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Alloimmune Activation Enhances Innate Tissue Inflammation/Injury in a Mouse Model of Liver Ischemia/Reperfusion Injury[1]

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Abstract

The deleterious sensitization to donor MHC Ags represents one of the most challenging problems in clinical organ transplantation. Although the role of effector/memory T cells in the rejection cascade has been extensively studied, it remains unknown whether and how these "Ag-specific" cells influence host innate immunity, such as tissue inflammation associated with ischemia and reperfusion injury (IRI). In this study, we analyzed how allogeneic skin transplant (Tx) affected the sequel of host's own liver damage induced by partial warm ischemia and reperfusion. Our data clearly showed that allo-Tx recipients had increased inflammatory response against IR insult in their native livers, as evidenced by significantly more severe hepatocelluar damage, compared with syngeneic Tx recipient controls, and determined by serum ALT levels, liver histology (Suzuki's score) and intrahepatic pro-inflammatory gene inductions (TNF-α, IL-1β and CXCL10). The CD4 T cells, but neither CD8 nor NK cells, mediated the detrimental effect of allo-Ag sensitization in liver IRI. Furthermore, CD154, but not IFN-γ, was the key mechanism in allo-Tx recipients to facilitate IR-triggered liver damage. These results provide new evidence that alloreactive CD4 T cells are capable of enhancing innate tissue inflammation and organ injury via an Ag-non-specific CD154-dependent but IFN-γ independent mechanism.

Introduction

Transplant patients sensitized to a broad range of donor Ags through multiple blood transfusions, previous failed grafts or pregnancies, experience an increased rate of early rejection episodes, which are difficult to manage with currently used immunosuppressive agents (1–3). In experimental settings, graft rejection mediated by memory/effector T cells generated from previous exposure to allo-Ags or from heterologous immunity of the cross-reactive antimicrobial T cells, becomes resistant to the majority of newly developed immune response modifiers, including the costimulation blockers (4, 5). As rejection results from a complex immune cascade, with innate immune activation as the first line of host response, local organ injury due to ischemia and reperfusion (IRI) represents the key innate immune event in transplant recipients. Moreover, innate immunity plays a critical role in the activation of recipient adaptive responses and contributes to both acute and chronic rejection episodes (6–8). We and others have shown that the host sentinel innate Toll-like-receptor (TLR) system becomes activated during IR, leading to the local production of pro-inflammatory cytokine/chemokine programs, and upregulation of T cell costimulation molecules (9–15), essential for the adaptive T cell activation. Although IRI is traditionally

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thought as an innate immune-dominated tissue inflammation, it also involves the adaptive immune components (16, 17). The role of B cells has been identified in myocardial, skeletal muscle, and kidney reperfusion injury models (18–20). The role of T cells, initially demonstrated in liver IRI (21), has been later confirmed in kidney, cardiac, lung and brain ischemia models (22–25). Mechanistically, natural IgM Ab against self- Ags has been associated with B cell (18), whereas IFN-γ and CD154 appear necessary for CD4 T cell (26–28) function.

As a central paradigm of immune activation/regulation, the interaction between innate and adaptive cells is reciprocal. Innate antigen-presenting cells (APCs), such as DCs and macrophages, become activated by exogenous microbial/viral components or endogenous ligands, such as HMGB-1, to facilitate adaptive T cell activation by Ag-presentation, costimulation and cytokine/chemokine programs (29–31), whereas activated T cells may enhance or regulate innate cell function by T cell-derived cytokines or cell-cell contact (32, 33). Although IR-triggered innate immune activation affects alloreactive T cell activation in the rejection cascade (34, 35), whether and how Ag-activated T cells contribute to innate immune activation and function remains to be determined. The differentiation between allo-Ag-dependent and –independent mechanisms represents a challenging issue in dissecting alloreactive T cell function during the course of IRI in allo-transplant recipients.

The blockade of CD154, a key costimulatory molecule for CD4 and CD8 activation, inhibits alloreactive immune responses and prolongs allograft survival in otherwise normal hosts (36–39). However, in sensitized recipients, the role of CD154 signaling has been marginalized, with the activation and function of memory/effector T cells being largely CD154 independent, and allograft rejection becomes CD154 blockade resistant (40, 41). Although CD154 costimulation has been shown critically involved in the pathogenesis of IRI, whether it remains functional in allo-Ag-sensitized recipient in the liver innate immune activation remains to be determined.

The present study is the first to analyze the cross talk interactions between innate and adaptive immune mechanisms during the course of IR-mediated tissue damage. We utilized a murine model of liver partial warm IRI to determine the impact of allogeneic skin graft-induced “systemic” sensitization on local IRI immune cascade. In this setting, we were able to isolate the cross-talk between activated alloreactive T cells and allo-Ag independent innate immune response from the innate and adaptive integrated responses in the sensitized allograft rejection model. Results from this model may further our understanding of the pathophysiology of allograft rejection in sensitized recipients in which not only adaptive, but also innate immunity against the graft may be altered, as compared with those in naïve recipients.

Methods

Animals

Male wide-type (WT) and nude C57BL/6 (B6) and Balb/c (B/c) strains (8–12 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were housed in the UCLA animal facility under specific pathogen-free conditions, and received humane care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institute of Health (NIH publication 86-23 revised 1985).

Mouse warm hepatic IRI and its modulation in allo-Ag sensitized mice

Orthotopic full-thickness skin grafts (ca. 0.5 cm in diameter) from B/c (allogeneic) or B6 (syngeneic) donors were sutured bilaterally onto the flanks of WT B6 mice. Ten days later,

these mice were subjected to liver partial warm IR, as described (9, 28, 42). Briefly, mice anesthetized with sodium pentobarbital (60 mg/kg i.p) were injected with heparin (100 mg/Kg), and an atraumatic clip was used to interrupt the arterial and portal venous blood supply to the cephalad lobes of the liver. After 90 min of partial warm ischemia, the clip was removed, initiating hepatic reperfusion. Mice were sacrificed after 6 h of reperfusion. Sham WT controls underwent the same procedure, but without vascular occlusion.

The CD4 (clone GK1.5), CD8 (clone 2.43), NK (clone PK136) cell-depleting Abs were administered (0.5 mg/mouse i.v.) 24 h prior to liver IR insult. The anti-CD154 (MR1) or anti-IFN- γ (XMG1.2) Abs were given (0.5 mg /mouse) i.v. 1 h prior to the onset of ischemia.

For the nude mice reconstitution experiment: spleens and lymph nodes were pooled from 4–6 mice of either syngeneic or allogeneic skin transplanted recipient as described above at day 10 post Tx. CD4 T cells were isolated using the EasySep Negative Selection kit (StemCell Technologies, Vancouver, BC, Canada). Eight to ten millions of purified CD4 T cells were injected, i.v., into nude mice and these mice were subjected to liver IR at day 7 post T cell infusion.

Evaluation of the hepatocellular damage

Serum alanine aminotransferase (sALT) levels, indicator of hepatocellular injury, were measured using an auto analyzer (ANTECH Diagnostics (Los Angeles, CA). For histology, liver specimens were fixed in 10% buffered formalin and embedded in paraffin. Liver sections (4 μm) were stained with hematoxylin and eosin. The severity of IRI was graded blindly using modified Suzuki's criteria (43). In this classification, sinusoidal congestion, hepatocyte necrosis, and ballooning degeneration are graded on a scale of 0 to 4. No necrosis, congestion, or centrilobular ballooning is given a score of 0, whereas severe congestion /ballooning, and >60% lobular necrosis is given a value of 4.

Liver lymphocyte isolation and FACS analysis

To avoid enzyme digestion of lymphocyte cell surface markers, a mechanical method was used to separate intrahepatic lymphocytes from liver PCs. Livers, perfused *in situ* with 10 ml of cold PBS to remove circulating PBLs, were pressed through a sterile stainless steel screen in 30 ml RPMI media with 5% FBS and disperse cell aggregates with pippeting 4–5 times. The hepatocytes were removed by low-speed centrifugation at 50g at 4°C for 3 min. The supernatant was collected and centrifuged at 400g for 10 min, and the pellet was resuspended. The cell suspension was then layered on top of a density cushion of 25%/50% discontinuous Percoll (Pharmacia) and centrifuged at 900g for 20 min to obtain lymphocyte fraction at the interface.

Quantitative RT-PCR

Two and a half μg of RNA was reverse-transcribed into cDNA using SuperScriptTM III First-Strand Synthesis System (Invitrogen, Carlsbad, CA). Quantitative- PCR was performed using the DNA Engine with Chromo 4 Detector (MJ Research, Waltham, MA). In a final reaction volume of 25 μl, the following were added: 1×SuperMix (Platinum SYBR Green qPCR Kit, Invitrogen, Carlsbad, CA), cDNA and 0.5 mM of each primer. Amplification conditions were: 50 °C (2 min), 95 °C (5 min) followed by 50 cycles of 95 °C (15 s), 60 °C (30 s). Primers used to amplify specific gene fragments were described (9).

Statistical analysis

All values are expressed as mean ± SD. Data were analyzed with an unpaired two-tailed Student's t test. $P < 0.05$ was considered to be statistically significant.

Results

Liver IRI in allo-Ag sensitized recipients

To determine the impact of alloreactive immune activation on recipient's native tissue inflammation/injury, we studied liver IRI sequel in groups of allogeneic vs, syngeneic skin transplant recipients, and naïve mice. WT (B6) mice were first challenged with MHC-fully mismatched (B/c), or syngeneic (B6) skin grafts, and 10 days later, when both CD4 and CD8 T cells became fully activated by allo-Ag (41), the native livers were subjected to partial 90-min warm IR experiment. The hepatocellular damage was evaluated at 6 h and 24 h of reperfusion. Compared with naïve B6 mice, allo-sensitized recipients suffered significantly more severe liver injury, i.e., at 6 h, both sALT levels (IU/L; Fig. 1a: sALT: naive = 4660 ± 727.6; n=12, vs. allo = 14080 ± 2905; n=11; p=0.0093) and liver Suzuki's scores were significantly higher in allograft recipients, compared with naïve counterparts (Fig. 1b and 1c, Suzuki's scores: naïve = 6.75±0.75 vs. allo = 9.67±0.3, $p<0.05$). Although by 24h no significant differences in sALT levels were found between the two animal groups, liver Suzuki's scores still remained higher in allo-transplant recipients, compared with naïve hosts (Fig. 1c, Suzuki's scores: naïve = 2.5±0.5 vs. allo = 5.5±0.5, $p<0.05$), suggesting the persistence of the more severe liver tissue injury due to host allosensitization. The aggravated hepatocellular damage after skin-induced host sensitization was due to allo-immune activation, as syngeneic skin grafted and naïve mice had comparable sALT levels and liver Suzuki's scores (e.g., at 6 h, sALT: syn = 6855 ± 1229; n=14; p=0.15 vs. naïve; p=0.02 vs. allo; Suzuki's score: syn = 7.25 ± 0.85, p=0.6). In agreement with the degree of hepatocellular damage, IR-induced liver inflammation, as measured by local expression of mRNA coding for TNF-α, IL-1β and CXCL10, was selectively elevated in allo-sensitized recipients (Fig. 1d).

CD4 T cells are required for liver IRI in allo-sensitized hosts

Although allo-Ag sensitization results in activations of multiple types of host adaptive immune cells, these cells are not supposed to target to host own livers. To address the question of whether alloreactive T cells are responsible for the aggravated liver inflammation/injury in sensitized recipients. we adopted an Ab-mediated depletional approach in these mice.

First, we confirmed T cell activations by allogeneic skin grafts in both spleen and liver by FACS, as evidenced by increased number of pro-inflammatory effector ($CXCR3+CD62L^{low}$) CD4 and CD8 T cells in allogeneic, but not syngeneic skin transplant groups (Fig. 2a, b). The frequency of activated effector CD4 T cells, represented by percentage of $CD4+CXCR3+CD62L^{low}$ subset, nearly doubled in spleens of allo-sensitized mice (naïve: 14.47 ± 0.34 vs. allo: 21.57 ± 2.2; n=3, $p<0.05$). Interestingly, this CD4 effector subset was selectively enriched in liver compartment of naïve hosts (25.62 ± 0.68; n=3) as compared with the spleens, increased further following allo-Ag activation (32.38 ± 0.24; n=3; $p<0.02$ vs. naive). For CD8 T cells, the enrichment of $CXCR3+CD62L^{low}$ subset after allo-sensitization was also evident in both spleen (naïve = 10.71 ± 1.795, vs. allo: 42.45 ± 6.005; n=3; $p<0.02$) and liver (naïve = 28.95 ± 4.545 vs. allo: 51.97 ± 6.830; n=3; $p<0.05$) compartments.

To determine the functional significance of T cells in this model, we selectively depleted CD4 or CD8 T cells 24 h prior to the onset of liver ischemia (day +9 post-skin Tx to avoid the interference of host sensitization process). Controls consisted of pre-sensitized recipients depleted of NK cells, which do not contribute to the mechanism of liver IRI in otherwise normal mice (44). The hepatocellular damage was measured at 6 h post reperfusion. As shown in Fig. 3, the depletion of CD4 T cells, but not CD8 T cells or NK cells, protected

livers from IRI in sensitized mice. Indeed, sALT levels (IU/L) were significantly lower (allo/CD4: 2340 ± 716.0 n=8, vs. allo: 13060 ± 2843; n=12; $p<0.01$) and livers showed much better preserved histological architecture after CD4 depletion (Fig. 3a, b). In parallel, IR-induced liver pro-inflammatory TNF-α, IL-1b and CXCL10 expression remained suppressed in CD4-, but not CD8- or NK- depleted groups (Fig. 1c).

To further establish the role of CD4 T cells as the key mediator of aggravated liver inflammation/injury in the allo-sensitized recipients, we reconstituted nude mice with purified CD4 T cells from either syngeneic or allogeneic skin Tx recipients. These T cells from different Tx recipients repopulated the peripheral blood of nude mice at similarly levels (Fig. 4. a, 1.7±0.4 vs. 1.3±0.5%), which are significantly lower than the normal CD4 levels in WT mice (15±3%). As demonstrated previously, nude mice themselves (Gr#1) were resistant to liver IRI. CD4 T cell-reconstituted nude mice became sensitive to the liver injury, as evidenced by liver pathology and pro-inflammatory gene TNF-α induction post IR (Fig. 4.c, d, e). CD4 T cells derived from allogeneic skin Tx recipients (Gr#3), indeed, had more potent effects than those from syngeneic skin recipients (Gr#2), to recreate liver IRI, as shown by the higher sALT levels (2353±755 vs. 588±60, n=4, $p=0.04$), worse liver pathology (Suzuki score=5.0±1.0 vs. 2.75±0.95, $p=0.04$) and higher proinflammatory gene (IL-1β and CXCL10) inductions (Fig. 4). Thus, CD4 T cells from allo-skin transplant recipients were sufficient to mediate the impact of allo-immune activation on liver IRI.

CD154, but not IFN-γ, is critical for allo-immune mediated liver IRI

Our model of allo-Ag induced sensitization provides a unique opportunity to address the question as to whether the costimulatory pathways act directly to stimulate T cell or alternatively they affect innate immune activation in the mechanism of liver IRI. Indeed, CD154 signaling is no longer required for CD4 or CD8 activation, and CD154 blockade fails to inhibit allograft rejection in the sensitized state (41, 45). Thus, we infused pre-sensitized mice with anti-CD154 MR1 Ab one hour prior to the onset of ischemia, and then evaluated the hepatocellular damage at 6 h of reperfusion. Interestingly, CD154 blockade effectively protected livers from IRI in sensitized mice, with serum ALT levels (IU/L) significantly lower (2559 ± 501.3; n=7, vs. control = 14530 ± 4936; n=5; $p<0.016$), and liver architecture better preserved, compared with controls (Fig. 5a, b). Moreover, CD154 blockade markedly inhibited IR-triggered liver pro-inflammatory IL-1β, TNF-α and CXCL10 gene induction profile (Fig. 5c). In marked contrast, although activated T cell-derived IFN-γ has been implicated in the mechanism of IRI (26), the neutralization of IFN-γ failed to protect livers from IR-triggered damage (sALT = 10980 ± 3175; n=8; p=NS). Thus, CD154 rather than IFN-γ, signaling is critical in the pathophysiology of liver inflammation and tissue injury in allo-Ag sensitized recipients.

Discussion

Although the concept of two-way cross talk between innate and adaptive immunity has been well recognized (46, 47), most evidence comes from Ag-specific disease models, such as infections and organ transplantation with T cell activation as the readout. This study is the first to analyze the reverse effects of alloimmune activation on the innate immunity, as measured by the outcome of Ag non-specific tissue inflammation/injury. As inflammation/injury occurred in host/recipient's own organs, which by definition would not be recognized by allo-Ag-activated T cells, these activated T cells interacted with liver innate immune cells in an Ag non-specific fashion. Indeed, our data shows that the activation of alloimmune system enhances liver innate immune response against IR; and that the CD154-CD40 pathway rather than IFN-γ signaling plays a critical role in this adaptive - innate immune cross talk. The latter finding is of particular interesting in the context of allograft rejection in sensitized recipients, as this costimulatory pathway is required for naïve but not effector/

memory T cell activation, and CD154 blockade fails to protect allografts from either CD4 or CD8 effector/memory T cell-mediated rejection (40, 41, 45). Here, by employing the very same allo-sensitized transplant model, we demonstrate, for the first time, that CD154-CD40 signaling between T cells and innate immune cells, such as macrophages, remains to be the key mechanism of T cell help for innate immune activation in both naïve and sensitized recipients. Hence, a two-way signaling pathway may have distinctive properties at different ends of the interaction: i.e., although CD154-CD40 signaling losses its impact on effector/memory T cell activation, it remains critical for the activation of innate immune cells in sensitized hosts. This finding further supports our hypothesis that CD154 functions in liver IRI by directly activating innate immune cells via CD40 rather than activating T cells (44). In addition, as Ag-activated T cells function in a Ag-non-specific manner, our results implicate that any Ag-activated T cells, such as those from infections, may also have the same effect on liver innate immune-mediated inflammation/injury.

The issue of T cell regulation of innate immune activation is re-emerged in recent years, partly because of the ability of natural regulatory T cells to suppress not only adaptive T cell-dependent, but also innate immune-mediated pathology (48, 49). The $CD4^+CD25^+$ Treg cells elaborate immune-regulatory cytokines, such as IL-10, TGF-b, IL-4 and IL-13, which do suppress pro-inflammatory cytokine production by macrophages. However, this regulation requires cognate T cell stimulation, and kinetically it occurs late at the innate activation/effector stage. More recently, conventional CD4 and CD8 T cells were also found to be able to downregulate innate immune response at the very early stage by MHC-dependent cell-cell contact mechanism and in Ag non-specific manner (32). In this particular case, naïve T cells exerted comparable suppressive effects as memory cells on TLR3-initiated innate immune response; the suppression of IFN-γ production was more prominent than that of TNF-α; and NK cells represented one of the important innate immune targets. These findings are distinctive from our present study in which neither IFN-γ nor NK cells played any significant role in liver IRI (44). In addition, effector CD4 T cells rather than naïve or CD8 T cells are functioning as the main regulators of TLR4-initiated liver innate immune response in our model in the absence of cognate Ag stimulations. Although our data fail to show the effect of CD8 depletion in allo-sensitized recipients, one potential reason could be the incomplete depletion of CD8 effector/memory T cells, which are known to be resistant to Ab-mediated depletion (50). Interestingly, it has been reported most recently that effector/memory CD4 T cells are able to function in a cognate manner to abolish macrophage caspase-1 activation triggered specifically by Nod-like receptor (NLR), but not TLR4 or TLR3 ligands. However, these T cells regulated other NLR-triggered pro-inflammatory pathways in the opposite way (51) that effector CD4 T cells, in fact, promote upregulation of MHC class II, CD40 and TNF-α gene expression programs in macrophages. CD154 is critical for this T cell regulation of NLR-mediated innate immune activation. Similar to the case of Treg, this conventional T cell-mediated innate immune regulation seems to act as a feedback loop to prevent excessive tissue inflammation, which kinetically occurs late during immune responses.

The distinctive roles of CD154-CD40 pathway in both innate and adaptive immune activation have been extensively studied. The CD40 is expressed on APCs, including DCs, activated macrophages and B cells. Its activation by CD154 ligation, in conjunction with TLR activation, results in the full maturation (or licensing) of APCs, leading to their increased proficiency of Ag presentation, cytokine/chemokine production and longer survival (52, 53). These result in clonal expansion and differentiation of the reciprocal cognate T cells. We have previously shown that CD40 upregulation induced by TLR4 ligands represents the triggering event for CD154-CD40 activation in liver immune response against IR (44). Liver CD4 T cells are highly enriched with CD154-expressing effector memory type cells. As shown here, skin sensitized recipients have increased numbers/

percentages of effector memory CD4 T cells in both liver and at the periphery, which in turn may increase CD154-CD40 interactions, ultimately leading to the enhancement of liver inflammation during IR in allotransplant recipients. Indeed, the ability of CD154 blockade to ameliorate liver IRI in the sensitized state provides evidence that CD154-CD40 signaling is the key mediator of allo-immune response in innate immunity-driven liver inflammation and injury.

In summary, our study shows that activated alloreactive CD4 T cells reciprocally regulate innate immune response by enhancing local organ inflammation/injury via Ag-non-specific CD154-dependent but IFN-γ independent mechanism.

Abbreviations

IRI	ischemia and reperfusion injury
TLR	toll-like receptor
APCs	antigen presenting cells
sALT	serum alanine aminotransferase

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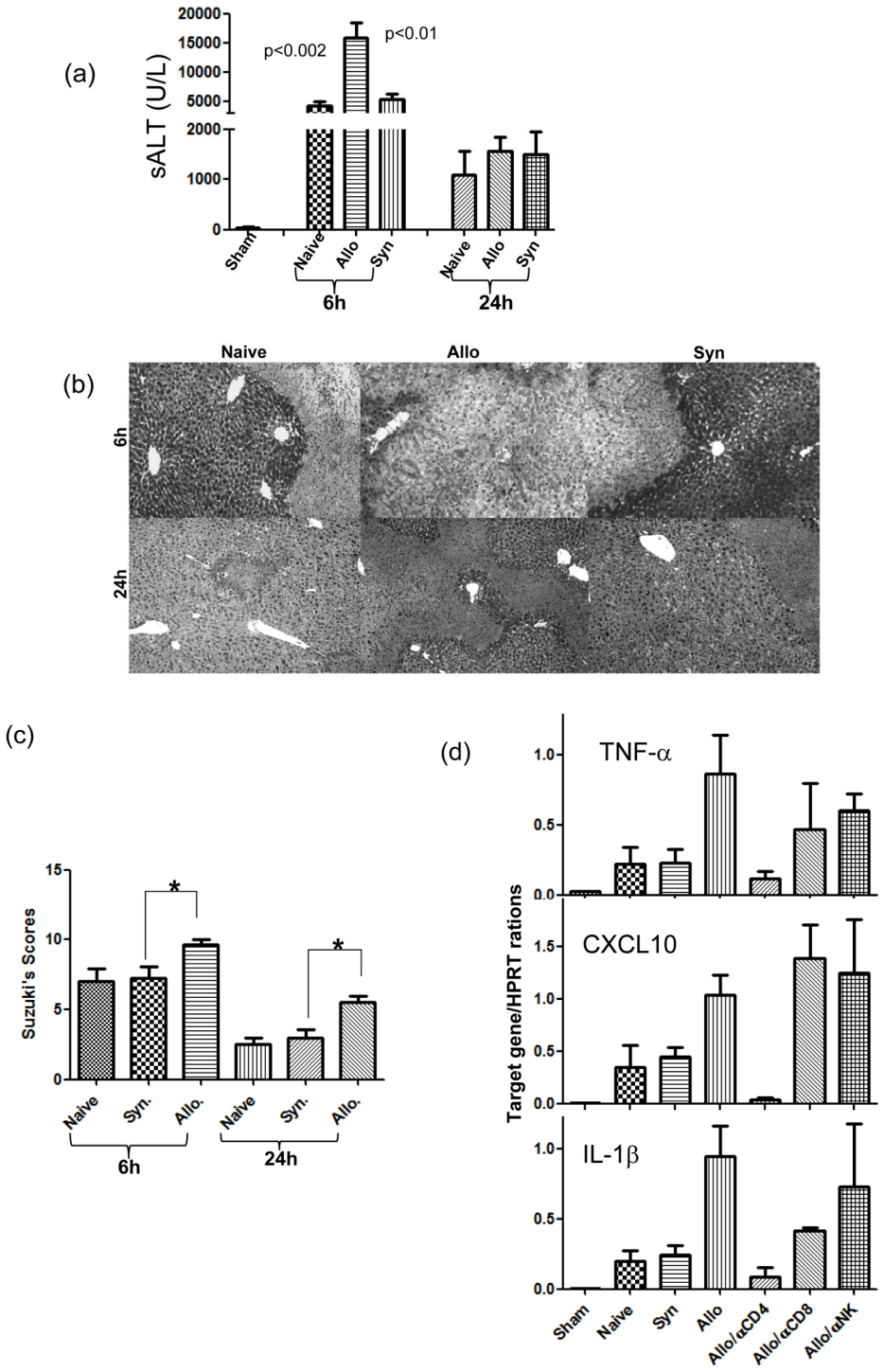
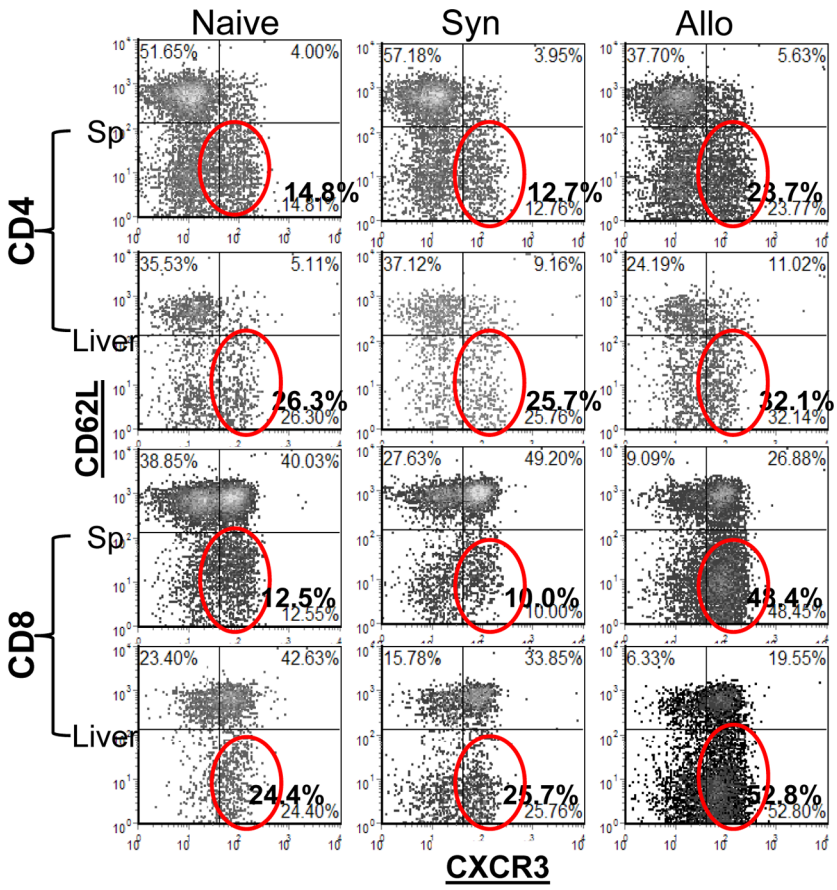


Figure 1.

Alloimmune activation enhances liver IRI. (a). WT C57BL/6 mice were either left untreated (naïve), or were challenged with a syngeneic (Syn) or MHC-fully-mismatched Balb/c (Allo) skin grafts 10 days prior to being subjected to liver IR, as described in Material and Methods. Liver injury was evaluated at 6 h and 24 h post-reperfusion by measuring sALT levels (n=11–14/group). Liver tissue was harvested at 6 h and 24 h post reperfusion for

histological and molecular analyses. Representative liver H/E sections (100x) from each experimental group are shown (b), as well as their average Suzuki's scores (c). Liver expression of TNF-α, CXCL10, and IL-1β genes was determined by target gene/HPRT ratios and measured by qRT-PCR (d, n=3–4/group).

(a)



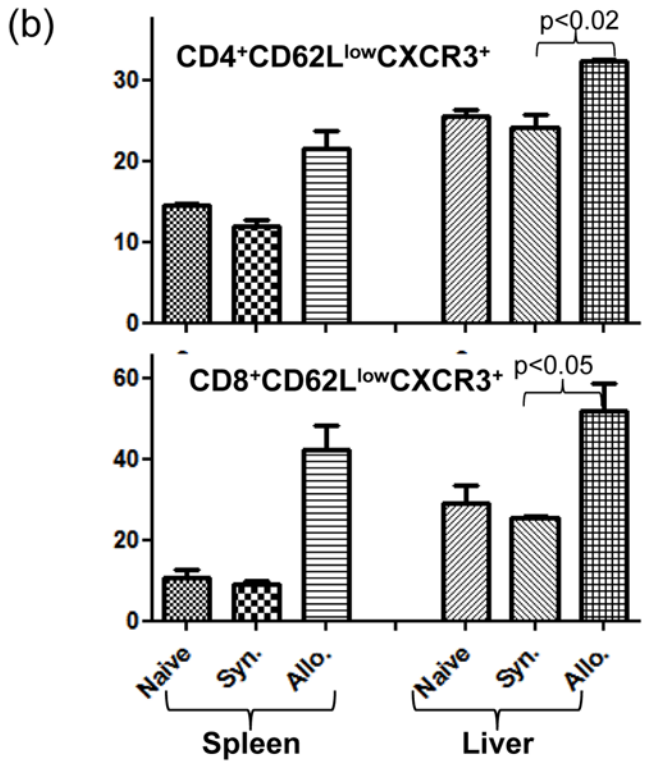


Figure 2.

Effector/memory T cells in sensitized recipients. T cell activation in naïve, syn- or allo-skin transplanted recipients was evaluated by FACS analysis of spleen or liver-infiltrating lymphocytes, as described Material and Methods. CD4+ or CD8+ lymphocytes were gated and further analyzed for their CD62L and CXCR3 expression. Effector (or effector memory) T cells were defined by the frequency of $CXCR3^{+}CD62L^{low}$ subpopulation (circled). Representative density plots of effector CD4 or CD8 T cells in spleens and livers are shown (a), and their average percentages in each experimental group are plotted (b). N=3/group

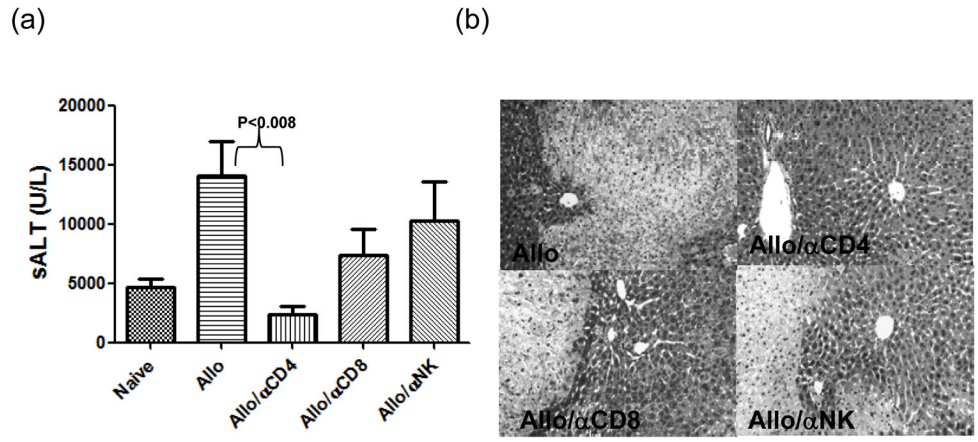


Figure 3.

CD4 T cells mediate the enhanced liver IRI in sensitized recipients. C57BL/6 mice were either left untreated (naïve), or transplanted with a MHC-fully-mismatched Balb/c (Allo) skin graft. At day 9 post skin challenge, recipient mice were treated with CD4, CD8, or NK cell-depleting Ab, as described in Material and Methods. At day 10, these mice were subjected to liver partial warm ischemia (90 min), followed by evaluation of the hepatocellular damage (sALT levels) at 6 h post-reperfusion (a, n=6–8/group). Liver tissue samples were also harvested, and representative H/E sections (100x) are shown (b). Liver expression of TNF-α, CXCL10, and IL-1β, as determined by target gene/HPRT ratios, and measured by qRT-PCR (c, n=3–4/group).

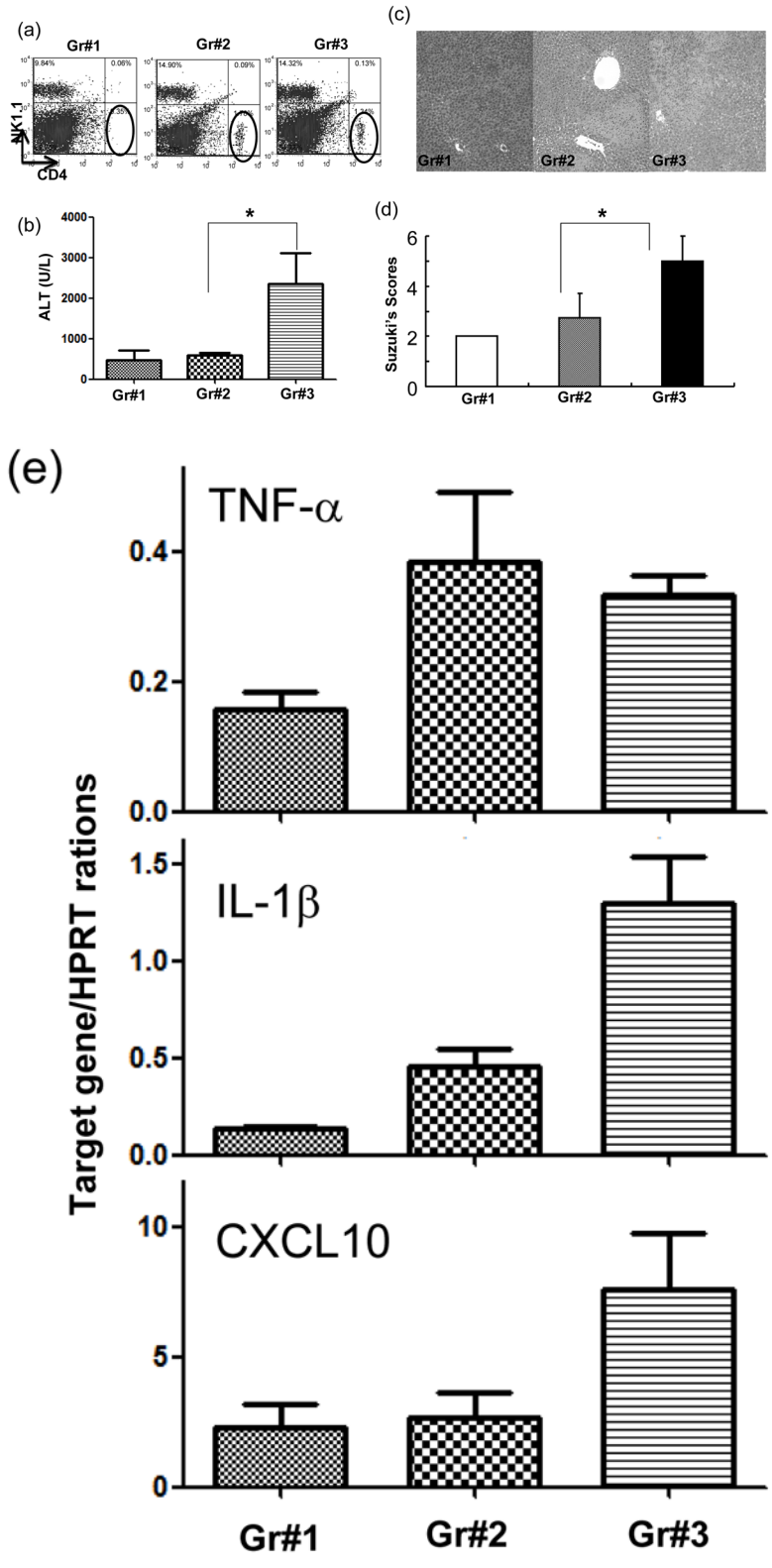


Figure 4.

Allogeneic-Ag activated CD4 T cells recreated liver IRI in nude mice. Nude mice were either untreated (Gr#1) or reconstituted with purified syngeneic CD4 T cells from either syn- (Gr#2) or allo-geneic (Gr#3) skin Tx recipients, as described in the material and methods. CD4 T cells were detected by FACS at similar levels in both groups of reconstituted nude mice (a). These mice were then subjected to liver partial warm ischemia (90 min), followed by measurement of sALT levels at 6 h post-reperfusion (b). Representative liver H/E sections (100x) from each experimental group are shown (c) and Suzuki scores for each groups were plotted (d). Liver expression of TNF-α, CXCL10, and IL-1β, measured by target gene/HPRT ratios, were determined by qRT-PCR (e). n=4/group.

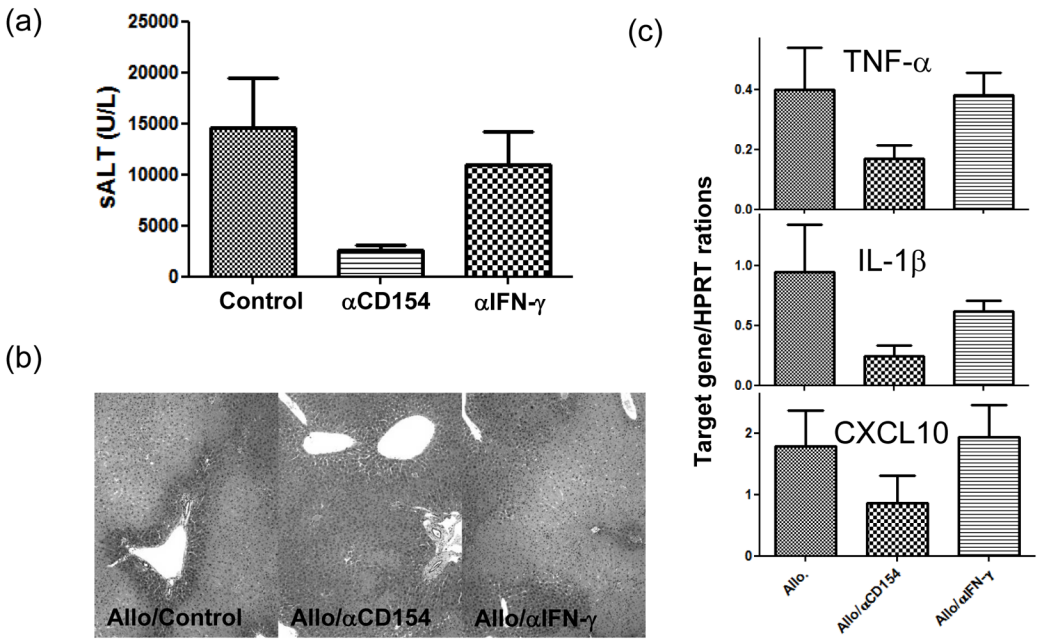


Figure 5.

CD154 signaling is critical for alloimmune-mediated liver IRI. C57BL/6 mice were transplanted with MHC-fully-mismatched Balb/c (Allo) skin grafts. At day 10, recipient mice were treated with either control Ig, or anti-CD154 or anti–IFN-γ Ab, as described in Material and Methods. These mice were then subjected to liver partial warm ischemia (90 min), followed by measurement of sALT levels at 6 h post-reperfusion (a, n=5–8/group). Representative liver H/E sections (100x) from each experimental group are shown (b). Liver expression of TNF-α, CXCL10, and IL-1β, determined by target gene/HPRT ratios, and measured by qRT-PCR (c, n=3–4/group).