# Type I interferon receptor-triggering delays Kupffer cell replenishment during acute fulminant viral hepatitis

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41 **Author contributions**

42 K.B. and T.F. designed, performed and analyzed all experiments and wrote the manuscript.
43 J.S., P.-K.T., C.C., J.S., L.G., and S.N. performed experiments, S.L. and M.K. provided
44 IFN-$\beta^{WT/\Delta\beta\text{-luc}}$ mice and Mx2-luc mice, M.H. designed histology experiments, G.S. provided
45 VACV, U.K. designed and supervised the study, wrote the manuscript and organized funding
46 of the project.

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48

49 **Abstract**

50 **Background and Aim:** Virus-induced fulminant hepatitis is a major cause of acute liver
51 failure. During acute viral hepatitis the impact of type I interferon (IFN-I) on myeloid cells,
52 including liver-resident Kupffer cells (KC), is only partially understood. Here we dissected the
53 impact of locally induced IFN-I responses on myeloid cell function and hepatocytes during
54 acute liver inflammation.

55 **Methods:** Two different DNA-encoded viruses, vaccinia virus (VACV) and murine
56 cytomegalovirus (MCMV), were studied. *In vivo* imaging was applied to visualize local IFN-β
57 induction and IFN-I receptor (IFNAR)-triggering in VACV infected reporter mice. Furthermore,
58 mice with a cell type-selective IFNAR-ablation were analyzed to dissect the role of IFNAR-
59 triggering in myeloid cells and hepatocytes. Experiments with Cx3cr1$^{+/gfp}$ mice revealed the
60 origin of reconstituted KC. Finally, mixed bone marrow chimeric mice were studied to
61 specifically analyze the effect of IFNAR-triggering on liver infiltrating monocytes.

62 **Results:** VACV infection induced local IFN-β responses, which conferred IFNAR-triggering
63 primarily within the liver. IFNAR-triggering was needed to control the infection and prevent
64 fulminant hepatitis. The severity of liver inflammation was independent of IFNAR-triggering of
65 hepatocytes, whereas IFNAR-triggering of myeloid cells protected from excessive
66 inflammation. Upon VACV or MCMV infection KC disappeared, whereas afterwards
67 infiltrating monocytes differentiated to KC. During the time of IFNAR-triggering such
68 replenished monocyte-derived KC comprised more IFNAR-deficient than -competent cells in
69 mixed bone marrow chimeric mice, whereas after the decline of IFNAR-triggering both
70 subsets showed an even distribution.

71 **Conclusion:** Upon VACV infection IFNAR-triggering of myeloid cells, but not of hepatocytes,
72 critically modulates acute viral hepatitis. During infection with DNA-encoded viruses
73 IFNAR-triggering of liver-infiltrating blood monocytes delays MoKC development, pointing
74 towards new therapeutic strategies of acute viral hepatitis.

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76

77 **Introduction**

78 Many different virus infections can cause acute hepatitis that may result in acute liver failure.
79 Besides infections with hepatotropic virus (e.g., hepatitis B virus (HBV) and hepatitis C virus
80 (HCV)) also other viruses such as cytomegalovirus (CMV) can cause acute hepatitis (*1-5*).
81 Yet, it is unclear to which extent virus pathogenicity or immunopathology cause liver damage.
82 The liver is the central metabolic organ, which is characterized by a tolerogenic environment.
83 Hepatic sinusoids are populated with antigen-presenting cells such as Kupffer cells (KC). KC
84 are self-maintaining liver-resident macrophages, which shape the local immune milieu (*6-8*).
85 During homeostasis KC display an overall anti-inflammatory phenotype (M2-like phenotype)
86 and primarily work as scavenger cells that eliminate insoluble macromolecules and antigens
87 from blood (*9-11*). Upon infection, KC express enhanced levels of scavenger receptors, take
88 up and kill invading pathogens, and express MHC-II molecules to present antigens (M1-like
89 phenotype) (*8, 12*). Macrophage-depleted mice show enhanced susceptibility to infection
90 with viruses and bacteria (*13, 14*). Furthermore, liver-infiltrating monocytes can contribute to
91 KC responses and differentiate to DC or monocyte-derived macrophages (MoMF) (*15-17*).

92 Type I interferon (IFN-I) is an anti-viral cytokine that is induced early after infection, confers
93 direct anti-viral effects, and modulates immune cell functions (*18, 19*). Previous studies
94 showed that IFN-I, which is produced in the liver by myeloid cells, significantly contributes to
95 the anti-viral defense (*13, 15, 20, 21*). Furthermore, in poly(I:C)-treated mice IFN-I receptor
96 (IFNAR)-triggering of myeloid cells results in IL-1RA expression, which protects mice from
97 severe liver damage (*22*). In contrast, upon lymphocytic choriomeningitis virus (LCMV)
98 infection IFNAR-triggering enhances immunopathology within the liver by inducing oxidative
99 damage in hepatocytes (*23, 24*).

100 Here we performed infection studies with two different DNA viruses, vaccinia virus (VACV)
101 and murine cytomegalovirus (MCMV), which both encode several potent IFN-I evasion
102 proteins (*25-27*). Both viruses efficiently infect the liver and cause acute hepatitis (*28, 29*).
103 Previous studies revealed that in VACV-infected mice IFN-I responses are not detectable in
104 the serum (*26, 30*), nevertheless, IFNAR$^{-/-}$ mice succumb to VACV infection (*31*).
105 Interestingly, we detected induction of local IFN-I expression in the liver of VACV-infected
106 mice. This local IFN-I not only conferred anti-viral effects but also modulated inflammatory
107 responses of myeloid cells and delayed differentiation of infiltrating monocytes to KC. Similar
108 observations were made upon MCMV infection highlighting the modulatory role of local IFN-I
109 responses during acute fulminant viral hepatitis.

110

## Material and Methods

### Mice and viruses

C57BL/6 (WT) (Harlan Winkelmann), IFNAR$^{-/-}$ (IFNAR1) (*31*), IFN-β$^{WT/\Delta\beta\text{-luc}}$ (*32*), Mx2-luc (*33*), Cx3cr1$^{+/gfp}$ (*34*), Mx-Cre$^{+/-}$Rosa26eYFP$^{ST/ST}$ (*35*), (*36*) Alb-Cre$^{+/-}$IFNAR$^{fl/fl}$ (*37*), and LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice (*38, 39*) were bred under specific pathogen free conditions at the central animal facility of TWINCORE or the Helmholtz Center for Infection Research (HZI), Brunswig, Germany. For the generation of 1:1 mixed bone marrow (BM) chimeras 5 x $10^5$ BM cells from CD45.1$^+$ WT and CD45.2$^+$ IFNAR$^{-/-}$ donors were mixed. One day after lethal irradiation of CD45.1$^+$ WT recipients, 1 x $10^6$ mixed BM cells were transplanted intravenously (i.v.). Chimerism was determined by FACS analysis. Mouse experimental work was carried out using 8 to 16 week old mice in compliance with regulations of the German animal welfare law. VACV strain Western Reserve (originally provided by Bernard Moss, NIH, Bethesda, MD) was propagated on BHK-21 cells. MCMV-Δm157 (MCMV) (*40*) was provided by Stefan Jordan (Icahn School of Medicine, New York). Virus stocks were purified by sucrose density gradient ultracentrifugation. To determine virus loads, organ homogenates were titrated on RK13 cells and plaque-forming units (pfu) per gram tissue were determined. In all infection experiments mice were i.v. infected with 2 x $10^6$ pfu VACV or 5 x $10^5$ pfu MCMV.

### *In vivo* imaging

Reporter mice were i.v. injected with 3 mg of D-luciferin (PerkinElmer) diluted in PBS and anesthetized using 2 to 2.5% isoflurane (Abbott). The emitted light signals were measured in an IVIS SpectrumCT (Caliper) and the photon flux was analyzed with Living Image 4.5 software (Caliper).

### Quantification of enzyme-activity

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine levels were determined using commercially available kits from Fuji DRI-CHEM NX500.

### Histology

For paraffin staining, livers were fixed in 4% paraformaldehyde and paraffin-embedded. Two µm sections were cut and deparaffinized, rehydrated, and boiled at 100°C. Liver sections were stained with hematoxylin-and-eosin or immunolabeled with F4/80, caspase-3, or iNOS specific antibodies (automated BOND-MAX stainer with Bond Polymer Refine Detection Kit (Leica)). Images were acquired with a Leica SCN400 Slide Scanner and quantified using Leica Digital Image Hub software. For cryosections, livers were fixed in 4% paraformaldehyde and dehydrated in 30% sucrose solution before freezing in Tissue-Tek

144 (Sakura). 6 μm sections were cut and immunolabelled with primary antibodies specifically
145 binding F4/80, albumin, or VACV. Photographs were taken on an Axiovert microscope
146 (Zeiss) and AxioVision software. Original pictures were adjusted for brightness, contrast, and
147 color balance and post processed using Fiji software (NIH). Cell counting was performed
148 using Fiji software.

149 **Cell isolation, flow cytometry, and cell sorting**

150 Mononulcear cell isolation from the liver was performed as previously described (*41*). After *in*
151 *situ* perfusion and *in vitro* digestion of the liver, cells were filtered through 70 μm cell strainers
152 and centrifuged at 300 g for 8 min at 4°C. The cell pellet was resuspended in 33% Percoll
153 and centrifuged at 500 g for 15 min at room temperature. Following red blood cell lysis
154 (Sigma) cells were immunolabeled with fluorochrome-conjugated antibodies (Biolegend and
155 BD) for flow-cytometry analysis (LSR II Sorb, Becton Dickinson) or cell sorting (MoFlo XDP,
156 Beckman Coulter or FACSAria fusion sorter Becton Dickinson).

157 **Statistical analysis**

158 Statistical analyses were performed using GraphPad Prism 6 software. If not indicated
159 otherwise, all data shown was pooled from 2-4 independent experiments.

160

161 For further information on single mouse strains, antibodies, and software used please refer to
162 supplementary CTAT table and supplementary material and methods section.

163

164 **Results**

165 Previously we found that VACV-infected mice do not mount IFN-I responses in the serum
166 (*26, 30*). Nevertheless, *in vivo* imaging experiments with VACV-infected IFN-$\beta^{WT/\Delta\beta\text{-luc}}$ reporter
167 mice revealed that local IFN-β expression was induced within secondary lymphoid organs as
168 well as in the liver (Fig. 1A/B). In order to test whether this locally expressed IFN-β also
169 translated into biologically active protein, we analyzed Mx2-luc mice reporting on IFN-I
170 receptor (IFNAR)-triggering. Indeed, upon VACV infection of such mice a dominant luciferase
171 signal was detected within the liver (Fig. 1C/D). WT mice survived systemic VACV infection
172 with mild signs of disease, whereas IFNAR$^{-/-}$ mice showed significant body weight loss, high
173 viral titers in the liver, and succumbed to the infection within 5 days post infection (dpi) (Fig.
174 2A/B/C). In VACV-infected IFNAR$^{-/-}$ mice the liver damage was further illustrated by elevated
175 ALT and AST serum levels (Fig. 2D). Normal creatinine serum levels indicated that VACV
176 infection did not impair the kidney function (Fig. 2D).

177 To determine cell types that were directly infected by VACV, we performed
178 immunohistochemical analysis of the liver. Interestingly, in both WT and IFNAR$^{-/-}$ mice
179 hepatocytes were infected (Fig. 2E), which indicates that IFNAR deletion does not change
180 the viral tropism. In addition, primary hepatocytes isolated from WT mice were heavily
181 infected *in vitro* by VACV and released infectious particles into the supernatant (Fig. S1). 5
182 dpi WT mice showed leukocyte infiltration within the liver and mild signs of steatosis (Fig. 3A
183 and S2A). In contrast, infected IFNAR$^{-/-}$ mice displayed strong leukocyte infiltration that was
184 associated with loss of cellular integrity, endothelium disruption, and formation of
185 inflammatory foci (Fig. 3A and S2A). Additionally, caspase-3 expression and the pro-
186 inflammatory myeloid cell mediator iNOS were enhanced within inflammatory foci in the liver
187 of IFNAR$^{-/-}$, but not WT mice (Fig. 3A/B and S2B-D). These data indicate that upon VACV
188 infection local IFNAR-triggering contributes to the control of infection, modulates
189 inflammation, and prevents acute liver failure.

190 Next we determined whether upon VACV infection IFNAR-triggering affected the composition
191 and function of local myeloid cell subsets within the liver. In flow-cytometric analysis of
192 liver-derived CD11b$^{+}$ mononuclear cells, numbers of infiltrating CD11c$^{+}$I-A/I-E$^{-}$ MoMF and
193 CD11c$^{+}$I-A/I-E$^{+}$ DC were significantly enhanced in WT mice at 2 and 4 dpi when compared
194 with uninfected controls (Fig. 4A/B). In contrast, numbers of F4/80$^{hi}$CD11b$^{+}$ KC were
195 significantly reduced on 2 dpi, whereas by 4 dpi the KC pool was replenished (Fig. 4A/B). Of
196 note, replenished F4/80$^{+}$ KC also expressed the specific KC marker CLEC4F (Fig. S3) (*42*).
197 Infected IFNAR$^{-/-}$ mice showed a similar increase of DC when compared with WT mice,
198 whereas in contrast to WT mice MoMF were not dramatically enhanced (Fig. 4A/B).
199 Furthermore, IFNAR$^{-/-}$ mice also showed significantly decreased KC numbers 2 dpi, whereas

200 4 dpi KC replenishment was severely impaired (Fig. 4A/B). Similarly, in immunohistological
201 analysis, 2 dpi KC numbers were strongly decreased in WT and IFNAR$^{-/-}$ mice and
202 reappeared 4 dpi only in WT mice, but not in IFNAR$^{-/-}$ mice (Fig. 4C/D). Thus, despite VACV
203 readily infects hepatocytes, liver inflammation leads to a complete loss of KC, which are
204 replenished in WT, but not IFNAR$^{-/-}$ mice. In infected WT mice replenished KC showed
205 unaffected iNOS (M1-like) and YM-1 (M2-like) expression, whereas Arg-1 (M2-like)
206 expression was reduced (Fig. S4A). In infected IFNAR$^{-/-}$ mice KC showed enhanced iNOS as
207 well as YM-1 expression, and Arg-1 expression was unaffected (Fig. S4A). Thus, KC from
208 infected IFNAR$^{-/-}$ mice show reduced replenishment and a combined M1 and M2 phenotype.

209 To specify liver-resident cell subsets that modulated viral hepatitis upon IFNAR-triggering, we
210 analyzed mice with a selective IFNAR deletion on hepatocytes (Alb-Cre$^{+/-}$IFNAR$^{fl/fl}$) or
211 myeloid cells, including KC (LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$) (*43*). Although VACV readily infected
212 hepatocytes (see Fig. 2E and Fig. S1), VACV-infected Alb-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice developed
213 only mild liver inflammation similar to that observed in WT mice (Fig. 5A-C and S2A/C). In
214 contrast, LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice developed hepatitis, as indicated by increased leukocyte
215 infiltration and caspase-3 immunolabeling in histology (Fig. 5A/B and S2A-C), as well as
216 elevated ALT and AST serum levels (Fig. 5C). Of note, in VACV infected
217 LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice viral titers were elevated 1 and 2 dpi, and were comparable with
218 those of IFNAR$^{-/-}$ mice, before they decreased by 4 dpi to similar levels as also detected in
219 WT mice (Fig. S5). These results indicated that IFNAR-triggering of local KC was needed for
220 early control of virus infection. However, flow-cytometric analysis demonstrated that KC
221 replenishment was normal in Alb-Cre$^{+/-}$IFNAR$^{fl/fl}$ and LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice (Fig. 5 D/E).
222 KC sorted from uninfected and infected mice revealed that in LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice KC
223 showed a very similar M1/M2 expression profile as observed in WT mice (Fig. S4A). Hence,
224 modulation of viral hepatitis is independent of IFNAR-triggering of hepatocytes, while
225 IFNAR-triggering of myeloid cells, including KC, plays a critical role. In contrast, KC
226 replenishment is not promoted by direct IFNAR-triggering of hepatocytes or KC. Importantly,
227 LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice showed only partial IFNAR-depletion of CD115$^{+}$Ly6C$^{+}$ blood
228 monocytes (Fig. 5F/G). This could explain why in LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice MoKC
229 replenishment was similar to that observed in WT mice.

230 KC replenishment might be conferred either by locally proliferating KC precursors or by
231 infiltrating monocytes. The immunohistological analysis of liver 5 dpi of WT and IFNAR$^{-/-}$ mice
232 revealed that F4/80$^{+}$ cells neither showed a KC-typical spindle-like morphology nor strong
233 F4/80 expression. Rather, they had a round appearance and were weakly F4/80$^{+}$, implying
234 that they represented another cell subset than KC (Fig. S6A). Indeed, 4 dpi of WT mice
235 MoMF, DC, and KC showed a significantly increased expression of the monocyte marker

236 Ly6C (Fig. S6B/C). To further resolve the origin of replenished KC, Cx3cr1$^{+/gfp}$ mice
237 expressing GFP in monocytes, but not in KC, were VACV-infected (*6*). Interestingly, the
238 restored KC pool showed significantly enhanced GFP expression, whereas MoMF and DC
239 showed unchanged GFP expression (Fig. 6A/B). Cell proliferation could not be detected in
240 replenished KC, as indicated by absence of Ki67 staining (data not shown). These data
241 support the hypothesis that while KC are depleted upon virus infection, Ly6C$^{+}$ inflammatory
242 monocytes infiltrate the liver and differentiate to MoMF, DC, and monocyte-derived KC
243 (MoKC).

244

245 To investigate the role of direct IFNAR-triggering of blood monocytes for MoKC
246 replenishment under conditions of controlled VACV infection (Fig. S7A), mixed BM chimeric
247 mice were studied. 4 dpi similar percentages of WT and IFNAR$^{-/-}$ MoMF were detected in the
248 liver of mixed BM chimeric mice, while the percentage of IFNAR$^{-/-}$ DC was enhanced (Fig.
249 6C/D). Most interestingly, before the infection of BM marrow chimeric mice WT and
250 IFNAR$^{-/-}$ MoKC showed a similar abundance, whereas after infection IFNAR$^{-/-}$ MoKC were
251 significantly increased (Fig. 6C/D). Of note, similar percentages of IFNAR$^{-/-}$ and WT blood
252 monocytes were detected in uninfected as well as infected mixed BM chimeric mice (Fig.
253 6E). These data indicate that IFNAR-ablation on liver infiltrating blood monocytes promotes
254 their differentiation to MoKC. Interestingly, in infected mixed BM chimeric mice the imbalance
255 of WT and IFNAR$^{-/-}$ MoKC was still detectable on 8 dpi, whereas it reversed after 14 dpi (Fig.
256 6F). Thus, the preferred formation of IFNAR$^{-/-}$ MoKC was a transient effect and we
257 hypothesized that it was conferred by direct IFNAR-triggering of infiltrating monocytes. To
258 test this we performed long-term kinetics of hepatic IFNAR-triggering by analyzing VACV
259 infected Mx2-luc mice. Indeed, luciferase expression indicating IFNAR-triggering was
260 detected on 2, 4, and 8 dpi, wheres at later time points it decreased to background levels
261 (Fig. S7B). Thus, MoKC development was impaired by direct IFNAR-triggering of developing
262 MoKC. Additionally, in infected MxCre$^{+/-}$Rosa26YFP$^{ST/ST}$ mice, MoKC showed YFP
263 expression 4 dpi supporting the hypothesis that developing MoKC were directly triggered by
264 local IFN-I expression (Fig. S7C/D).

265 To address whether also in other viral infections local IFN-I responses modulated MoKC
266 replenishment, next we performed infection studies with murine cytomegalovirus (MCMV).
267 We and others found that despite MCMV encodes IFN-I evasion factors such as m27, this
268 virus still induces IFN-I responses (*44, 45*) and readily infects hepatocytes (*29*). Similar to
269 VACV infection, also upon MCMV infection KC numbers significantly decreased in the liver
270 (Fig. S8A/B). Upon MCMV infection of mixed BM chimeric mice the ratio of WT and
271 IFNAR$^{-/-}$ MoMF and DC was not affected as indicated by similar percentages of WT and

272 IFNAR$^{-/-}$ cells (Fig. 6G). Nevertheless, numbers of IFNAR$^{-/-}$ MoKC were dramatically
273 increased when compared with WT MoKC. As similarly observed upon VACV infection, also
274 upon MCMV infection similar percentages of IFNAR$^{-/-}$ and WT monocytes were detected in
275 the blood (Fig. 6H). Thus, also during MCMV-induced acute hepatitis direct IFNAR-triggering
276 of liver infiltrating blood monocytes inhibits their differentiation to MoKC.

277 **Conclusion**

278 Although upon VACV infection of mice serum IFN-I responses are sequestered by
279 virus-encoded evasion proteins (*26, 30*), here we found that local IFN-I responses were still
280 induced within secondary lymphoid organs and the liver. The hepatic IFN-I was biologically
281 active and essential to reduce virus replication and to modulate the inflammatory response.
282 While IFNAR-triggering of hepatocytes was dispensable for modulation of liver inflammation,
283 IFNAR-triggering of myeloid cells was of key relevance. Specifically, IFNAR-triggering of
284 liver-resident macrophages was essential to control virus replication, while IFNAR-triggering
285 of infiltrating monocytes delayed MoKC development. Similar results were obtained after
286 MCMV infection. Thus, in acute viral hepatitis local IFNAR-triggering of myeloid cells
287 balances anti-viral immunity and immunopathology.

288 Many viruses modulate IFN-I function (*18*). VACV as well as CMV encode several factors
289 that sequester IFN-I expression or IFNAR-triggering (*27, 46*). In particular, the VACV-
290 encoded soluble factor B18 inhibits IFNAR-triggering of cells *in vitro* and *in vivo* (*25, 26*).
291 Here we showed that upon VACV infection IFN-β expression was locally induced, and that
292 IFN-I was biologically active mainly within the liver. Direct IFNAR-triggering of hepatocytes
293 has been suggested to play a key role in inducing immunopathology in the liver of LCMV-
294 infected mice (*23*). This is in contrast to our data, which show that IFNAR-triggering of
295 hepatocytes had no detrimental effect on the hepatocytes during VACV infection, although
296 these cells were readily infected by VACV. The anti-viral function of local IFN-I that was
297 conferred by IFNAR-triggering of KC was highlighted by VACV infection experiments with
298 LysMCre$^{+/-}$IFNAR$^{fl/fl}$ mice, which showed elevated viral titers 1 and 2 dpi, before on day 4 the
299 infection was controlled. Additionally, LysMCre$^{+/-}$IFNAR$^{fl/fl}$ mice showed enhanced apoptosis
300 and ALT levels when compared with WT mice, also indicating increased early viral replication
301 in the absence of KC-specific IFNAR-triggering.

302 Interestingly, 2 days after VACV infection the liver showed a severe KC loss, as indicated by
303 the complete absence of F4/80 and CLEC4F positive cells. KC were subsequently
304 replenishment in WT, but not in IFNAR$^{-/-}$ mice. Importantly, also upon MCMV infection a
305 similar KC loss was detected, which implied that KC loss during viral hepatitis is a rather
306 common phenomenon upon infection with DNA-encoded viruses. This notion is of particular
307 relevance because human CMV is a major cause of acute lethal hepatitis in immune-
308 competent as well as immune-compromized patients (*5, 47*).

309 KC replenishment was primarily conferred by differentiation of blood-derived CX3CR1$^{+}$
310 monocytes to KC (MoKC). In line with this, previous studies showed that upon bacterial
311 infection KC undergo rapid IFNAR-dependent necroptosis (*48*). In that case, KC loss was

312 followed by monocyte influx, which lead to anti-microbial inflammation as well as
313 anti-inflammatory tissue repair. As previously detected in other settings (*16*), also in VACV-
314 infected IFNAR$^{-/-}$ mice KC showed simultaneous upregulation of M1 and M2 genes,
315 presumably due to concurrent signals deriving from inflammation and organ pathology.

316 While IFNAR$^{-/-}$ mice showed impaired KC replenishment, mice with a conditional myeloid
317 cell-specific IFNAR-deletion showed KC replenishment which was comparable with that of
318 WT mice. This difference might be due to the deregulated cytokine milieu and massively
319 enhanced viral loads in IFNAR$^{-/-}$ mice. Therefore we addressed whether direct
320 IFNAR-triggering was needed for KC replenishment under conditions of controlled virus
321 replication, i.e., in mixed BM chimeric mice. Surprisingly, in such mice IFNAR-deficient
322 monocytes differentiated even more efficiently to MoKC than IFNAR-competent ones. This
323 phenomenon is in accordance with the hypothesis of Guilliams and Scott (*49*), that only the
324 most competitive progenitors can access the available niche at a certain time point.
325 Interestingly, by day 14 the imbalance of IFNAR-competent and -deficient MoKC was
326 reversed. The presence of local IFN-I expression correlated directly with the imbalance of
327 WT and IFNAR$^{-/-}$ MoKC, which indicates that the niche occupation is influenced by local
328 IFNAR-triggering and can be reverted after resolution of an infection. In line with this,
329 immunohistology revealed that VACV-infected LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice showed increased
330 F4/80 immunolabeling when compared with WT and IFNAR$^{-/-}$ mice. This could be due to the
331 partial IFNAR-deletion on monocytes in LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice, which might lead to an
332 even enhanced MoKC reconstitution. IFNAR-competent and -deficient monocytes sorted
333 from infected mixed BM chimeric mice 2 dpi showed similar iNOS, Arg-1, and YM-1 gene
334 expression (Fig. S4B). Therefore, direct IFNAR-triggering of infiltrating myeloid cells or MoKC
335 does not affect the determination of a pro- or anti-inflammatory phenotype of MoKC.

336 In the pathogenesis of hepatitis the beneficial or detrimental role of hepatic IFNAR-triggering
337 is discussed controversially: In poly(I:C)-induced hepatitis myeloid-derived suppressor cells
338 are recruited in an IFNAR-dependent manner to mount anti-inflammatory IL-1RA responses
339 (*22*). Furthermore, IFNAR-triggering is essential to mediate efficient CMV clearance by
340 IFNAR-dependent CCL-2 induction, macrophage recruitment, and NK-cell activation (*21*). In
341 contrast, upon LCMV infection of STAT-1 deficient mice IFN-I induces lethal
342 immunopathology (*24*) and oxidative damage of hepatocytes (*23*). However, other studies
343 showed that upon LCMV infection KC are necessary to prevent liver pathology in an
344 IFNAR-dependent manner (*13*). Our data clearly indicate a protective and
345 immunomodulating role of local IFN-I responses within the liver during acute viral hepatitis.

346 Here we showed that IFNAR-triggering directly delays MoKC differentiation, but does not
347 influence the fate of the resulting MoKC. Since it was shown previously that after induction of

348 liver damage M2-like macrophages are recruited to promote organ reconstitution (*16*), in
349 acute hepatitis the development of MoKC with a M2-like phenotype might be advantageous
350 for liver restoration. Thus, it is important to better understand the balance between
351 pro-inflammatory and anti-inflammatory signaling of liver-resident KC and liver-infiltrating
352 MoMF as a basis for the development of new therapeutic strategies for acute viral hepatitis.

353

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362

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526

527 **Figure legends**

528 **Figure 1: Upon VACV infection, local IFN-I responses are induced which confer**
529 **IFNAR-triggering primarily within the liver.** IFN-$\beta^{WT/\Delta\beta\text{-luc}}$ or Mx2-luc mice were
530 intravenously (i.v.) infected with 2 x $10^6$ pfu VACV. At the indicated time points luciferin was
531 injected i.v. and luciferase activity was determined by *in vivo* imaging. *In vivo* imaging of (A)
532 IFN-$\beta^{wt/\Delta\beta\text{-luc}}$ and (C) Mx2-luc mice mice. (B/D) Light signals detected in the regions of interest
533 (ROI) equivalent to spleen, liver and cervical lymph nodes (cLN) were quantified (n ≥ 9).
534 Error bars indicate mean ± SEM, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05; one-way ANOVA.

535

536 **Figure 2: IFNAR-triggering protects against VACV-induced liver damage and promotes**
537 **survival.** C57BL/6 mice and IFNAR$^{-/-}$ mice were i.v. infected with 2 x $10^6$ pfu VACV. (A/B) In
538 case body weight decreased by more than 20% of the initial bodyweight, or when the overall
539 health status was dramatically reduced, mice were sacrificed. (n ≥ 10) Mantel Cox test. (C)
540 Virus load in livers was determined 5 days post infection (dpi) by plaque assay. (n ≥ 7) (D)
541 Serum samples were drawn at the indicated time points and analyzed for ALT, AST, and
542 creatinin levels (n = 6-11). (E) F4/80 (red), VACV (green), and albumin (blue)
543 immunolabeling of livers 18 hpi. Scalebars 100 µm; inlay-scalebars 20 µm. Error bars
544 indicate mean ± SEM, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05; one-tailed Mann-Whitney U test.

545

546 **Figure 3: IFNAR-triggering protects against VACV-induced fulminant hepatitis.**
547 C57BL/6 mice and IFNAR$^{-/-}$ mice were i.v. infected with 2 x $10^6$ pfu VACV. (A)
548 Hematoxylin-and-eosin staining and immunohistochemical analysis of caspase-3 (Casp-3)
549 and iNOS of uninfected or infected livers 5 dpi. Scalebars 100 µm. (B) Quantification of (A) (n
550 = 3-5). Error bars indicate mean ± SEM, **p ≤ 0.01, *p ≤ 0.05; one-tailed Mann-Whitney U
551 test.

552

553 **Figure 4: Local IFN-I responses modulate Kupffer cell loss and myeloid cell infiltration**
554 **of the liver.** C57BL/6 mice and IFNAR$^{-/-}$ mice were i.v. infected with 2 x $10^6$ pfu VACV. (A)
555 Myeloid cells were isolated from liver at the indicated time points and analyzed by
556 flow-cytometry. MoMF = monocyte-derived marcrophages (CD11b$^+$ CD11c$^+$I-A/I-E$^-$), DC =
557 dendritic cells (CD11b$^+$CD11c$^+$I-A/I-E$^+$), KC = Kupffer cells (F4/80$^{hi}$CD11b$^+$). (B)
558 Quantification of myeloid cell subsets in the liver of (A) (n ≥ 7). (C) Mice were perfused at the
559 indicated time points, livers were fixed, and fluorescent immunolabeling was performed. (C)
560 Albumin (blue) and F4/80 (red) immunolabeling of livers 0, 2, and 4 dpi. Scale bars 100 µm;

561 inlay-scalebars 20 µm. (D) Quantification of (C) (n ≥ 4). Error bars indicate mean ± SEM; ***p
562 ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05; one-tailed Mann-Whitney U test.

563

564 **Figure 5: VACV-induced liver inflammation is controlled by direct IFNAR-triggering of**
565 **myeloid cells.** C57BL/6, Alb-Cre$^{+/-}$IFNAR$^{fl/fl}$, and LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice were i.v. infected
566 with 2 x $10^6$ pfu VACV. (A) Immunohistochemical analysis of Casp-3 expression in mice 5 dpi
567 (data shown for WT samples are identical with those shown in Fig. 3). (B) Quantification of
568 (A) (n = 3-5). (C) Serum samples were drawn and analyzed for ALT and AST levels (n = 3-6).
569 (D) Myeloid cells were isolated from livers 4 dpi and analyzed by flow-cytometry. (E)
570 Quantification of KC in livers of (D) (n ≥ 6). (F) Blood of uninfected C57BL/6, IFNAR$^{-/-}$, and
571 LysM-Cre$^{+/-}$IFNAR$^{fl/fl}$ mice was analyzed for IFNAR expression by flow-cytometry. (G)
572 Quantification of IFNAR expression (n ≥ 3, one out of two similar experiments is shown)..
573 Error bars indicate mean ± SEM; ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05; one-tailed
574 Mann-Whitney U test.

575

576 **Figure 6: Virus-induced IFN-I responses delay differentiation of infiltrating blood**
577 **monocytes to MoKC.** (A) Cx3cr1$^{+/gfp}$ mice were i.v. infected with 2 x $10^6$ pfu VACV. Myeloid
578 cells were isolated from livers 0 and 4 dpi and GFP expression of liver MoMF, DC, and KC
579 was analyzed by flow-cytometry. (B) Quantification of GFP expressing cells in (A) (n ≥ 5).
580 Mixed bone marrow (BM) chimeric mice (CD45.1$^+$WT and CD45.2$^+$IFNAR$^{-/-}$> CD45.1$^+$WT)
581 were i.v. infected with (C/D/E/F) 2 x $10^6$ pfu VACV or (G/H) 5 x $10^5$ pfu MCMV. Myeloid cells
582 were isolated from blood and livers at the indicated time points and analyzed by
583 flow-cytometry. (D/F/G) CD45.2$^+$ (IFNAR$^{-/-}$) MoMF, DC, and KC isolated from livers of
584 infected mice (n = 5-10). (E/H) Percentage of CD45.2$^+$ (IFNAR$^{-/-}$) monocytes isolated from
585 blood (n = 5-10). One out of two similar experiments is shown. Error bars indicate mean ±
586 SEM; ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05; one-tailed Mann-Whitney U test.