Supplemental Information

Lymphocytes Negatively Regulate NK Cell Activity via Qa-1b following Viral Infection

Haifeng C. Xu, Jun Huang, Aleksandra A. Pandyra, Elisabeth Lang, Yuan Zhuang, Christine Thöns, Jörg Timm, Dieter Häussinger, Marco Colonna, Harvey Cantor, Karl S. Lang, and Philipp A. Lang
Figure S1: High dose infected animals demonstrate elevated IFN-γ production, related to Figure 1.

C56BL/6 were infected with low dose or high dose LCMV WE. Virus titers were measured from spleen, liver, lung, and kidney tissue (a) 1 day post infection (n=4) (b) 2 days post infection (n=4) (c) 4 day post infection (n=4). (Error bars show SEM, **p < 0.001, ns indicates statistically not significant between the indicated groups)
Figure S2: Qa-1b expression is mediated by type I interferon on B cells, related to Figure 2.

(a) C57BL/6 mice were infected with low or high dose of LCMV WE. 1 day after infection (p.i.) H2-t23 expression levels in sorted CD3-CD19+ B cells, CD3+CD8+ T cells, CD3+CD4+ T cells, and CD3-CD19-CD11b+ cells from spleen tissue were determined (n=3-4). (b-d) CD8+ T cell-depleted or non-depleted C56BL/6 mice were infected with high dose LCMV WE. (b) H2-t23 expression levels were measured in spleen tissue 1 day after infection (n=4). (c-d) CD4+ T cell-depleted or non-depleted C56BL/6 mice were infected with high dose LCMV WE. 1 day p.i., spleen tissue was analyzed for Qa-1b, CD19, CD8 and F4/80 expression (n=4). (e-f) Control (C56BL/6) or Ifnar1−/− animals were infected with high dose LCMV WE. 1 day p.i., spleen tissue was analyzed for (e) H2-t23 gene expression and (f) Qa-1b, CD19, CD8 and F4/80 expression (n=6, scale bar indicates 50µm). (Error bars show SEM, **p < 0.01, ***p < 0.001, ns indicates statistically not significant between the indicated groups)
Figure S3: Expression of surface molecules, granzyme B, and perforin is not affected by Qa-1b, related to Figure 3.

(a-c) Control or Qa-1b<sup>-/-</sup> mice were infected with high dose LCMV WE. (a) NK or ILC1 surface molecule expression is shown as indicated in spleen tissue 1 day p.i. (One representative FACS blot of n=4-5 is shown). (b) Ganzyme B and perforin expression was determined on splenic cNK cells 1 day p.i. One representative FACS blot of n=4-5 is shown. (c) C56BL/6 mice were infected with low dose or high dose LCMV WE. NKG2 expression were determined on indicated ILC subsets (n=4). (d) Control or NKG2A deficient animals were infected with high dose LCMV WE. 1 day p.i. NKG2 expression were determined on indicated ILC subsets (n=4).
Figure S4: Normal IFN-I response triggers similar early virus replication between Control and Qa-1b−/− mice, related to Figure 4.

(a-e) Control or Qa-1b−/− mice were infected with a high dose of LCMV WE. (a) IFN-α concentrations from sera of infected animals were measured at indicated time points (n=3). (b) Number of conventional DC (cDC) were determined by CD11c and MHCII staining in spleen tissue of control and Qa-1b−/− mice at indicated time points post infection (n=3). (c) Expression of co-stimulatory molecules CD40 (left panel), CD80 (middle panel), and CD86 (right panel) was determined on CD11c+ MHCII+ splenic cDC (n=3). (d-e) Virus titers were measured from spleen, liver, lung, and kidney tissue (d) 1 day post infection (n=6) and (e) 2 days post infection (n=6). (f) Control and Qa-1b−/− mice were infected with high dose LCMV WE. 12 days post infection, proportion of effector (CD62L−IL7R−), effector memory (CD62L−IL7R+), central memory (CD62L+IL7R+) of blood and splenic gp33-specific tetramer are shown (n=7-9). (Error bars show SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ns indicates statistically not significant between the indicated groups).
**Figure S5**

(a-f) Control and Qa-1b<sup>−/−</sup> mice were infected with high dose LCMV WE. (a) Gp33-specific tetramer was measured in the spleen 4 days post infection (n=4). (b) Gp33-specific tetramer was measured in spleen (left) and liver tissue (right) 8 days p.i. (n=3). (c) At day 8 post infection, splenocytes (left panel) or liver cells (right panel) were re-stimulated with the LCMV-specific epitope gp33, followed by measurement of IFN-γ production (n=3). (d) One representative histogram of surface molecule expression on day 8 splenic gp33-specific tetramer is shown (n=3). (e) Virus titers were determined in spleen, liver, lung, and kidney tissue 8 days p.i. (n=3). (f) Bone marrow cells from control or Qa-1b<sup>−/−</sup> mice were mixed at 1:1 ratio with bone marrow cells from Cd8<sup>−/−</sup> mice were transferred into lethally irradiated WT mice. One month later, these mixed chimeric animals were infected with 2×10<sup>5</sup> pfu LCMV WE. 12 days after infection gp33-specific tetramer in the spleen (left panel). Splenocytes were re-stimulated with the LCMV-specific epitope gp33, followed by staining for IFN-γ (n=4-5, right panel). (g) Control and Qa-1b<sup>−/−</sup> mice were infected with high dose LCMV WE. 4 days post infection, expression of NKG2A/C/E was determined on splenic gp33-specific tetramer (n=4). (h) Control and Klrc1<sup>−/−</sup> mice were infected with high dose LCMV WE, 4 days post infection, expression of NKG2A/C/E was determined on splenic gp33-specific tetramer (n=4). (i) Bone marrow cells from control or Klrc1<sup>−/−</sup> mice were mixed at 1:1 ratio with bone marrow cells from Cd8<sup>−/−</sup> mice were transferred into lethally irradiated WT mice. One month later, these mixed chimeric animals were infected with 2×10<sup>5</sup> LCMV WE. 12 days after infection gp33-specific tetramer in the spleen (left panel). Splenocytes were re-stimulated with the LCMV-specific epitope gp33, followed by staining for IFN-γ (n=4-5, right panel) (Error bars show SEM, *p < 0.05, **p < 0.01, ***p < 0.001, ns indicates statistically not significant between the indicated groups).
Figure S6: NK cell depletion partially restores defective T cell immunity, related to Figure 7.

(a) C57BL/6 mice were treated with or without NK cell depletion antibody. 2 days after treatment, NK cell number was determined from spleen tissue (n=3). (b) NK cell-depleted and non-depleted control and Qa-1b-/- mice were infected with high dose LCMV WE. 12 days after infection surface molecules on splenic gp33-specific CD8+ T cells were measured (n=8-10) (Error bars show SEM, ***p < 0.001, ns indicates statistically not significant between the indicated groups)