against the NP396-404 epitope derived from the identical LCMV-NP protein of both viruses (Figure 1). CTL against the LCMV-GP derived epitope GP33-41 served as control (Figure S1). LCMV-ARM immune splenocytes efficiently lysed NP396-404 pulsed target cells upon 5 h incubation while comparably weaker specific CTL activity of rLCMV/INDG immune splenocytes required around 15 h for detectable activity (Figure S1A,C). Due to the lack of the LCMV-GP gene in rLCMV/INDG (Figure 1), GP33-41 specific CTL activity was only observed in LCMV-ARM immune splenocytes (Figure S1B,D). Early elimination of rLCMV/INDG by the nAb response (Figs. 2,3) and limiting antigen available for CTL induction offered a likely explanation for this observation. Analysis of CTL responses in B cell deficient μMT mice could however not demonstrate a positive effect in the absence of nAb production. Analysis of blood cells (Figure S1E) or splenocytes (Figure S2) from rLCMV/INDG and LCMV-ARM infected mice by MHC class I tetramers revealed similar NP396-404 specific $CD8^+$ T cell frequencies on day 6-8. rLCMV/INDG induced CTL were also functional as

documented in the protection of immune mice from lethal choriomeningitis after intracerebral challenge with LCMV-ARM (not shown). As predicted (Figure 1), GP33-41 specific $CD8^+$ T cells were only detected in blood of LCMV-ARM infected mice (Figure S1F).

Unimpaired CTL response in LCMV-ARM plus rLCMV/INDG coinfection

We assessed the $CD8^+$ T cell response in C57BL/6 mice infected i.v. with $2x10^4$ PFU of either rLCMV/INDG or LCMV-ARM or with a combination of both (Figure S2). Specific CTL activity directed against either the NP396-404 (Figure S2A) or the GP33-41 (Figure S2B) epitope were largely unaffected by coinfection as compared to LCMV-ARM infection alone. As shown before (Figure S1), rLCMV/INDG infection alone induced very low NP396-404 specific CTL activity despite frequencies of epitope specific $CD8^+$ T cell that were comparable to LCMV-ARM infected animals (Figure S2C). NP396-404 specific $CD8^+$ T cell frequencies in animals coinfected with rLCMV/INDG plus LCMV-ARM were even slightly increased as compared to LCMV-ARM infection or to rLCMV/INDG infection alone (Figure S2C). This vigorous CTL response had however no clear effect on the production of VSV neutralizing total Ig (Figure 3F) and VSV-IND neutralizing IgG was only slightly reduced (Figure 3G). Hence, rapid and potent induction of nAbs by rLCMV/INDG but not by LCMV-ARM were unlikely due to differences in specific CTL activity elicited.

Unimpaired nAb response and isotype class switch in rLCMV/INDG infected animals transfused with LCMV-ARM effector splenocytes

To evaluate the possibility that the nAb response to rLCMV/INDG was only susceptible to T cell mediated suppression during the earliest phase of nAb production and to clarify the mechanisms underlying reduced isotype class switch in rLCMV/INDG plus LCMV-ARM coinfected animals (Figure 3G), we performed adoptive transfer experiments. Splenocytes from C57BL/6 mice infected with $2x10^4$ PFU LCMV-ARM eight days previously exhibited high NP396-404 and GP33-41 specific CTL activity (Figure S3A). Transfer of $5x10^6$ of these donor splenocytes into recipients infected with $2x10^4$ PFU rLCMV/INDG 4 days previously had no effect on the VSV neutralizing total Ig and IgG titers mounted (Figure S3B). Identical adoptive cell transfer to naïve animals conferred however full protection from lethal choriomeningitis upon intracerebral challenge with $3x10^3$ PFU LCMV-ARM (15) (data not shown), confirming the unimpaired *in vivo* effector capacity of the transferred cell population. Thus, nAb production to rLCMV/INDG was refractory to T cell mediated suppression even in the earliest phase of the response and reduced isotype class switch in rLCMV/INDG plus LCMV-ARM coinfected animals was most likely the result of LCMV induced immunopathology disturbing lymphoid microarchitecture and compromizing B cell / T cell cooperation (20).

Supplemental methods

Mice

B-cell deficient μMT mice (44) on the C57BL/6 background were obtained from the Institut für Labortierkunde, University of Zurich.

Cytotoxicity assays and enumeration of epitope specific $CD8^+$ T cells

Virus specific cytotoxic activity of spleen cells was assayed as described previously (45). Briefly, single-cell suspensions were prepared from the spleens of mice at day 6 to 8 after infection and were used directly in a primary in vitro ^{51}Cr release assay. Target cells were EL-4 cells coated with GP33-41 or NP396-404 (10^{-6}M) and uncoated control cells. Background killing on uncoated control target cells was insignificant in all experiments shown and was subtracted to obtain specific lysis (%). Spontaneous Cr^{51} release of target cells was between 12-18% and 27-31% for 5h and 15h incubations, respectively. The immunodominant H-$2D^b$ binding LCMV peptides GP33-41 and NP396-404 were purchased from Neosystem Laboratoire (Strasbourg, France). Epitope specific $CD8^+$ T cells were stained with allophycocyanin conjugated MHC class I tetramers (46) containing either of the above peptides, were costained with anti-CD8α-PerCP or –PE conjugate (clone 53-6.7) and/or with anti-B220-PerCP conjugate obtained from BD PharMingen (San Diego, CA) and were measured on a Facscalibur (Becton Dickinson, San Diego, CA). The frequency of $tetramer^+CD8^+$ cells was calculated as percentage of the entire $CD8^+$ lymphocyte population (Figure S1) or as percentage of the $CD8^+B220^-$ lymphocyte population (Figure S2).

Supplemental References

44. Kitamura, D., Roes, J., Kuhn, R., and Rajewsky, K. 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. *Nature* 350:423-426.

45. Zinkernagel, R.M., Haenseler, E., Leist, T., Cerny, A., Hengartner, H., and Althage, A. 1986. T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class I-restricted virus-specific cytotoxic T cells as a physiological correlate of the 51Cr-release assay? *J Exp Med* 164:1075-1092.

46. Altman, J.D., Moss, P.A., Goulder, P.J., Barouch, D.H., McHeyzer-Williams, M.G., Bell, J.I., McMichael, A.J., and Davis, M.M. 1996. Phenotypic analysis of antigen-specific T lymphocytes. *Science* 274:94-96.

Supplemental figure legends

Fig. S1

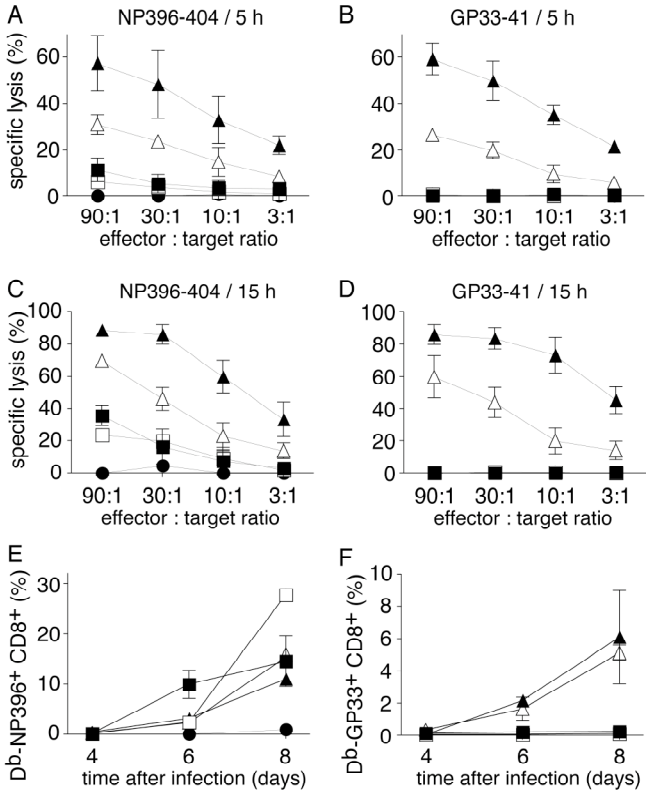

Figure S1: Normal frequencies but reduced cytolytic capacity of rLCMV/INDG induced CD8$^+$ T cells independent of virus nAb.

A-D: C57BL/6 (■,▲) and µMT mice (❑,△) were infected with 2x10^4 PFU of rLCMV/INDG (■,❑) or of LCMV-ARM (▲,△) i.v. Cytolytic activity of splenocytes was measured 6 days later in a primary *ex vivo* CTL assay (A-D) against NP396-404 (A,C) or GP33-41 (B,D) labeled EL-4 target cells. Specific Cr^{51} release was determined after 5 h (A,B) or 15 h (C,D), incubation, respectively. One representative experiment of two similar ones is shown (A-D). E,F: Mice were infected as above and the frequency of circulating NP396-404 and GP33-41 specific CD8$^+$ T cells in blood was determined by MHC class I tetramers. Except for naïve controls (●, single mouse), symbols represent

the mean of three mice per group +/- SD.

Fig. S2

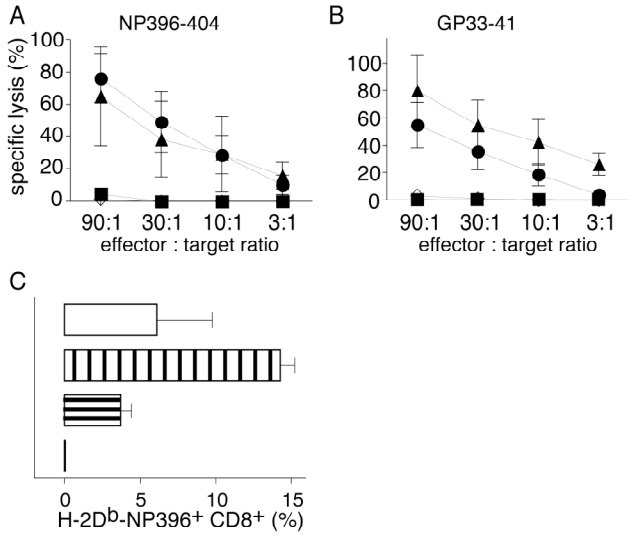

Figure S2: Unimpaired CTL response in LCMV-ARM plus rLCMV/INDG coinfection.

C57BL/6 mice were infected i.v. with $2x10^4$ PFU LCMV-ARM (▲, white bar), $2x10^4$ PFU rLCMV/INDG (■, horizontally hatched bar), with both above inocula (●, vertically hatched bar) or were left uninfected (◊ in A,B; black bar in C). NP396-404 (A) and GP33-41 specific (B) primary ex vivo CTL activity of splenocytes and the frequency of NP396-404 specific $CD8^+$ T cells were measured 8 days later. For A and B, one representative experiment of two is shown. Except for naïve controls, symbols represent the mean of three mice per group +/- SD.

Fig. S3

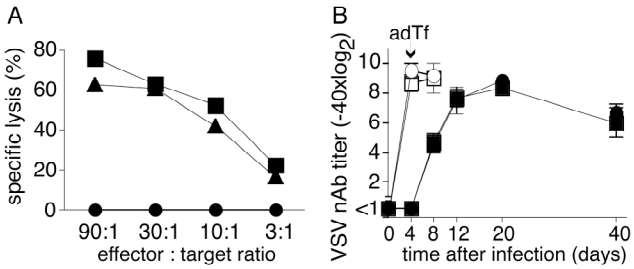

Figure S3: Unimpaired nAb response and isotype class switch in rLCMV/INDG infected animals transfused with LCMV-ARM effector splenocytes

Three C57BL/6 mice were infected with $2x10^4$ PFU LCMV-ARM i.v. . A: Eight days later, pooled splenocytes were tested for primary *ex vivo* CTL activity against NP396-404 (■) and GP33-41 (▲). Naïve splenocytes exhibited <1% killing on either target (●). B: C57BL/6 immunized with $2x10^4$ PFU rLCMV/INDG four days previously received $5x10^6$ of the above effector splenocytes (❐,■) or no adoptive transfer (●,❍). Serum samples collected at the indicated time points were tested for VSV-IND neutralizing total Ig (❑,❍) and IgG (■,●). One representative experiment of two is shown. Symbols in B represent the mean of three mice per group +/- SD while symbols in A are single samples.