The role of BAFFR and Qa-1b during viral infection

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2 Abbreviations

APCs: Antigen presenting cells

APRIL: A proliferation-inducing ligand

BAFF: B-cell activating factor

BAFFR: B-cell activating factor receptor

BCMA: B-cell maturation antigen

cSMA: Central supramolecular activation cluster

cART: Antiretroviral therapy

CTLs: Cytotoxic T lymphocytes

CpG: 5'-C-phosphate-G-3'

CMP: Common myeloid progenitor

CPL: Common lymphoid progenitors

CRTAM: Class I-restricted T cell-associated molecule

DCs: Dendritic cells

EBV: Epstein-Barr virus

GATA3: GATA binding protein 3

GMP: Granulocyte-monocyte progenitor

HBsAg: Surface antigen of Hepatitis B virus

HSV: Herpes simplex virus

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

HLA-DR: Human leukocyte antigen-antigen D related

HLA-E: Human leudocyte antigen-E

IBD: Inflammatory bowel disease

ICAM-1: Intercellular adhesion molecule 1

Id2: Inhibitor of DNA binding 2

ISGs: IFN-stimulated genes

IRFs: IFN regulatory factors

IFN-Is: Type I interferons

IFN-α: Interferon-alpha

IFN-γ: Interferon-gamma

IgG: Immunoglobulin G

IgM: Immunoglobulin M

IL-21: Interleukin-21

ILCs: Innate lymphoid cells

iNKT: Invariant natural killer T cells

Jak1: Janus kinase 1

KIR: Killer-cell immunoglobulin-like receptor

KLRG1: Killer cell lectin-like receptor subfamily G member 1

Ltα: Lymphotoxin alpha

Ltβ: Lymphotoxin beta

LCMV: Lymphocytic choriomeningitis virus

LAG-3: Lymphocyte-activation gene3

LFA-1: Lymphocyte function-associated antigen-1

LTi: lymphoid tissue inducer cell

MICA-B: MHC class I chain-related protein A and B

MSV: moloney sarcoma virus

MAPK: Mitogen-activated protein kinases

NK cells: Natural killer cells

NKR-P1A (KLRB1): Killer cell lectin-like receptor subfamily B, member 1

NFIL3: Nuclear factor interleukin-3 regulated

PBMC: Peripheral blood mononuclear cells

pMHC: Peptide-loaded major histocompatibility complex

PI3K: Phosphatidylinositide 3-kinase

PD-1: Programmed cell death protein 1

RNP: Ribonucleoprotein

TACI: Transmembrane activator and calcium modulator cyclophilin ligand interactor

TIM-3: T-cell immunoglobulin and mucin-domain containing-3

Tox: Thymocyte selection-associated high mobility group box

Tyk2: Tyrosine kinase 2

TNFα: Tumor necrosis factor alpha

TLRs: Toll-like receptors

RORγt: Retinoid-related orphan receptor γt

Stat2: Signal transducer and activator of transcription

ULBP1: UL16 binding protein 1

Usp18: Ubiquitin Specific Peptidase 18

VSV: Vesicular stomatitis virus

3 Summary

The first part of the thesis demonstrates that the B cell-activating factor receptor (BAFFR), as a type III transmembrane protein in the TNF receptor family, is essential for B cell development and furthermore for the generation of antibodies for the protection against infections and maintain homeostasis.

Patients who have BAFFR deficiency have been identified within cohorts with common variable immunodeficiency, the most prevalent symptomatic primary immunodeficiency in adult patients. Usually these patients display impaired immunoglobulin production and severe B cell lymphopenia. Furthermore, the lack of BAFF signaling in BAFFR deficient animals is also related with severe B cell lymphopenia. However, whether the lack of BAFF/BAFFR signaling may impact the immune responses following viral infection has not yet been studied.

Normally, effective immune activation can help body defense against viral infection successfully. In mice, $CD169^+$ macrophages in the marginal sinus of the spleen and in the subcapsular space of the lymph node filter pathogens from the blood and the lymph respectively. These $CD169^+$ macrophages also play critical role in the production of innate cytokines and the presentation of viral antigens to B cells and represent an important link to bridge innate and adaptive immunity.

In our study, we use C57BL/6 and *Baffr*$^{-/-}$ mice to investigate the role of BAFFR in the immune system after VSV and LCMV infection. And we found that the absence of BAFFR caused reduced lymphotoxin expression, decreased number of $CD169^+$ cells, delayed and impaired innate and adaptive immune responses. Moreover, after 10^5pfu VSV infection, BAFFR deficient mice also develop fatal disease.

The secondary part of this thesis shows that a high dose of virus infection can result in

inhibition of NK cell activation, which is associated with enhanced expression of Qa-1b. Qa-1b, as an MHC Ib molecule, can be served as the ligand for inhibitory receptor on NK cells. And the expression level of Qa-1b on B cells is significantly upregulated after LCMV infection, plus this upregulation is dependent on type I interferons.

NK cells, as the member of type I ILCs, express Eomesodermin and T-bet and can induce perforin, granzyme and IFN-γ. And NK cells can control infection induced by herpes simplex virus-1, influenza virus and ectromelia virus and murine cytomegalovirus via the production of IFN-γ or cytotoxicity. Nevertheless, NK cells play a dual role following viral infection. On the one hand, they display anti-viral functions; on the other hand, they can also target virus-specific T cells after viral infection and contribute to the establishment of chronic viral infection.

In addition, the murine Qa-1b molecule and its human homolog HLA-E can interact with NKG2 family members. However, the affinity of HLA-E to the inhibitory receptor CD94/NKG2A is higher than to the activating receptor CD94/NKG2C, indicating that the inhibitory signals might be dominating. But NKG2A can be detected on naïve NK cells and activated T cells in contrast to NKG2C or NKG2E with an anti-NKG2A/C/E antibody. Moreover, deletion of NKG2A leads to over-activation of T cells. Despite these findings demonstrate the important interaction between Qa-1b and the receptors of NK cell, the role of Qa-1b in viral infection still needs to be explored.

Taken together, large virus load causes enhanced expression of Qa-1b on B cells. Lack of Qa-1b leads to increased NK-mediated regulation toward T cells in LCMV infection. Therefore, Qa-1b and NKG2A deficient mice show decreased T cell response and defective viral control. Additionally, NK cell depletion could partially restore anti-viral T cell immunity and alleviate pathology in the absence of Qa-1b.

4 Zusammenfassung

Im ersten Teil der Arbeit konnte gezeigt werden, dass der Rezeptor des B-Zellaktivierungsfaktors (BAFFR), ein Typ-III Transmembranprotein welches der großen Familie der TNF-Rezeptoren angehört, maßgeblich zur B-Zellentwicklung beiträgt. Die Signaltransduktion über die Bindung von BAFF an seinen Rezeptor spielt eine wichtige Rolle bei der Entwicklung, Reifung und dem Überleben von B-Zellen. Darüber hinaus reguliert er die Antikörperproduktion zum Schutz vor Pathogenen und ist damit an dem Erhalte der Homöostase beteiligt.

Patienten mit einer genetischen Defizienz des BAFF-Rezeptors leiden häufig an dem Krankheitsbild des variablen Immundefektsyndroms (CVID), dessen charakteristisches Merkmal ein Mangel an Immunglobulinen und B-Zellen sind. Ähnlich dem humanen System zeigen auch BAFFR-defiziente Mäuse eine deutliche B-Zell-Lymphopenie. In wie weit der BAFF/BAFFR- vermittelte Signalweg möglicherweise die Immunreaktion während einer viralen Infektion beeinflussen könnte ist jedoch bisher noch nicht untersucht worden.

Normalerweise erfolgt die Abwehr von viralen Infektionen über eine effiziente Aktivierung des Immunsystems. Im murinen Organismus können $CD169^{+}$ Makophagen in der Marginalzone der Milz und in dem subkapsulären Sinus von Lymphknoten identifiziert werden. Funktionell sind sie sowohl an der Produktion von Zytokinen als auch der Präsentation viraler Antigene gegenüber B-Zellen beteiligt und tragen damit bedeutend zur Interaktion zwischen dem angeborenen und adaptiven Immunsystem bei.

In der vorliegenden Arbeit wurden *Baffr*$^{-/-}$-Mäuse im Vergleich zu C57BL/6-Mäusen zur Analyse der BAFFR-vermittelten Signaltransduktion während der VSV und LCMV Infektion verwendet. Dabei konnte neben einer verminderten Lymphotoxin-

Expression und reduziertem Vorkommen von $CD169^+$ Makrophagen ferner eine verzögerte und beeinträchtigte Reaktion des angeborenen und adaptiven Immunsystems detektiert werden. Des Weiteren wurde in BAFFR-defizienten Tieren in Folge der VSV-Infektion mit 10^5 PFE ein massiver Infektionsverlauf im Vergleich zu WT Tieren induziert werden.

Der zweite Teil der vorliegenden Arbeit zeigt, dass eine starke Virusinfektion NK-Zellen inhibieren kann, welches mit einer verstärkten Expression von Qa-1b assoziiert ist. Qa-1b kann als MHC-Ib-Molekül ferner als Ligand für inhibitorische Rezeptoren auf NK-Zellen dienen. Während einer Infektion mit LCMV steigt das Expressionsniveau von Qa-1b abhängig von Typ-I Interferon signifikant an.

NK-Zellen exprimieren als ILC-Typ I Eomasodermin und T-bet und können die Sekretion von Perforin, Granzym und IFN-γ induzieren. Des Weiteren konnten diverse Studien zeigen, dass NK-Zellen Infektionen des Herpes-Simplex-Virus-1, Influenzavirus und Ectromelia-Virus sowie des Maus-Cytomegalievirus über die Produktion von IFN-γ oder Zytotoxizität kontrollieren können. Dennoch besitzen NK-Zellen während einer Virusinfektion eine duale Funktion. Auf der einen Seite sind sie Bestandteil der antiviralen Immunantwort auf der anderen Seite können sie jedoch über die Interaktion mit Virus-spezifischen T-Zellen die Induktion einer chronischen Virusinfektion begünstigen.

Zusätzlich können das murine Qa-1b-Molekül und sein humanes homolog HLA-E mit Mitgliedern der NKG2-Familie interagieren. Die Affinität zwischen HLA-E und dem inhibitorischen Rezeptor CD94/NKG2A sind dabei höher als zu dem aktivierenden Rezeptor CD94/NKG2C, welches darauf hindeutet, dass die inhibitorischen Signale dominieren. Unterstützt wird diese Hypothese über die Expression von NKG2A auf nai-

ven NK- und aktivierten T-Zellen, im Gegensatz zu den anderen NKG2-Familienmitgliedern. Darüber hinaus resultiert die Deletion von NKG2A in hyperreaktiven T-Zellen.

Zusammenfassend zeigen die Ergebnisse der vorliegenden Arbeit, dass eine große Viruslast eine verstärkte Expression von Qa-1b auf B-Zellen induziert. So entwickeln Qa-1b defiziente Mäuse eine reduzierte T-Zell vermittelte Immunantwort und weisen eine signifikant reduzierte Kontrolle über den Virus auf. Ferner induzierte die Depletion von NK-Zellen eine verbesserte antivirale T-Zellantwort und reduzierte Pathologie in Qa-1b defizienten Tieren.

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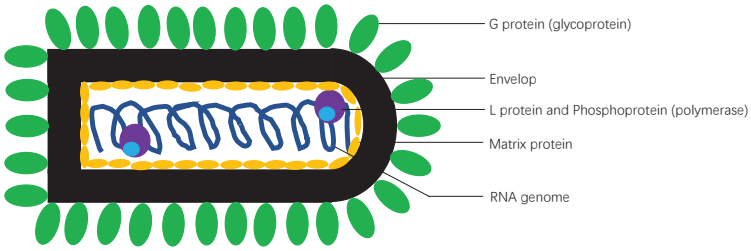
6 Introduction

6.1 Viruses

6.1.1 Vesicular Stomatitis Virus

Vesicular stomatitis virus (VSV), as a member of the *Rhabdoviridae* family, is an enveloped, bullet-shaped virion containing a prototypic, non-segmented negative sense RNA. Its 11kb genome encodes five genes: nucleocapsid protein, phosphoprotein, matrix protein, glycoprotein and large polymerase. These viral proteins assemble to an approximately 185nm x 75nm virion (Ge, Tsao et al. 2010) (Graphical fig 1).



Graphical fig 1 The structure of VSV: The figure shows the structure of vesicular stomatitis virus, G protein (green), envelop (black), L protein and phosphoprotein (purple and blue), matrix protein (yellow) and RNA genome (dark blue) (Fuchs, Frank et al. 2015)

There are two major VSV serotypes: Indiana and New Jersey. Normally, pigs, horses, cattle, other mammals and their insect vectors can be infected by VSV as the natural hosts (Hansen, Thurmond et al. 1985, Drolet, Campbell et al. 2005). However, cases of VSV infection in humans are rare, and these infections mainly occur among veterinarians and researchers who are exposed to VSV (Reif, Webb et al. 1987).

Understanding how VSV infects a host is an essential approach to eliminating the virus. The process of VSV infection includes viral entry, viral replication, viral assembling and viral exit. During the viral entry, VSV glycoprotein G plays a significant role in the interaction between the viral protein and the receptor on the host cell. Since VSV attaches to the host cell, it triggers membrane fusion through a pH-dependent approach

(Roche, Albertini et al. 2008). Besides VSV G protein, the membrane lipid phosphatidylserine and the endoplasmic reticulum chaperone Gp96 are also critical for VSV entry (Coll 1995, Coll 1997, Hall, Burson et al. 1998, Bloor, Maelfait et al. 2010). After VSV enters the host cell, it begins the process of replication. To maintain virus replication successfully, it is necessary for host cells to provide an appropriate environment for viral genome transcription, replication and translation. The VSV polymerase is similar to other RNA viruses in that it lacks a proofreading function and usually shows high mutation frequencies; this characteristic makes VSV enable to adapt to different environments (Steinhauer and Holland 1986, Steinhauer, de la Torre et al. 1989, Holland, Domingo et al. 1990). In the cytoplasm of the host cell, VSV L protein and P protein form a complex to start primary transcription of viral mRNAs (Gao and Lenard 1995, Green and Luo 2009). After transcription, VSV mRNAs take advantage of host cell ribosomes to translate viral proteins, while the process of host protein synthesis is inhibited in the infected cells (Whitlow, Connor et al. 2006, Whitlow, Connor et al. 2008). Then viral proteins assemble to new virions, and the virion then exits from the host through the site in the host plasma membrane which enriched in VSV G protein by budding (Swinteck and Lyles 2008).

Furthermore, in order to infect host successfully, VSV developed strategies to impact the immune systems of the host. For example, wild-type VSV Indiana can inhibit interferon (IFN) induction, while the VSV Indiana with a mutant T1026R1 in M protein is a good inducer of IFN. This result indicates that the M protein of VSV plays a critical role in IFN gene regulation (Ferran and Lucas-Lenard 1997). Similarly, mice with a deficiency of interferon alpha/beta receptor gene, but not mice with the ablation of the interferon gamma receptor gene, are highly susceptible to VSV infection (Steinhoff, Muller et al. 1995, van den Broek, Muller et al. 1995). Because of the importance of IFN in the early stage of the anti-viral response, taking advantage of interferon to treat mice following VSV intranasal inoculation could increase the survival rate in mice (Gresser, Tovey et al. 1975).

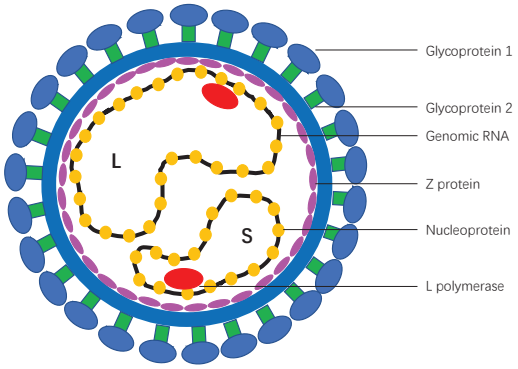
Besides the interferon system as the key for virus defense, nitric oxide (NOS) is also important for anti-viral response. The expression of nitric oxide synthetase increases in olfactory bulb neurons after VSV infection, and blocking the nitric oxide signal with 7-nitroindazole causes a 10-fold higher titer of virus in the brain (Komatsu, Bi et al. 1996).

Even though VSV can result in diseases as severe vesiculation or ulceration of the tongue, oral tissues, feet and teats (Letchworth, Rodriguez et al. 1999), the characteristics of VSV, such as rapid replication, high virus yields and a small, easily manipulated genome, make it a popular model virus for many basic research applications. Additionally, its antigenic distinction from common human pathogens and its inability to transform host cells make VSV a widely used experimental platform for vaccine vectors (Rose, Roberts et al. 2000, Reuter, Vivas-Gonzalez et al. 2002, Bukreyev, Skiadopoulos et al. 2006). Finally, VSV also plays a significant role in oncolytic virus therapy of anti-cancer approaches because of its inherent oncolytic virus qualities (Hastie and Grdzelishvili 2012).

6.1.2 Lymphocytic Choriomeningitis Virus

Lymphocytic Choriomeningitis Virus (LCMV) belongs to the *Arenaviridae* family, which was found by Armstrong and his colleagues when they researched the samples from a St. Louis encephalitis epidemic (Muckenfuss, Armstrong et al. 1934). *Mus musculus* and *Mus domesticus* can be infected by LCMV as the natural rodent host (Laposova, Pastorekova et al. 2013). LCMV is an enveloped, negative-strand RNA virus with two segments of genome, one large and one small, covered by viral glycoprotein. The size of an LCMV virion is around 40nm to 200nm (Knipe and Howley 2013). Viral glycoprotein precursor and nucleoprotein are encoded by viral S RNA, while the viral small RING-finger-containing Z protein and viral RNA-dependent RNA polymerase are encoded by viral L RNA (Laposova, Pastorekova et al.

2013). The viral nucleoprotein and RNA-dependent RNA polymerase are critical and necessary for efficient RNA synthesis, including transcription and replication (Lee, Novella et al. 2000) (Graphical fig 2).



Graphical fig 2 The structure of LCMV: the figure shows the structure of Lymphocytic Choriomeningitis Virus, glycoprotein 1 (blue), glycoprotein 2 (green), genomic RNA (black), Z protein (purple), nucleoprotein (yellow), L polymerase (red). (Laposova, Pastorekova et al. 2013)

Moreover, LCMV has already been applied to many viral infection studies as a popular virus model. For example, in the research of T-cell activity and their roles in viral infection. One study demonstrated that spenocytes from mice which were infected with LCMV WE around day 2-5 showed enhanced cytotoxic activity against viral infected targets *in vitro* and *in vivo*; additionally, spleen cells (memory populations) from mice which were infected more than 13 days with LCMV showed increasingly potent secondary effectors when they were co-cultured with infected peritoneal cells, or transferred to irradiated and infected recipients (Dunlop, Doherty et al. 1977). Furthermore, in the study of major histocompatibility complex restriction in T-cell recognition, scientists found that cytotoxic T cells from adult mice infected with LCMV, which only interacted with H-2K or H-2D compatible LCMV-infected target cells in an *in vitro* ^{51}Cr-release killer assay (Blanden, Doherty et al. 1975, Doherty and Zinernagel 1975, Zinkernagel, Dunlop et al. 1975); similar phenotype was also observed *in vivo* - effector T cell activity was restricted by the H-2 gene complex (Doherty and Zinkernagel 1975). Besides these, researchers also used LCMV to find

that during chronic infection, the program of the memory T cell was altered, the functions of effector T cells was lost and the expression of multiple inhibitory receptors was upregulated (Wherry 2011, Doering, Crawford et al. 2012, Schietinger and Greenberg 2014).

In the process of LCMV infection, LCMV binds to a molecular mass of 120-140kDa membrane glycoprotein on infected cell (Borrow and Oldstone 1992), and the virus envelope glycoprotein initiates its entry into the cell. Then the ribonucleoprotein (RNP) of LCMV enters the cytoplasm of the infected cells through a pH-dependent membrane fusion method (Borrow and Oldstone 1994), and the viral polymerase mediates RNP as a template for viral transcription and replication (Perez and de la Torre 2003). Furthermore, the results of mapping multiple sites within the distal side of the noncoding intergenic region (IGR) indicate that IGR serves as a transcription termination signal for LCMV polymerase (Meyer and Southern 1994, Tortorici, Albarino et al. 2001). Finally, assembled viral RNPs associated at the surface of infected cells with membranes that are enriched with mature viral glycoprotein for the formation and release of LCMV. In this step, the RING-finger Z protein play a key role and functions as a matrix protein in viral particle assembly (Perez, Craven et al. 2003). Apart from RING-finger Z protein, the correct proteolytical processing of LCMV glycoprotein precursor is also critical for its incorporation into virions and to produce infectious particles (Kunz, Edelmann et al. 2003, Burri, da Palma et al. 2012).

Depending on the virus inoculums and the age of the infected animal, LCMV can establish different states of antiviral immunity. Normally, 10^2-10^5 pfu of LCMV infection causes systemic infection of all organs excluding the brain. Significant $CD8^+$ T cells expands, but fewer $CD4^+$ T cells emerge in this kind of infection. Antibodies are detected after seven days of LCMV infection, but they have no ability to neutralize the virus. The virus is cleared completely in 14 days. But in persistent infection models (LCMV Docile, Clone13 and Traub strain), a higher virus dose is required, resulting in

T-cell exhaustion in immunocompetent mice. Additionally, it also establishes persistent infection in newborn mice after 10^3 pfu of LCMV infection. However, 1-50 pfu of LCMV directly inoculated to adult mice intracerebrally causes choriomeningitis and death in seven to nine days (Oldstone 2002, Zinkernagel 2002). The LCMV strains that can lead to persistent infections are immunosuppression characterized by T-cell dysfunction and loss of virus-specific T cells (Ng, Sullivan et al. 2011). Moreover, persistent LCMV infection also causes decreased accumulation of viral glycoproteins at the surface of infected cells, reduced replication, dysfunction of dendritic cells, and higher expression levels of negative immune molecules including PD-1, TIM-3 and LAG-3 (Ng, Sullivan et al. 2011). For elimination of persistent LCMV infection, $CD4^+$ T cells play an essential role in helping $CD8^+$ T cells with $CD4^+$ T cell-produced interleukin 21, which can directly impact the generation of functional $CD8^+$ T cells, regulate the development of T-cell exhaustion--a state of T-cell dysfunction which is defined by sustained expression of inhibitory receptors plus poor effector function--and effectively control chronic virus infection (Yi, Du et al. 2009).

Humans can also be infected by direct contact with rodents and material contaminated with rodent excreta, or through rodent bites and mucosal exposure to aerosols. Furthermore, pet mice and hamsters have also been thought to be important sources of infection (Dykewicz, Dato et al. 1992, Buchmeier and Zajac 1999, Emonet, Retornaz et al. 2007). LCMV infections are usually asymptomatic or lead to a mild self-limiting illness in immunocompetent individuals. Symptoms include chills, fever, headache, nausea, myalgia and vomiting, which appear one to three weeks after exposure. In most cases, the disease recovers without treatment within a few days, but illness can develop to aseptic meningitis or meningoencephalitis in a minority of patients (Buchmeier, de La Torre et al. 2007). Infection with LCMV during the first trimester of pregnancy is related to an enhanced risk of spontaneous abortion (Barton and Mets 1999, Barton, Mets et al. 2002). When infection occurs in the second and third trimester, it has been associated with congenital intrauterine infection (Barton and Mets 1999, Barton, Mets

et al. 2002, Jamieson, Kourtis et al. 2006, Bonthius and Perlman 2007). Transplacental infection of the fetus is considered to happen during maternal viremia in mid- to late pregnancy (Kunz and de la Torre 2008).

6.2 Immune system

6.2.1 The innate immune system

6.2.1.1 The role of type I Interferons in immune responses

Type I Interferons (IFN-Is) contain one IFNβ gene, either 13 (humans) or 14 (mice) IFNα genes and IFN-κ, IFN-δ, IFN-ε, IFN-τ, IFN-ω, and IFN-ζ. Normally, almost all cells express IFNαR. Through a dimeric chain IFNαR1 and IFNαR2, IFN-I signal activates the Janus kinase 1 (JAK1) and tyrosine kinase 2 (Tyk2) to initiate transduction of IFN-I signaling by signal transducer and activator of transcription (Muckenfuss, Armstrong et al.) phosphorylation. Additionally, IFNβ can stimulate IL-10 production via activation of signaling pathway mediated by JAK1 and phosphoinositide 3-kinase (PI3K) (Gonzalez-Navajas, Lee et al. 2012).

IFN-Is are critical for inflammation and immunosuppression in chronic infection, They have abilities to induce multiple anti-viral response genes to inhibit virus replication in the infected cells and send a signal to nearby cells to prevent infection rapidly (Ng, Mendoza et al. 2016). Moreover, direct IFN-Is signaling is also necessary for T-cell expansion and protection from natural killer (NK) cell-mediated killing at the onset of viral infection (Kolumam, Thomas et al. 2005, Wiesel, Crouse et al. 2012, Crouse, Bedenikovic et al. 2014, Xu, Grusdat et al. 2014). However, prolonged IFN-Is signaling can also result in immune dysfunction (Klatt, Chomont et al. 2013). Therefore, IFN-Is act as a double-edged sword in the field of anti-viral infection.

In addition to the effects on APCs and T cells, IFN-Is are also essential to regulatory

$CD4^+$ T cells (Tregs) in viral infection, Tregs usually suppress or downregulate induction and proliferation of effector T cells. Direct IFN-I signaling on Tregs can suppress their numbers and activation, leading to enhanced $CD4^+$ and $CD8^+$ anti-viral responses and a slightly decreased virus titer (Srivastava, Koch et al. 2014). A similar phenotype is observed when *ex vivo* depletion of Treg cells or their suppressive factors in peripheral blood mononuclear cells (PBMCs) from patients with human immunodeficiency virus (Urbani, Amadei et al. 2006) and hepatitis C virus (HCV) can enhance $CD8^+$ T-cell function and activation (Veiga-Parga, Sehrawat et al. 2013). Taking advantage of the LCMV model, many research results indicate that many of the immune dysfunctions and suppressive programs associated with sustained virus infections are eliminated when IFN-Is signaling is inhibited, such as a decrease in expression levels of IL-10 and PD-L1 in dendritic cells and macrophages, lowered chronic inflammation and enhanced multiple anti-viral cell populations (Teijaro, Ng et al. 2013, Wilson, Yamada et al. 2013, Cunningham, Champhekar et al. 2016).

Like the effects of IFN-Is on anti-viral infection, the role of IFN-Is is also beneficial and necessary to promoting T-cell responses, preventing proliferation and metastases of tumor cells in cancer. For instance, through enhanced IDO expression levels on DCs and macrophages or upregulated expression level of immune checkpoints, IFN-Is can attenuate anti-tumor T-cell responses (Dai and Gupta 1990, Terawaki, Chikuma et al. 2011, McGaha, Huang et al. 2012, Benci, Xu et al. 2016, Jiang, Wang et al. 2017). Furthermore, the combination of cycle arrest and cell death by IFN-Is can also inhibit tumor cell expansion (Hall, Bates et al. 1995, Sangfelt, Erickson et al. 1999). For example, caspase 8-dependent apoptotic sensitivity is regulated by TNFα-family member TRAIL (TNF-related apoptosis inducing ligand), which is driven by IFN-Is (Chawla-Sarkar, Leaman et al. 2001, Bernardo, Cosgaya et al. 2013).

Since IFN-Is are critical for anti-virus and anti-tumor work, IFN-Is has already been utilized as a therapy in clinic research. When IFN-Is are used in combination with cART

antiretroviral therapy in HIV patients, the results indicate reduced latent reservoir and longer time to HIV rebound (Azzoni, Foulkes et al. 2013). In cancer treatment, a similar variable outcome of IFN-Is therapy is also observed (Zitvogel, Galluzzi et al. 2015). Furthermore, from another study, researchers focused on a recently defined IFN-I negative regulator 2`-5` oligoadenylate synthetase-like 1 (OASL1), they found that Oasl1 deficient mice showed a sustained level of serum IFN-I, enhanced anti-viral $CD8^+$ T cells and accelerated elimination of virus after LCMV Cl-13 (Wang, Swiecki et al. 2012, Lee, Park et al. 2013). Comparable results are also observed in the administration of IFN-Is at the onset of Simian immunodeficiency virus (SIV) infection, when it can increase resistance to infection (Sandler, Bosinger et al. 2014). Taken together, the efficacy of IFN-Is therapy is highest before the infection, but once infection is established, IFN-Is therapy alone is less effective. Furthermore, because IFN-Is can drive chronic inflammation to promote HIV progression, the idea that blocking IFN-Is signaling could rescue the immune response has been suggested (Bosinger, Li et al. 2009, Jacquelin, Mayau et al. 2009, Harris, Tabb et al. 2010). Scientists used an anti-IFNAR2 blocking antibody on humanized mice more than 10 weeks after HIV infection, the results demonstrated that the anti-IFNAR2 group displayed highly reduced expression levels of PD-1, TIM3, CD38 on the $CD8^+$ T cells, enhanced production of IFN-γ and IL-2 by $CD8^+$ T cells and reduced HIV titer when compared with the control group (Zhen, Rezek et al. 2017).

6.2.1.2 The role of Innate lymphoid cells in immune responses

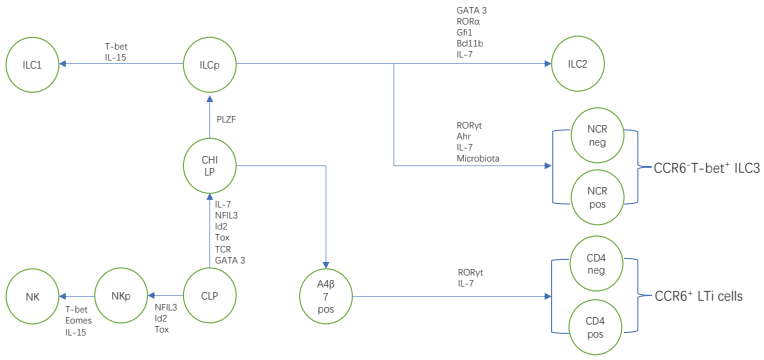
Compared with adaptive lymphocytes, innate lymphoid cells (ILCs) are relatively rare in lymphoid tissues; however, they are enriched in the skin, and lung and intestine, and adipose and some mucosal-associated lymphoid tissues. The features of ILCs are that they can respond to microbial signals and cytokines rapidly and produce multiple pro-inflammatory and immunoregulatory cytokines as potent innate cellular sources. Furthermore, according to current research, ILCs play an essential role in modulating

adaptive immunity (Sonnenberg and Artis 2012, Sonnenberg, Mjosberg et al. 2013, Spits, Artis et al. 2013, Walker, Barlow et al. 2013).

Initially, ILCs develop in the fetal liver and later in the adult bone marrow from common lymphoid progenitors (CLPs) (Cherrier, Sawa et al. 2012, Constantinides, McDonald et al. 2014, Klose, Flach et al. 2014). The requirements of the differentiation of all ILCs from CLPs are the transcription factors Id2 (inhibitor of DNA binding 2), NFIL3 (nuclear factor interleukin-3 regulated) (Spits, Artis et al. 2013, Walker, Barlow et al. 2013, Geiger, Abt et al. 2014, Klose, Flach et al. 2014, Kobayashi, Steinbach et al. 2014, Seillet, Rankin et al. 2014, Yu, Wang et al. 2014, Xu, Domingues et al. 2015) and Tox (thymocyte selection-associated high mobility group box) (Aliahmad, de la Torre et al. 2010, Seehus, Aliahmad et al. 2015). Mature ILCs can be further classified into groups: cNKs, ILC1s, ILC2s, ILC3s and LTi cells, which are based on different expression of cell surface markers, transcription factors and effector cytokines (Artis and Spits 2015, Serafini, Vosshenrich et al. 2015). ILC1s include NK cells and ILC1, and both can express transcription factor T-bet, activation receptors NKp46 and NK1.1, and produce interferon γ after stimulation with proinflammatory cytokines IL-12 and IL-18 (O'Sullivan, Sun et al. 2015). Moreover, as cytotoxic innate lymphocytes, NK cells are constitutive expression of the transcription factor Eomesodermin and the integrin CD49b, which can circulate through the vasculature to perform host immune surveillance against virus and tumors (O'Sullivan, Saddawi-Konefka et al. 2012, Daussy, Faure et al. 2014, Gasteiger, Fan et al. 2015, Cerwenka and Lanier 2016). ILC1, compared with NK cells, lack Eomesodermin and CD49b expression, but expresses CD49a. Plus, ILC1 is also necessary to protect the host from bacterial infection at barrier sites, such as the intestine, and is a long-term tissue resident in peripheral organs (Gordon, Chaix et al. 2012, Peng, Jiang et al. 2013, Daussy, Faure et al. 2014, Klose, Flach et al. 2014, Sojka, Plougastel-Douglas et al. 2014, Gasteiger, Fan et al. 2015, O'Sullivan, Rapp et al. 2016). In contrast to ILC1s, the characteristics of ILC2s are the expression of the transcription factor GATA3 and that they can trigger type 2 cytokines

such as IL-4, IL-5 and IL-13 (Moro, Yamada et al. 2010, Neill, Wong et al. 2010, Price, Liang et al. 2010, McKenzie, Spits et al. 2014, Monticelli, Osborne et al. 2015). Finally, ILC3s consist of NKp46^{+} ILC3, NKp6^{-} ILC3 and lymphoid tissue inducer cell (Cohen, Gandhi et al.) which express RORγt (retinoid-related orphan receptor γt), ILC3s have the functions of maintaining tissue homeostasis and antibacterial immunity in the intestine by the production of IL-17A, IL-22 and lymphotoxin (Mebius, Rennert et al. 1997, Satoh-Takayama, Vosshenrich et al. 2008, Sawa, Cherrier et al. 2010, Sawa, Lochner et al. 2011, Sonnenberg, Monticelli et al. 2011, Sonnenberg and Artis 2012) (Graphical fig 3).

As we mentioned at the beginning of this section, ILCs are critical in responding to pathogen infection and inflammation (Artis and Spits 2015, Serafini, Vosshenrich et al. 2015). ILC1 populations, as the main innate source of IFNγ and TNFα in mice, play important roles in recruiting inflammatory myeloid cells to control infection following



Graphical fig 3 The development of ILCs: the figure shows the development of innate lymphoid cells, ILCs develop in the fetal liver and later in the adult bone marrow from common lymphoid progenitors (CLP). Furthermore, the figure also displays that the cytokines and transcription factors that the ILCs need during the process of development. (Sonnenberg and Artis 2012)

infection with the oral pathogen *Toxoplasma gondii* (Klose, Flach et al. 2014). After exposure to extracellularly multicellular parasites, ILC2s can be stimulated to response. They can produce IL-13 in the intestine following infection of mice with the parasite *Nippostrongylus brasiliensis*. Epithelial cell-derived IL-25 and IL-33 can also promote the ILC2 population expansion and cytokine production during parasite infection (Moro,

Yamada et al. 2010, Neill, Wong et al. 2010, Price, Liang et al. 2010). After influenza virus infection and subsequent immune-mediated tissue damage, ILC2s are activated in response to IL-33 (Monticelli, Sonnenberg et al. 2011). Besides that, ILC2s can also respond to dietary stress, and the type 2 immune response might have adapted to support anti-parasite immunity in human with malnutrition. In contrast to ILC1s and ILC2s, ILC3s are particularly important in resist infection of mice with either extracellular bacteria or fungi. Following the infection of Gram-negative enteric pathogen *Citrobacter rodentium*, ILC3s, as the dominant and essential source of IL-22, are rapidly stimulated to protect host (Satoh-Takayama, Vosshenrich et al. 2008, Zheng, Valdez et al. 2008, Cella, Fuchs et al. 2009, Sonnenberg, Monticelli et al. 2011).

Additionally, ILC3s can also be promoted by DC-derived IL-23 in human and mice (Cella, Fuchs et al. 2009) and promote tissue repair through several distinct mechanisms. For example, the architecture of secondary lymphoid organs can be impaired after $CD8^+$ T cell-mediated killing of infected stromal cells following systemic viral infections. Lymphoid tissue induced cells which express LTα1β2 can be activated to act on LTβR-expressing stromal cells to enhance their proliferation and survival (Scandella, Bolinger et al. 2008). Moreover, many studies have demonstrated that there are reduced numbers of ILC3s in intestinal tissues from inflammatory bowel disease (IBD) patients relative to non-IBD controls (Takayama, Kamada et al. 2010, Ciccia, Accardo-Palumbo et al. 2012, Bernink, Peters et al. 2013), indicating that ILC3s also have a vital role in the regulation of tissue repair. However, persistent inflammatory signals can also result in unrestrained activation of certain ILCs at barrier surfaces to exacerbate colitis and dermatitis, and even contributing to tumorigenesis (Buonocore, Ahern et al. 2010, Fuchs, Vermi et al. 2013, Kirchberger, Royston et al. 2013, Salimi, Barlow et al. 2013, Chan, Jain et al. 2014).

Above all, these studies illustrate the impact of ILCs in tissue microenvironments and highlight the delicate balance between ILC-mediated immune protection and pathology.

6.2.2 The adaptive immune system

6.2.2.1 The role of antibodies in immune responses

The antibody response not only plays an essential role in preventing viral infection but also may contribute to resolution of infection. However, usually only a small part of these antibodies are neutralizing antibodies which have ability to neutralize viral infection by interfering with virion binding to receptor, blocking uptake into cells, preventing uncoating of the genomes in endosomes. binding and cell entry, these antibodies impact viral infection (Bizebard, Gigant et al. 1995, Smith, Chase et al. 1996, Saphire, Parren et al. 2001). Hence, neutralizing antibodies develop a way by steric obstruction to bind to structures that interfere with the interaction of viral surface proteins and their receptors on cells (Roben, Moore et al. 1994, Fleury, Barrere et al. 1999).

As mentioned above, most virus-specific antibodies have no neutralizing activity. That is because they are stimulated by viral fragments or by viral proteins that are released from infected, dying cells (Battegay, Kyburz et al. 1993, Parren, Burton et al. 1997). Natural antibodies, referred to as low affinity and polyreactive antibodies, are the first line of viral defense in a host. These antibodies can be detected even under germ-free conditions in mice. According to recent research, the self-renewing $CD5^+$ B1 B-cell compartment can secrete a large amount of these antibodies (Haury, Sundblad et al. 1997, Baumgarth, Herman et al. 1999). Natural antibodies also provide an essential link between the innate and adaptive immune systems through restricting initial viral dissemination (Ochsenbein and Zinkernagel 2000), and are critical for the recruitment of viral antigens to secondary lymphoid organs (Ochsenbein, Pinschewer et al. 2000). Furthermore, some natural antibodies have abilities to neutralize viruses directly. Spontaneous pre-immune neutralizing antibody titers are generally high for cytopathic viruses, such as VSV, but are relatively low or below detection levels for poorly cytopathic viruses, such as LCMV (Hangartner, Senn et al. 2003).

Additionally, rapidly induced neutralizing antibodies are significant for the survival of the host. Because the priming of $CD4^+$ T helper cell (Th) responses takes several days, the rapid secretion of early neutralizing immunoglobulin M (IgM) overcomes this delay, and much research has demonstrated that most cytopathic viruses can induce T-cell independent, neutralizing IgM antibody responses (Burns, Billups et al. 1975, Fehr, Naim et al. 1998, Szomolanyi-Tsuda and Welsh 1998, Bachmann, Ecabert et al. 1999). Furthermore, neutralizing antibodies are also important for protection against reinfection by viruses (Graham, Bunton et al. 1991, Ahmed and Gray 1996, Seiler, Brundler et al. 1998, Harada, Muramatsu et al. 2003). The applications of effective and efficient vaccines relate closely with neutralizing antibody titers (Plotkin 2001, Zinkernagel 2003). For example, as a poorly cytopathic virus, HBV stimulates the typical antibody responses, but the production of neutralizing antibodies is delayed (Alberti, Cavalletto et al. 1988, Neurath, Seto et al. 1989, Jung and Pape 2002). But through vaccination against HBV surface antigens, the delayed antibodies provide excellent protection against this virus. Finally, immunization with haptens has also indicated that somatic hypermutation (SHM) improves the affinity and avidity of the antibody response in persistent, poorly-cytopathic viral infections (Griffiths, Berek et al. 1984, Weiss, Zoebelein et al. 1992). For instance, the analysis of gp120-specific antibodies in an HIV-infected, long-term non-progressor demonstrates that all isotype class-switched neutralizing antibodies are clonally related and display extensive SHM (Toran, Kremer et al. 1999).

Nevertheless, viruses also have developed strategies to prevent neutralizing antibody production. Recent studies indicate that four factors can impair an early neutralizing-antibody response against a poorly cytopathic virus. These factors are active modification of the B-cell repertoire by depletion of certain B-cell subsets, a low frequency of germline-encoded immunoglobulin V-regions that encode neutralizing antibodies, induction of abnormal Th-cell function that causes polyclonal B-cell activation, and immunopathological changes in secondary lymphoid organs

(Hangartner, Zinkernagel et al. 2006). Moreover, cytopathic viruses also develop approaches to escape the responses of neutralizing antibodies by diversifying their antigenic and protective determinants in several closely related varieties, such as serotypes or subtypes (Hangartner, Zinkernagel et al. 2006). For example, influenza A viruses can change serotypes dynamically to escape immunity within a population (Hilleman 2002). Additionally, sera from HIV-infected patients usually fail to neutralize viruses that are isolated concomitantly from the same patient, while the same sera frequently do neutralize viruses that are isolated 6 to 12 months earlier or from different persons, which indicates that the refinement of the antibody response continuously selects for new virus-escape variants within a single host (Richman, Wrin et al. 2003, Wei, Decker et al. 2003).

In conclusion, firstly the rapid induction of neutralizing antibodies is significantly important for surviving infections by acutely cytopathic viruses, but the induction of neutralizing antibodies is less essential for the control of acute infections by viruses where infection is usually poorly cytopathic or controlled by cytotoxic T lymphocytes (CTLs) in adults. In addition, SHM is critical for the control of persisting, poorly cytopathic viruses.

6.2.2.2 The role of B cells in immune responses

The essential role of B cells in immune responses is the generation of antibodies to protect a host from infectious diseases and maintain homeostasis. The production of antibodies depends on the spatial and temporal development of B cells. In bone marrow, B cells develop from hematopoietic stem cells, and they undergo a development process which includes rearrangement of genes to produce a large number of B-cell receptors (BCR) for antigen recognition. Moreover, through somatic hypermutation of the BCR, further development and interaction with antigens cause affinity maturation of the B-cell response, and B cells start to secret soluble BCRs as antibodies at the immature

stage of development. These are initially pentameric IgM; nevertheless, as affinities increase and T cells help, class-switching occurs and leads to the production of IgG. Finally, B cells also have other functions and may act as APCs and regulatory cells (Hickling, Chen et al. 2014, Yam-Puc, Zhang et al. 2018).

Because of the size of a virus, usually around 20 to 200nm, this feature makes viruses have the ability to transport to lymphoid tissues for stimulation of B-cell responses (Manolova, Flace et al. 2008, Batista and Harwood 2009). The highly repetitive structure of viral particles can cross-link B-cell receptors efficiently, which can cause B-cell activation directly without Th cells (Bachmann and Zinkernagel 1997). Furthermore, repetitiveness of viral particles is also a strong stimulus for B cells and can sometimes override B-cell tolerance, resulting in activation of anergic B cells (Bachmann, Rohrer et al. 1993, Chackerian, Durfee et al. 2008). This characteristic of viruses helps complements fixation. Complements also can directly interact with B cells to drive more efficient antibody responses (Bottazzi, Doni et al. 2010, Link, Zabel et al. 2012). Besides binding to multimeric components of the innate humoral immune system, repetitive viral particles are also able to engage the B-cell receptors of low affinity B cells. This is particularly essential for induction of antibody responses upon intranasal immunization, because it allows the interaction between viral particles and B-cell receptors of B cells in the lung that will transport viral particles to B-cell follicles of the spleen for deposition on follicular dendritic cells and subsequently induction of GC responses (Bessa, Zabel et al. 2012).

Additionally, specific toll-like receptors (TLRs) within endosomes of antigen presenting cells and B cells have the ability to recognize the nucleic acids of viruses. For instance, the double-stranded RNA of a virus can activate TLR3, single-stranded RNA can activate TLR7/8 and the DNA of virus can activate TLR9, but TLR9 is only stimulated by DNA rich in non-methylated CG motifs (CpGs) which are found in bacteria and some viruses (Barton, Kagan et al. 2006). However, the TLR signaling is

not necessary for the antibody production (Gavin, Hoebe et al. 2006). For instance, mice that are deficient in TLR-signaling display enhanced IgG responses following viral infection (Heer, Shamshiev et al. 2007, Meyer-Bahlburg, Khim et al. 2007). Moreover, other research indicates that TLR-signaling in B cells is important for IgG class-switching rather than dendritic cells (Jegerlehner, Maurer et al. 2007, Bessa, Jegerlehner et al. 2009, Hou, Saudan et al. 2011). Mice that are immunized with RNA-loaded or CpG-loaded virus-like particles show strong IgG responses dominated by IgG2a, while mice immunized with virus-like particles devoid of nucleic acids or mice lacking TLR-signaling in B cells specifically have reduced IgG responses dominated by IgG1 isotypes. Therefore, B cells are stimulated directly to undergo isotype-switching to IgG2a through interacting with viral particles carrying RNA or DNA. This finding also demonstrates that efficacy of antiviral vaccines may be enhanced by inclusion of these TLR-ligands. In clinical application, formulation of HBsAg for vaccination against the hepatitis B virus with CpGs leads to enhanced antibody responses in humans (Barry and Cooper 2007). Finally, strong IgA responses that are induced following systemic exposure to viral particles are dependent on TLR-signaling in B cells and independent of Th cells or TLR-signaling in DCs (Bergqvist, Gardby et al. 2006, Bessa, Schmitz et al. 2008, Bessa, Jegerlehner et al. 2009, Bergqvist, Stensson et al. 2010).

In conclusion, B cells play an essential role in the anti-viral responses of a host. Therefore, modeling B-cell activities and understanding the mechanism of B-cell responses offer us more opportunities to increase our knowledge of the immune system and make us enable to predict and design effective vaccination strategies for infectious diseases such as HIV and influenza.

6.2.2.3 The role of T cells in immune responses

The interaction between T cell receptor (TCR) and peptide-loaded major histocompatibility complex (pMHC) represents the central step in T cell antigen recognition. Dependent on the protein antigen fragments presented in the cell surface of expressed MHC molecules, T cells discriminate between host and pathogen (Zinkernagel and Doherty 1974, Bjorkman, Saper et al. 1987, Bjorkman, Saper et al. 1987, Simpson 1988). This interaction of TCR and pMHC begins in the thymus and continues into the periphery, and it is regulated by numerous cellular molecules and can direct the expansion, differentiation and function of T-cell subsets.

According to *in vitro* initial studies, peptide-loaded MHC is critical to establishing cell signaling to T cells. Numerous costimulatory molecules in and around the developing synapse regulate APCs to authorize expansion, arming and execution of T-cell effector functions after pMHC engagement (Kupfer, Swain et al. 1986, Kupfer, Swain et al. 1987, Kupfer, Mosmann et al. 1991) . Furthermore, the study of Monks and Kupfer illustrates that the peripheral supramolecular activation cluster (pSMAC) surrounds the central supramolecular activation cluster (cSMAC) to form the mature immunological synapse structure (Monks, Freiberg et al. 1998). Importantly, adhesion molecules of pSMAC, for instance leukocyte function-associated antigen-1 (LFA-1), play an essential role in the physical stabilization of long-term cell-cell interaction, and LFA-1 also provides a link between synapse formation and dramatic changes in T-cell cytoskeletal structure (Simonson, Franco et al. 2006, Billadeau, Nolz et al. 2007, Suzuki, Yamasaki et al. 2007).

TCR-pMHC signaling is also regulated by many molecules, for example, CD28 is expressed constitutively on naïve T cells and is recruited along with TCR to the signaling synapse following TCR engagement. The interaction between CD28 and CD80/86 expressed on APCs amplifies TCR-pMHC signaling and permit T-cell

activation via only a small number of triggered TCRs (Viola and Lanzavecchia 1996). Additionally, LCMV provides an excellent model for understanding the mechanism of antiviral immune responses. For instance, the generation of LCMV glycoprotein (GP)-specific $CD8^+$ and $CD4^+$ TCR transgenic mice has provided transferable and traceable populations of virus-specific cells which allows further characterization of antiviral T-cell responses (Pircher, Burki et al. 1989, Oxenius, Bachmann et al. 1998). In addition, OT-I mice were adoptively transferred with 10^3 naïve LCMV-specific $CD8^+$ P14 T cells following LCMV infection, which can rescue from lethal meningits completely. This demonstrates that CLT-pMHC interaction is critical for mediating this disease (McGavern and Truong 2004).

Besides the significant role of $CD8^+$ T cells during the infection, the functions of $CD4^+$ T cells are associated with regulation of cytokine and chemokine secretion by the interaction between TCR and pMHC. Moreover, regulatory T cells (Tregs), as a subset of $CD4^+$ T cells, can suppress autoimmune upon transfer into day-3 thymectomized mice and inhibit transplant rejection (Shevach 2000, Josefowicz, Lu et al. 2012). Furthermore, Tregs are also vital for survival (Brunkow, Jeffery et al. 2001) and influence the immune responses to many viral infections (Belkaid 2007).

To resist the immune responses in the host, viruses also develop several strategies; for example, during chronic viral infection, virus-specific T cells display various levels of exhaustion. $CD8^+$ T-cell exhaustion has several stages: partial exhaustion I and II, full exhaustion and deletion (Fuller and Zajac 2003, van der Most, Murali-Krishna et al. 2003, Wherry, Blattman et al. 2003). During the stage of exhaustion, $CD8^+$ T cells lose the ability to produce effector cytokines such as IL-2, TNF-α and IFN-γ, and the capability of *ex vivo* cytotoxicity. Moreover, exhausted $CD8^+$ T cells also show reduced antigen-independent proliferation, are poorly responsive to IL-7 and IL-15 (Wherry and Ahmed 2004) and display metabolic and bioenergetic deficiencies (Wherry, Ha et al. 2007).

During HIV infection, persistent antigen load is considered the main reason for upregulation of PD-1 and impairment of the ability of HIV viral-specific $CD8^+$ T cells to produce effector cytokines (Day, Kaufmann et al. 2006, Petrovas, Casazza et al. 2006, Trautmann, Janbazian et al. 2006). In HIV patients, TIM-3 is also dramatically increased in both $CD4^+$ and $CD8^+$ T cells; these TIM-3^+CD8^+ T cells are also positively related with viral load and the number of $CD4^+$ T cells during progressive HIV infection as PD-1^+CD8^+ T cells (Jones, Ndhlovu et al. 2008). Additionally, a blockade of the PD-1/PD-1L pathway *in vitro* has been demonstrated to enhance the effector function of not only HIV-specific $CD8^+$ T cells (Day, Kaufmann et al. 2006, Petrovas, Casazza et al. 2006, Trautmann, Janbazian et al. 2006), but also HCV-specifc (Urbani, Amadei et al. 2006, Penna, Pilli et al. 2007, Radziewicz, Ibegbu et al. 2007), and HBV (Boni, Fisicaro et al. 2007)-specific $CD8^+$ T cells.

Therefore, therapeutic interventions are utilized to boost T cell responses during chronic viral infection. However, the effectiveness of single therapeutic vaccines for HBV, HCV and HIV is not as high as initially expected. Consequently, the combination of therapeutic vaccination with another immune-based approach may prove to be a more effective way to achieve additive efficacy. For example, compared to single therapy, a LCMV GP33-encoding vaccinia in combination with an anti-PD-L1 blocking antibody shows significantly enhanced viral-specific $CD8^+$ T-cell immunity and reduced viral load following chronic LCMV infection (Ha, Mueller et al. 2008).

Above all, understanding the role of T cells in the immune system can help to broaden the horizon of the functional dynamics of T cells during pathogen infection, and will continue to play a key role in the therapeutic advancement of anti-viral immunotherapy.

6.3 The biological functions of BAFFR in B cells and the immune system

The B cell-activating factor receptor (BAFFR), which is a type III transmembrane

protein in the TNF receptor family (Smith, Farrah et al. 1994, von Bulow and Bram 1997), is important for B-cell development (Schiemann, Gommerman et al. 2001, Thompson, Bixler et al. 2001). In humans, BAFFR is encoded by the *TNFRSF13C* gene which is localized to chromosome 22q13.1-13.31. A sequence motif "PVPAT" within the cytoplasmic domain of BAFFR mediates the specific function of BAFFR (Morrison, Reiley et al. 2005). Furthermore, BAFFR can be detected on all B cells except for bone marrow plasma cells in humans. However, BAFFR is expressed on transitional and mature B cells in mice (Mackay and Schneider 2009). A recent study also found that a small subset of resting murine and human T cells bind soluble BAFF via BAFFR and following *in vitro* stimulation the expression of BAFFR enhances (Tangye, Bryant et al. 2006).

B-cell activating factor (BAFF) is also a member of the TNF superfamily and can be produced by macrophages, dendritic cells, monocytes, neutrophils, follicular dendritic cells, activated T cells, splenic radiation-resistant stromal cells, astrocytes, osteoclasts and ductal epithelia cells (Kalled 2006). It also plays an essential role in the regulation of normal B-cell proliferation, activation and differentiation. Moreover, BAFF can bind to three receptors: BAFFR, B-cell maturation antigen (BCMA) and transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI). Among these three receptors, TACI can be detected on both B cells and activated T cells, while the expression of BAFFR and BCMA are only on B cells (Marsters, Yan et al. 2000, Thompson, Bixler et al. 2001). Moreover, TACI and BCMA can also bind to a proliferation-inducing ligand (APRIL), whereas BAFFR binds to BAFF specifically (Mackay and Schneider 2009). In animal models, BAFF-deficient mice display a complete loss of follicular and marginal zone B cells (Schiemann, Gommerman et al. 2001); however, over-expressing BAFF in transgenic mice show a different phenotype: enlarged spleens, a significant increase in mature B cells, enhanced levels of autoantibodies and high plasma cell numbers (Mackay, Woodcock et al. 1999). In humans, under different pathological conditions, elevated levels of soluble BAFF are observed in patients (Doreau, Belot et al. 2009).

BAFF/BAFFR signaling is significantly important to B-cell survival, growth and metabolic regulation, but because immature B cells have low expression of BAFFR, it has no impact on central B-cell selection in the bone marrow (Liu and Davidson 2011). Recent studies indicate that when BAFF levels are enhanced, the steady-state numbers of quiescent primary B cells are observed to increase (Moore, Belvedere et al. 1999, Batten, Groom et al. 2000). Furthermore, via the PI3K signaling pathway the signaling of B cell receptor crosslinking in naïve cells triggers the expression of BAFFR (Stadanlick, Kaileh et al. 2008), then BAFFR engagement upregulates CD19 expression by regulating the transcription factor Pax5 to enhance BCR signaling (Mackay and Schneider 2009). Finally, BAFFR and B cell receptor inhibit apoptotic pathways through altering the expression of different pro-survival and pro-apoptotic proteins (Mackay, Figgett et al. 2010). Downstream of the BAFFR signaling, the NF-κB pathway is also important for whole functions of BAFFR. Once BAFF binds to BAFFR, the signaling induces the processing of p100 to p52 through the non-canonical NF-κB pathway in a NF-κB-inducing kinase (NIK) and IκB kinase (IKK) dependent approach. Then the p52/RelB active heterodimers translocate to the nucleus and regulate gene expression (Claudio, Brown et al. 2002). Moreover, through activating the IKK complex, BAFFR can also activate NF-κB in the canonical pathway approach (Ramakrishnan, Wang et al. 2004).

Additionally, an increased level of BAFF has also been observed in many viral infections including HCV, EBV and HIV infection (Stohl, Cheema et al. 2002, He, Raab-Traub et al. 2003, Rodriguez, Valdez et al. 2003). For example, HIV patients have an increased level of IL-10 and BAFF in their serum, and the BAFF levels enhance with disease progression (Stohl, Cheema et al. 2002, Rodriguez, Valdez et al. 2003). The elevated levels of BAFF in the serum of HCV patients are associated with the presence of arthritis/arthralgia or vasculitis, and high values at the beginning of acute HCV infection are also a hint to its evolution to chronic infection (Tarantino, Marco et al. 2009). Another relationship between enhanced serum BAFF levels and liver fibrosis

was found in HCV-infected patients, indicating that patients with cirrhosis have more BAFF expression than non-cirrhotic patients (Toubi, Gordon et al. 2006, Sene, Limal et al. 2007). The induction of BAFF expression might also explain the association between viral infection and the occasional development of autoimmunity following EBV, HCV and HIV infection. Persistent viral infection may result in increased cell apoptosis and the release of various nuclear antigens such as heat shock proteins and the binding of toll-like receptors (He, Qiao et al. 2004, Machida, Cheng et al. 2006). Moreover, mice that are deficient in BAFFR following LCMV infection also show reduced T-cell responses and low survival rate after VSV infection (Xu, Huang et al. 2015).

Besides the key role of BAFF in viral infections, an increased level of BAFF is also present in autoimmune diseases including Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, myasthenia gravis, mixed cryoglobulinemia, coeliac disease, autoimmune hepatitis, primary biliary cirrhosis, bullous pemphigoid and localized scleroderma (Stohl, Metyas et al. 2003, Jonsson, Szodoray et al. 2005, Asashima, Fujimoto et al. 2006, Matsushita, Hasegawa et al. 2006, Fabris, Visentini et al. 2007, Matsushita, Hasegawa et al. 2007, Migita, Abiru et al. 2007, Ragheb, Lisak et al. 2008, Migita, Ilyassova et al. 2010).

Since the BAFF/BAFFR signaling shows important biological functions in both human and animals, B-cell directed therapies potentially become a new treatment for autoimmune diseases like system lupus erythematosus and rheumatoid arthritis (Sun, Lin et al. 2008, Levesque 2009, Looney 2010). Atacicept is a BAFF receptor-IgG fusion protein which shows good tolerability and clinical efficacy in patients with rheumatoid arthritis and patients with systemic lupus erythematosus (Ryan and Grewal 2009). Additionally, Belimumab, as another fully human IgG1 monoclonal antibody against BAFF, can reduce total peripheral B-cell numbers, immunoglobulin levels and the frequency of lupus flares to improve disease activity in patients with systemic lupus

erythematosus (Baker, Edwards et al. 2003, Sun, Lin et al. 2008). Both candidates show great clinical efficacy, but further studies are still warranted.

In conclusion, BAFF/BAFFR signaling is critical not only in the B-cell development, but also in regulation of innate and adaptive immune responses.

6.4 The biological role of CD169$^+$ macrophage in immune responses

Three macrophage populations can be marked by anti-CD169 antibodies in mouse secondary lymphoid organs such as marginal zone metallophilic macrophages in the spleen and subcapsular sinus and medullary macrophages in lymph nodes (Crocker and Gordon 1989). CD169$^+$ macrophages are specialized to process antigens recognized by mannose receptors depending on their location (Taylor, Gordon et al. 2005). Furthermore, CD169, as a macrophage-restricted marker, is a member of the Siglec superfamily which comprises 17 immunoglobulin-like domains and a short cytoplasmic tail that lacks signaling motifs (Crocker and Gordon 1986, Crocker and Gordon 1989, Crocker, Mucklow et al. 1994, Crocker, Paulson et al. 2007, Crocker and Redelinghuys 2008).

According to recent studies, marginal zone metallophilic macrophages are critical for humoral responses and are especially necessary for the initiation of antibody responses against T-cell dependent antigens (Buiting, De Rover et al. 1996). In the process of the immune response, following lipopolysaccharide stimulation CD169$^+$ cells are found within B-cell follicles in the spleen (Groeneveld, Erich et al. 1986, Yu, Wang et al. 2002) and at the boundary between the T-B cell areas in the initial stages of the germinal center reaction (Martinez-Pomares, Kosco-Vilbois et al. 1996, Berney, Herren et al. 1999, Mueller, Cremer et al. 2001). Moreover, B cell-derived lymphotoxin can impact CD169$^+$ macrophages and induce the expression of ligands for the cysteine-rich domain of mannose receptor in marginal zone metallophilic macrophages in the spleen. It is

also important for the presence of subcapsular sinus macrophages in the lymph nodes (Phan, Green et al. 2009). These features of $CD169^+$ macrophages, they have abilities to facilitate B cell activation via capture and display of naïve antigens delivered in the form of viral particles, particular antigen and immunocomplexes (Carrasco and Batista 2007, Junt, Moseman et al. 2007, Martinez-Pomares and Gordon 2007, Phan, Grigorova et al. 2007, Phan, Green et al. 2009, Gonzalez, Degn et al. 2011).

Besides the interaction between $CD169^+$ macrophages and B cells, $CD169^+$ macrophages are also capable of activating $CD8^+$ T cells by two potential approaches: antigen transfer to $CD8\alpha^+$ DCs in the spleen (Backer, Schwandt et al. 2010) and direct antigen presentation to $CD8^+$ T cells (Chtanova, Han et al. 2009, Asano, Nabeyama et al. 2011). Recent research indicates that following infection with *Plasmodium chabaudi* $CD169^+$ macrophages can be targeted by activated $CD8^+$ T cells (Beattie, Engwerda et al. 2006). Furthermore, subcapsular sinus macrophages are able to limit virus spreading and invasion of the central nervous system following peripheral infection with VSV (Iannacone, Moseman et al. 2010). In this situation, subcapsular sinus macrophages control the infection via production of IFNα but do not impact $CD4^+$ and $CD8^+$ T cell responses (Iannacone, Moseman et al. 2010). Additionally, $CD169^+$ metallophilic macrophages can also express Usp18 which is a potent inhibitor of IFN-I signaling cascade to allow locally restricted VSV replication (Honke, Shaabani et al. 2011). This enforced VSV replication in subcapsular sinus macrophages is vital for host survival (Iannacone, Moseman et al. 2010, Moseman, Iannacone et al. 2012). Subcapsular sinus macrophages secrete a large amount of IFN-I which can establish a local antiviral environment before being eventually killed by cytopathic VSV. In the example of the neurotropic VSV, the localized production of IFN-I is necessary to protect the host from central ascending nervous system paralysis and death (Iannacone, Moseman et al. 2010). The increased viral antigen may stimulate adaptive immunity (Hickman, Takeda et al. 2008, Honke, Shaabani et al. 2016).

CD169$^+$ macrophages play a significant role not only in antiviral responses, but also in anti-tumor immunity. By using animal models in which CD169$^+$ macrophages can be specifically depleted by diphtheria toxin, the scientists found that CD169$^+$ subcapsular sinus macrophages are involved in antigen-presentation and the induction of CTLs in lymph nodes, but the results of CTL responses to tumor cells are not different between wild-type animals and CD169-deficient animals (Asano, Nabeyama et al. 2011). Furthermore, according to the statistical analysis of the post-operative survival rate and clinic-pathological factors of groups where the Lymph nodes had high and low CD169 expression levels, an increased density of CD169$^+$ macrophages in the sinus area and higher percentage of CD169$^+$ sinus macrophages are significantly associated with higher CD8$^+$ lymphocyte infiltration into primary tumor tissues. Patients with a higher level of CD169$^+$ macrophages display better overall survival rate (Komohara, Ohnishi et al. 2017). Comparable results are also observed in the patients with melanoma and endometrial tumors (Saito, Ohnishi et al. 2015, Ohnishi, Yamaguchi et al. 2016). Based on the studies of CD169$^+$ macrophages in the anti-tumor area, new anti-tumor therapies have begun to be developed. For instance, taking advantage of sialic acid conjugated liposomes that deliver antigen to CD169$^+$ macrophages can stimulate the production of antigen-specific T cells (Chen, Kawasaki et al. 2012). Another strategy is CD169-targeted liposomes with lipid antigen, which can induce activation of iNKT cells in a CD1d-dependent approach (Barral, Polzella et al. 2010).

Above all, CD169$^+$ macrophages are extremely important for antigen acquisition, delivery to lymphocytes and anti-tumor responses. Future studies on CD169$^+$ macrophages will benefit from how the nature of the antigen impacts its targeting and processing in draining lymphoid tissues. It is also critical to find a solution to translate the research achievements on CD169$^+$ macrophages to the development of the therapies for associated human diseases.

6.5 The biological role of Qa-1b in immune responses

Qa-1b is an MHC class Ib molecule and is encoded by the *H2-T23* gene in the T region of the major histocompatibility complex (Lalanne, Transy et al. 1985, Transy, Nash et al. 1987, Wolf and Cook 1995). Besides the *H2-T23* gene, there are other class Ib genes presenting in the regions of MHC, such as *H-2Q*, *H-2T* and *H-2M*. Normally, compared with the expression of MHC class Ia molecules, the class Ib genes show tissue-specific patterns of expression and relatively little polymorphism.

Taking advantage of an antiserum against the defined class Ib molecules, thymus leukemia antigen (TL), the Qa-1b antigen was first found as a serological determinant expressed on peripheral T cells (Jensen, Sullivan et al. 2004). According to the sequence, Qa-1b is quite similar to the MHC class Ia molecule, which is expressed in tissues widely in a pattern similar to MHC class Ia molecules. But the expression level on the cell membrane is lower than MHC class Ia molecules (Jensen, Sullivan et al. 2004). Furthermore, Qa-1 can act as an MHC ligand for recognition by T cells through the capacity to induce allogeneic responses that are not restricted by H-2D and H-2K (Forman 1979, Kastner, Rich et al. 1979, Lindahl, Hausmann et al. 1982). H-2D region alleles impact the recognition of target cells via a subset of Qa-1b-specific alloreactive cytolytic $CD8^+$ TCRαβ T-cell clones (Aldrich, Rodgers et al. 1988). The majority of alloreactive Qa-1-specific CTL clones recognize Qdm, which is a specific Qa-1 bound peptide derived from the leader sequence of H-2D (Aldrich, DeCloux et al. 1994), and this peptide is highly conserved in H2D/L leader peptides and is not present in H-2K molecules.

Many studies indicate that Qa-1 molecules are not only predominantly loaded with a single peptide but also have the capacity to present antigens other than Qdm to T cells. For example, the expression of Qa-1 on the membrane of transfected cells could be stabilized by a tryptic digestion of *Mycobacterium bovis* heat-shock protein 65, which

illustrate that peptides from hsp65 can bind to Qa-1(Imani and Soloski 1991). Moreover, following infection with *Listeria monocytogenes* (Bouwer, Lindahl et al. 1994, Bouwer, Seaman et al. 1997, Bouwer, Bai et al. 1998, Seaman, Perarnau et al. 1999, Bouwer, Barry et al. 2001) and *Salmonella typhimurium* (Lo, Ong et al. 1999, Lo, Woods et al. 2000, Soloski and Metcalf 2001), pathogen-specific Qa-1 restricted TCR αβ CTL have been found. According to a recent study, in a deficient source of Qdm cell line, the hsp60 epitope is the dominant peptide associated with Qa-1 (Davies, Kalb et al. 2003).

A number of studies have indicated that Qa-1 is a restriction factor to regulate T cells. For instance, suppressor $CD8^+$ T cells could recognize Qa-1^+ B cells to regulate antibody responses (Noble, Zhao et al. 1998) and ocular anterior chamber-related immune deviation (D'Orazio, Mayhew et al. 2001).

Despite evidence that Qa-1b is important in adaptive immunity, research has also indicated that Qa-1b plays an essential role in innate immunity. Qa-1b can interact with CD94/NKG2 on mouse NK cells (Salcedo, Bousso et al. 1998, Vance, Kraft et al. 1998, Vance, Jamieson et al. 1999, Kraft, Vance et al. 2000, Salcedo, Colucci et al. 2000), NK-T cells and subsets of T cells (Vance, Kraft et al. 1998, McMahon, Zajac et al. 2002, Meyers, Ryu et al. 2002, Miller, Peters et al. 2002, Moser, Gibbs et al. 2002, Braud, Aldemir et al. 2003). The class Ib molecule HLA-E in human is the functional homolog of Qa-1b and also serves as the ligand for CD94/NKG2 receptors (Borrego, Ulbrecht et al. 1998, Braud, Allan et al. 1998, Lee, Llano et al. 1998). However, HLA-E and Qa-1b have serine at positions 143 and 147, which might reduce their capacity to form stable complexes with a diverse population of peptides. Therefore, both these molecules have a special role in the immune system in regulation of NK cells.

As illustrated above, HLA-E is a functional homolog of Qa-1b in humans. The interaction between HLA-E and CD94/NKG2A/C/E has important clinical significance, such as the capacity to inhibit the function of NK cells to prevent autoimmune incidents.

Furthermore, during viral infection, the virus developed many mechanisms to evade immune cell recognition and viral lysis. For instance, the HCMV UL40 protein has the ability to be identified by HLA-E, which interacts with CD94/NKG2A to inhibit NK cell function (Lin, Xu et al. 2007). A similar phenotype is also observed during HIV infection. HLA-E is upregulated (Nattermann, Nischalke et al. 2005) and HLA-A and HLA-B are downregulated (Cohen, Gandhi et al. 1999). This strategy of HIV infection leads to infected cells being unable to present viral peptides to activate CTLs; however, elevated levels of HLA-E on the surface of infected cells result in the inhibitory signal to NK cells via CD94/NKG2A. The HCV also has a similar way to impact host immune responses. The HCV peptide aa35-44 can bind to HLA-E and be recognized by CD94/NKG2A on NK cells (Nattermann, Nischalke et al. 2005). Moreover, a reduced expression level of HLA class Ia proteins along with enhanced levels of HLA-E during human papillomavirus infection was reported, and this virus is one of the main causes of cervical cancer (Goncalves, Le Discorde et al. 2008).

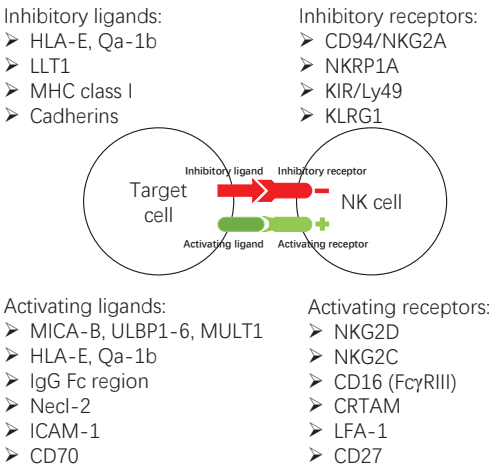
Above all, the Qa-1b in mice and HLA-E in humans are critical to innate and adaptive immune responses.

6.6 The biological role of CD94/NKG2A in immune responses

Natural killer (NK) cells, as one important subset of immune cells in the immune system, have the ability to kill target cells without prior immunization of the host (Moretta, Bottino et al. 2002). However, many normal cells also express the ligands for the inhibitory receptors of NK cells. In order to avoid autoimmune killing, there are some inhibitory receptors on the surface of NK cells which can transfer the inhibitory signals to override activation signals (Borrego, Kabat et al. 2002, Natarajan, Dimasi et al. 2002). NK cells express several different activating and inhibitory receptors, such as Ly49, KIR, CD94/NKG2 heterodimers and natural cytotoxicity receptors, as well as co-stimulatory receptors (Graphical fig 4). But the detailed mechanism for how inhibitory

signals interfere with activating signals is not yet clear. Normally, NK cells transfer inhibitory signals through intracellular immunoreceptor tyrosine-based inhibitory motifs, while activating signals can be transferred by immunoreceptor tyrosine-based activating motifs on the NK cells.

This section focuses more on the role of CD94/NKG2 in the immune system. CD94/NKG2 heterodimers are conserved between mice and humans. The gene coding for CD94 is located at the beginning of the sequence of genes in the CD94/NKG2 cluster, and the CD94 protein contains a short cytoplasmic domain with the extracellular portion



Graphical fig 4 The activating and inhibitory receptors and ligands of NK cells: the figure shows the activating (green) and inhibitory (red) receptors on NK cells, as well as the activating (green) and inhibitory (red) ligands on target cells. (Yokoyama and Plougastel 2003)

having a variation of the classical C-type lectin fold (Boyington, Riaz et al. 1999). The NKG2 family of genes all display great similarity in the structures of their exon-intron sequences (Sobanov, Glienke et al. 1999). The structure of NKG2 proteins contains an extracellular lectin-like domain, a transmembrane segment and cytoplasmic segments. NKG2 proteins have seven family members: NKG2A, NKG2B, NKG2C, NKG2D, NKG2E, NKG2F and NKG2H. NKG2D and NKG2F do not form a heterodimer with CD94. NKG2A and NKG2B show inhibitory functions and NKG2C, NKG2E, NKG2F

and NKG2H have activating functions (Takei, McQueen et al. 2001). Besides NK cells, $CD8^+$ T cells and NKT cells also express CD94/NKG2A in mice, and the expression of CD94/NKG2A is also observed on a subset of $CD8^+$ T cells in humans (Mingari, Moretta et al. 1998).

In mice, the interaction between CD94/NKG2A and Qa-1b can transfer an inhibitory signal to inhibit the cytotoxicity of NK cells (Vance, Kraft et al. 1998), while in humans the cytotoxicity of NK cells is inhibited through the engagement of CD94/NKG2A with its ligand HLA-E (Iwaszko and Bogunia-Kubik 2011). Furthermore, the expression of CD94 on NK cells in humans is also associated with the increase of apoptosis in NK cells (Ida, Robertson et al. 1997), while murine NK cells show lower levels of apoptosis in culture (Gunturi, Berg et al. 2003). This difference may be the result of different expression of activating and inhibitory CD94/NKG2 molecules in mice and humans. In HIV patients, the increased expression of CD94 is observed on some T cells (Galiani, Aguado et al. 1999, Tarazona, DelaRosa et al. 2002). A similar phenotype is also displayed in mice, following *Listeria monocytogenes* and other infectious agents, the expression of CD94 is dramatically increased on $CD8^+$ T cells (McMahon, Zajac et al. 2002, Miller, Peters et al. 2002, Moser, Gibbs et al. 2002, Gunturi, Berg et al. 2003). Moreover, IL-10, IL-12, IL-15 and TGF-β also play critical roles in CD94/NKG2 expression (Mingari, Ponte et al. 1998, Bertone, Schiavetti et al. 1999, Galiani, Aguado et al. 1999, Derre, Corvaisier et al. 2002). Furthermore, because of the activating and inhibitory functions of CD94/NKG2, the expression of CD94/NKG2 on T cells could also impact the cell survival. For example, after *Listeria monocytogenes* infection in mice, $CD94^{hi}CD8^+$ T cells display less apoptosis. This is unique to the CD94/NKG2 receptors, because the expression of Ly49 on these cells does not show the same effect (Gunturi, Berg et al. 2003). In addition, enhanced levels of CD94 may also help a subset of $CD8^+$ effector T cells survive better than others and eventually become memory cells (Gunturi, Berg et al. 2004).

The interaction of CD94/NKG2 and HLA-E also has important clinical significance. For examples, following HCMV infection, viral US6 protein inhibits expression of all class I HLA proteins, while the US2 protein and the US11 protein preserve expression of HLA-E which can interact with NK cell inhibitory receptors and transfer inhibitory signals to inhibit the cytotoxicity of NK cells. In addition, the HCMV UL16 protein can prevent the expression of MIC or ULBP which can interact with the activating receptors on NK cells (Lin, Xu et al. 2007). In addition, HIV infection can also impact the HLA gene expression on the cell surface. For instance, it can induce the downregulation of HLA-A and HLA-B to impact the antigen presentation of these molecules (Nattermann, Nischalke et al. 2005). During HCV infection, the homozygous HLA-E*0101/HLA-E*0101 genotype is associated with enhanced resistance to HCV infection (Schulte, Vogel et al. 2009). Besides the essential role of HLA-E CD94/NKG2 in virus infection, the interaction of these molecules also plays a critical role in immune responses during pregnancy. Fetal trophoblast cells express HLA class Ib, HLA-C and HLA-E in the process of human pregnancy, and HLA-E on trophoblast cells has high affinity to CD94/NKG2 receptor to transfer inhibitory signal to protect from NK cytotoxicity (Ishitani, Sageshima et al. 2003).

In summary, according to recent studies, the interaction of HLA-E (human) or Qa-1b (mouse) and CD94/NKG2 receptors is of critical importance in the process of signal transduction between CTLs or NK cells and their target cells.

7 Aim of the thesis

The acute and chronic infections which are caused by virus in humans lead to millions of deaths each year. Innate and adaptive immune system plays a critical role in antiviral infection. Especially the type I interferon in the innate immune system enables to limit viral replication. Furthermore, adaptive immune priming by innate immune cells could induce pathogen-specific immunity with long-term protection.

In the first part of the thesis, we investigate the role of BAFFR following VSV and LCMV infection. BAFFR can bind BAFF and is also known as tumor necrosis factor receptor superfamily member 13C, which is important for B cell development. It is also associated with many human diseases including rheumatoid arthritis and lupus erythematosus when the BAFF/BAFFR signaling is increased. While the role of BAFFR in B cells has been widely described, its role in innate immunity remains unclear. Therefore, we use BAFFR deficient mice to characterize BAFFR related innate and adaptive immune responses after viral infection.

Based on the results of the study, we found that BAFFR deficient mice display impaired production of type I interferon, reduced viral amplification and presentation, and reduced adaptive immunity. Above all, this study indicates that BAFFR signaling can impact both innate and adaptive immune responses after viral infection.

Furthermore, anti-viral T cell immunity is important for overcoming viral infection, and dysfunction of T cells leads to viral persistence. Previous studies have showed that NK cells play a dual role during viral infection. On the one hand, NK cells can be activated through type I interferon receptor signaling and exhibit perforin-mediated cytotoxicity; On the other hand, they also enable to target virus-specific T cells during viral infection and contribute to the establishment of chronic viral infections. Murine Qa-1b and its human homolog HLA-E can bind to receptors of the NKG2 family on

NK cells. This interaction can transfer inhibitory signals to reduce the activity of NK cells. Therefore, Qa-1b plays an essential role in the regulation of the activity of NK cells in viral infection.

In the second part of the thesis, the results indicate that the expression of Qa-1b can be stimulated by a large dose of LCMV. The deficiency of Qa-1b leads to enhanced NK cell-mediated regulation towards T cells after LCMV infection, persistent viral replication and elevated pathology. However, the depletion of NK cells could rescue these phenotypes partially in *Qa-1b*$^{-/-}$ mice.

8 Material and Methods

8.1 Material

8.1.1 Mice

CD45.1^{+} mice were purchased from Jackson Laboratory, *Baffr*$^{-/-}$, *Ifnar*1$^{-/-}$ and *Ltb*$^{fl/fl}$x*CD19Cre*$^{+}$ mice were described previously (Muller, Steinhoff et al. 1994, Tumanov, Kuprash et al. 2002, Sasaki, Casola et al. 2004). *Qa-1b*$^{-/-}$, *Klrc1*$^{-/-}$, *JhT*$^{-/-}$, *Rag1*$^{-/-}$ and CD45.1^{+}P14^{+} mice maintained under specific pathogen-free conditions. All mice were bred on a C57BL6 genetic background. The experiments were carried out in single ventilated cages. Furthermore, in accordance with the German law for animal protection, all experiments concerning animals were performed with the authorization of the Veterinäramt of Nordrhein Westfalen, Recklinghausen, Germany as well as the institutional guidelines of the Ontario Cancer Institute.

8.1.2 Virus

Vesicular Stomatitis Virus-Indiana strain (VSV-IND, Mudd-Summers isolation), was originally obtained from Prof. D. Kolakofsky (University of Geneva, Geneva, Switzerland). Then VSV was propagated on BHK-21 cells at a multiplicity of infection (Zou, Chastain et al.) of 0.01 and was then plaqued onto Vero cells. Lymphocytic Choriomeningitis Virus (LCMV) stain WE was originally obtained from F. Lahmann-Grube (Heinrich Pette Institute). LCMV strain Docile was originally obtained from Dr. C.J. Pfau (Troy, NY). Viruses were propagated in L929 cells as previously described (Welsh and Seedhom 2008). And viruses were administrated to mice through intravenous injection.

8.1.3 Chemicals

2-beta-mercecaptoethanol (2-ME) (Sigma)

Aceton (Merck)

BD FACS Lysing Solution (Becton Dickinson)

BDTM ACCUDROP (Becton Dickinson)

Brefeldin A (ebioscience)

CaliBRITE Beads (Becton Dickinson)

Citric acid (Sigma)

DEPC Treated Water (Ambion)

dNTPs (Promega)

DPBS powder (PAN BIOTECH)

EDTA Tetrasodium Salt (Calbiochem)

Ethanol 100% (Merck)

FACS Flow (Becton Dickinson)

FACS Rinse (Becton Dickinson)

FACS Shutdown (Becton Dickinson)

FCS (Biochrom)

Fluorescent Mounting Medium (Dako)

Formalin (Sigma)

H_2O_2 (Sigma)

HCL 37% (Sigma)

Isopropanol (Merck)

IL-2 (Miltenyi Biotec)

L-Glutamine-Penicillin-Streptomycin solution (Sigma)

Methylcellulose (FLUKA)

MHC Class I Monomer (NIH Tetramer Core Facility)

o-Phenylenediamine dihydrochloride (Sigma)

PBS, pH7.4 (flow cytometry grade (Life technologies)

Peptides (JPT Peptide Technologies)

Proteinase K (Sigma)

Qdm peptide (AMAPRTLLL)

Saponin (Sigma)

Taq Polymerase (Promega)

Tissue Tek (Sakra)

TRITON X-100 (Fluka)

Trizol (Invitrogen/Peqlab)

Trypsin EDTA Solution (Biochrom)

8.1.4 Kits

B220 B cell isolation kit (Miltenyi)

Cell counting beads (BD)

Foxp3 mouse regulatory T cell (Treg) staining kit (ebioscience)

IFN-α ELISA kit (PBL)

IFN-γ ELISA kit (eBioscience)

ImmPACT NovaRED Peroxidase Substrate kit (Vector Laboratories)

NK cell (DX5) MACS kit (Miltenyi)

Taqman One-step RT-PCR Master Mix Reageants Kit (Applied biosystem)

8.1.5 Medium

Medium VLE RPMI 1640 (Biochrom)

Alpha MEM (Biochrom)

DMEM (PAN BIOTECH)

DPBS (PAN BIOTECH)

Medium IMDM (Lonza)

RPMI 1640 with 20mM HEPES (Biochrom)

8.1.6 Antibodies

Anti-B220 (ebioscience)

Anti-CD11c (ebioscience)

Anti-CD11b (ebioscience)

Anti-CD16/32 (ebioscience)

Anti-CD19 (ebioscience)

Anti-CD3 (ebioscience)

Anti-CD45.2 (ebioscience)

Anti-CD5 (ebioscience)

Anti-CD8 (ebioscience)

Anti-CD90.2 (ebioscience)

Anti-CD117 (ebioscience)

Anti-CD169 (AbD Serotec)

Anti-Eomes (ebioscience)

Anti-F4/80 (ebioscience)

Anti-FceR1 (ebioscience)

Anti-GATA-3 (ebioscience)

Anti-Gr1 (ebioscience)

Anti-IL-7R (ebioscience)

Anti-IFNγ (ebioscience)

Anti-Ly6G (ebioscience)

Anti-MHC II (ebioscience)

Anti-MOMA-1 (Abcom)

Anti-NK1.1 (self-made)

Anti-RORγT (ebioscience)

Anti-PD-1 (ebioscience)

Anti-TCRb (ebioscience)

Anti-TNFα (ebioscience)

Streptavidin (ebioscience)

8.1.7 Plastics

1.5ml Eppendorf Tube (Eppendorf)

10ml Syringe (Strong, Holmes et al.)

15ml Pipet (Corning)

15ml Falcon Tube (BD Falcon)

2.0ml Eppendorf Tube (Eppendorf)

24 Well plate (TPP)

25ml Pipet (Corning)

29G 0.5ml Syringes (Becton Dickinson)

5ml Pipet (Corning)

50ml Centrifuge tube (TPP)

50mm Filter Unit (Thermo Scientific)

5ml Polystyrene Tube (BD Faclon)

70µm Cell strainer (BD Falcon)

96 Well Flat bottom plate (TPP)

96 Well U bottom plate (TPP)

Filter Tips (Star Lab)

MicroAmp 384-Well RT-PCR plate (Applied Biosystems)

Reservoirs (VWR)

Tips (SARSTEDT)

Tips (Star Lab)

Tissue Culture Flasks (TPP)

8.1.8 Machines

-20°C (Bosch)

4°C (Bosch)

7900HT Fast Real-Time PCR machine (Applied biosystem)

QuantStudio 5 Real-Time PCR machine (Applied biosystem)

-80°C (Thermo Scientific)

Biosafety Cabinet (Antair BSK)

Centrifuge (Eppendorf)

Centrifuge (Thermo Scientific)

CO_2 Incubator (Thermo Scientific)

Crypstat (Leica)

FACSCanto II (Becton Dickinson)

FACS Aria III (Becton Dickinson)

BD LSRFortessa (Becton Dickinson)

Light Microscope (VWR)

LSM510 Laser Scanning Microscope (Zeiss)

Observer Z1 Microscope (Zeiss)

AXIO Lab A1 Microscope (Zeiss)

Microplate Spectrophotometer (Thermo Scientific)

NanoDrop (Thermo Scientific)

Shaker (Heidolph)

Thermomixer (Eppendorf)

Tissue Lyser II (Qiagen)

Vortex (Scientific Industries)

8.2 Methods

8.2.1 Survival experiment following VSV infection

Wildtype and *Baffr*$^{-/-}$ mice were infected with 10^5 pfu of VSV by i.v., then all mice were maintained under specific pathogen-free conditions;

The health status of the mice was checked twice per day, and according to the appearance of clinical signs of VSV replication in the CNS (central nervous system), mice were removed from the experiment when they are paralyzed.

8.2.2 VSV Neutralizing antibody assay

Day 0: The preparation of VERO cell monolayers

Treat confluent VERO cells with trypsin and collect cells, re-suspend cells in 2%FCS Alpha Medium and count the cells, then dilute cells to the concentration at $1.5x10^5$ cells/ml; add 100µl of cells suspension (~$1.5x10^4$ cells) to each well of 96-well flat bottom Tissue Culture plate; incubate VERO cells at 37°C 5% CO2 cell culture incubator overnight until monolayer is confluent;

Day 1: The preparation of serum samples

Firstly add 20µl serum samples into Eppendorf tube which contains 780µl 2%FCS alpha medium (1:40 dilution), then switch tubes on the H_2O water bath (56°C) to inactivate samples for 30min; secondly, use 96-well round bottom plates to prepare 2-fold serial serum dilutions: add 100µl 2%FCS alpha medium to row2-12, and then add 200µl each serum sample to row1, transfer 100µl from row1 to row2, mix well and continue to row12, discard the last 100µl medium; thirdly, dilute VSV in 2%FCS alpha medium to concentration 5000pfu/ml, then keep virus on ice, add 100µl of diluted VSV to each well of plates, and each well should contain 30-50pfu VSV; incubate samples with virus exactly 90min in the 37°C 5% CO_2 cell culture incubator; finally, transfer serum-virus mixture onto VERO cell monolayers and incubate 3h in the 37°C 5% CO_2 cell culture incubator; Take overlay out of fridge to warm to room temperature, and add 100µl to each well then incubate in 37°C 5% CO_2 cell culture incubator for 1 day;

Day 2: Fixing and staining of monolayers

Utilize light microscope to check for plaque formation, make sure plaques are visible clearly; then flick off medium and add 100-200µl of 0.5% crystal violet staining solution per well; thirdly, stain the cell for 1h at room temperature; Flick off 0.5% crystal violet staining solution and wash plates 5-6 times in running water and dry plates; finally, scoring and statistics: the highest dilution of serum that reduces the number of

plaques by 50% is recorded.

8.2.3 Tetramer staining

Spleen tissue was placed into 15ml tube which contains 5ml 10%FCS IMDM medium; then smash spleen tissue and transfer 50µl cell suspension into 96-well round bottom plate, centrifuge down and flick off the medium; add 50µl tetramer dilution (tet-gp33, tet-np396 or tet-gp66) in each sample and incubate samples in 37°C 5% CO_2 cell culture incubator for 15min (tet-gp33, tet-np396) or 30min (tet-gp66); after incubation, add 50µl antibody dilution in each sample, and incubate 30min in 4°C fridge; finally, wash samples one time with FACS buffer, centrifuge down samples and flick off FACS buffer, and re-suspend the cell pellet in 200µl FACS buffer to check the staining on the flow cytometry machine.

8.2.4 Intracellular cytokine staining

Add 100µl of peptide dilution in each sample to stimulate cells (gp33, np396 for $CD8^+$ cells; gp61 for $CD4^+$ T cells) in 37°C 5% CO_2 cell culture incubator for 1h; after one hour add 20µl Brefeldin A (1:1000 dilution) in each sample and then incubate samples in 37°C 5% CO_2 cell culture incubator for 5h; after 5h incubation add surface marker antibody (CD4 or CD8) to each sample and incubate in 4°C fridge for 30min; then wash one-time samples with FACS buffer, centrifuge down samples and flick off FACS buffer, add 200µl fixation buffer and incubate at room temperature for 10min; after 10min wash two times samples with perm wash buffer, centrifuge down samples and flick off perm wash buffer; add 50µl IFN gamma antibody dilution which is diluted in perm wash buffer in each sample and incubate samples in 4°C fridge for 30min; finally, wash samples one time with perm wash buffer, centrifuge down samples and flick off perm wash buffer, then re-suspend the cell pellet in 200µl perm wash buffer to check the staining on the flow cytometry machine.

8.2.5 LCMV plaque assay

Put frozen tubes containing orgains (spleen, liver, lung, kidney, spinal cord and brain) in air flow of tissue culture hood until samples thawed, homogenize in Qiagen Tissue-Lyser for 3min at 30Hz immediately; after tissue lysis, place samples on ice and proceed to incubation steps; add 130µl 2%FCS alpha medium to columns 2-12 of 96-well round bottom plate; and transfer 90µl of samples in duplicate (A-B, C-D, etc.) to the first column; serially dilute samples from left to right, transferring 60µl samples repeatedly and exchange tips at column 7; then transfer 200µl samples in sequence from columns 12, 10, 8, 6, 4, 2 of the 96-well round plate to columns 6, 5, 4, 3, 2, 1 of the 24-well plate; and add 200µl MC57 cells ($1x10^6$ cells /ml in 2%FCS alpha medium) to all rows of the 24-well plate containing diluted samples; put the 24-well plate to 37°C 5% CO_2 cell culture incubator for 2h; after 2h, add 200µl overlay (2XDMEM and 2%Methyl-cellulose), and then put plates to 37°C 5% CO_2 cell culture incubator for two days; after two days, flick off medium and add 200µl 4% Formalin-PBS to each well of the plate, then incubate at room temperature for 30min; after fixation flick off formalin and add 200µl Hanks BSS+1%Triton X-100 to each well of the plate, then incubate at room temperature for exactly 20min; after 20min flick off medium and wash plates with PBS twice; after washing add 200µl 10%FCS PBS to each well of the plate for blocking, and incubate for more than 1h at room temperature; after one hour incubation flick off medium and add 200µl VL4 rat anti-LCMV mAb supernatant diluted in 1%FCS PBS, and incubate for 1h at room temperature; one hour later flick off medium and wash the plate with PBS twice, then add 200µl secondary anti-rat HRP diluted in 1%FCS and incubate for 1h at room temperature; after secondary antibody incubation flick off medium and wash the plate with PBS twice, then add 200µl color reaction solution to each well, incubate for 15min at room temperature until sufficiently dark plaques are produced; after color reaction flick off color reaction solution and wash the plate with tap water to set color; finally, dry the plates and do the statistics.

8.2.6 VSV plaque assay

Trypsin and collect confluent VERO cells ($1x10^6$ cells/ml), then add 200µl to each well of 24-well plate; transfer the plates to 37°C 5% CO_2 cell culture incubator for next step; put frozen tubes in air flow of tissue culture hood until samples thawed, homogenize in Qiagen TissueLyser for 3min at 30Hz immediately; then place samples on ice and proceed to incubation steps; add 130µl 2%FCS alpha medium to columns 2-12 of 96-well round bottom plate; then add 90µl of samples in duplicate (A-B, C-D, etc.) to the first column; serially dilute samples from left to right, transferring 60ul samples repeatedly and exchange tips at column 7; transfer 200µl samples in sequence from columns 12, 10, 8, 6, 4, 2 of the 96-well round plate to columns 6, 5, 4, 3, 2, 1 of the 24-well plate containing prepared VERO cells; put the 24-well plate to 37°C 5% CO_2 cell culture incubator for 3h; after 3h, add 200µl overlay (2XDMEM and 2%Methylcellulose), and then put plates to 37°C 5% CO_2 cell culture incubator for one days; the next day use light microscope to check for plaque formation, make sure plaques are clearly visible; flick off medium and add 200µl 0.5% crystal violet solution to each well of the plate, then incubate for 1h at room temperature; after one hour flick off 0.5% crystal violet staining solution and wash plates 5-6 times in running water and dry plates; finally, dry the plates and do the statistics.

8.2.7 RNA isolation

Put frozen tubes in air flow of tissue culture hood until samples thawed, homogenize in Qiagen TissueLyser for 3min at 30Hz immediately; then add 400µl TRIZOL into a 1.5ml Eppendorf tube and transfer 100µl lysing sample to this tube, vortex it; add 100µl chloroform to the same tube and vortex sample for 30 seconds; spin down samples in the centrifuge at 4°C, 12000rpm/min for 10min; transfer the top 200µl into a new Eppendorf tube and add 400µl isopropanol, vortex and incubate samples at room temperature for 15min; spin down samples in the centrifuge at 4°C, 12000rpm/min for 8min

again; remove the supernatant and add 800µl 75% ethanol to wash the pellet; spin down samples in the centrifuge at 4°C, 12000rpm/min for 5min; finally, remove the supernatant and dry samples in the hood, then add 102µl DEPC-treated water to the tube.

8.2.8 Conventional staining of histology

Fix histology for 10min in acetone; then dry samples for 5min at room temperature and mark the area of histology with glue pen; after drying block binding sites with 10%FCS PBS for 45min at room temperature; transfer histology on staining dish and wash samples once with 0.05%Tween-20 PBS; then add 100µl diluted primary antibody on the slides, and incubate for 1h at room temperature; after 1h incubation wash slides three times with 0.05%Tween-20 PBS, and add streptavidin HRP (rose color without dilution 2-3 drops) and incubate for 1h at room temperature; wash slides three times with 0.05%Tween-20 PBS again, then add 100µl refresh prepared color solution (DAB substrate buffer 1ml+1 drop DAB40x or 1ml ImmPACT NovaRED Diluent+mixed ImmPACT NovaRED reagents) on the slides, observe the color reaction under microscope, since seeing the positive staining, put the slides into the water to stop the reaction; place slides to the glass basket and transfer samples to Hämalaun for 3 seconds, then wash slides with tap running water for 10min; finally, add mounting medium and cover slides with glass cover.

8.2.9 H&E staining of histology

Fix samples in 50%methanol+50%acetone for 10min at room temperature; dry slides for 5min at room temperature and transfer slides to dH2O for 2min; transfer slides to Hematoxylin to incubate 5min at room temperature; then wash slides in tap running water for 10min; after washing transfer samples to 1%HCl+70%ethanol shortly; wash slides in tap running water for 10min again; transfer slides to Eosin for 12 seconds at room temperature; put slides to 70%ethanol for 3min; transfer slides to 80%ethanol and

incubate for 3min; then transfer slides to 100%ethanol and incubate for 3min; finally transfer slides to Xylol and incubate for 5min, then add mounting medium and cover slides with glass cover.

8.2.10 Fluorescence staining of histology

Fix samples in acetone for 10min at room temperature; dry slides for 5min and mark the cycle of target histology area with glue pen; Block slides with 10%FCS PBS for 45min at room temperature; transfer slides to staining dish and add 100µl primary antibody on the slides, then incubate at room temperature for 1h; wash slides with 0.05%Tween-20 PBS for three times; add 100µl secondary antibody on the slides, then incubate at room temperature for 1h; wash samples with 0.05%Tween-20 PBS for three times; add fluorescence mounting medium and cover slides with glass cover.

8.2.11 Interferon alpha enzyme-linked immunosorbent assay (ELISA)

Prepare serum samples and dilute with sample buffer; at the same time prepare mouse IFN alpha standard and dilute into a series of concentrations with sample buffer; then prepare antibody solution and dilute with concentrate diluent; add 100µl standard, blank and diluted samples to the 96-well pre-coated plate, then add 100µl diluted antibody solution to the plate; Incubate samples with antibody at 450rpm shake for 1h; transfer plate to 4°C fridge and incubate 20-24h; dilute 5ml of wash solution concentrate to a final volume of 100ml of distilled or deionized water and thoroughly mix, then store at room temperature for next step; after 20-24h, take out the plate from 4°C fridge, flick off the contents of the plate and wash with diluted wash solution for 4 times; prepare HRP solution within 15min before to use, dilute HRP conjugate in recommended volume of assay diluent, then add 100µl diluted HRP solution to the plate and incubate 2h at room temperature with shaking at 450rpm; after 2h, flick off the contents of the plate

and wash the plate with diluted wash solution for 4 times; then add 100µl TMB substrate solution to the plate, incubate samples in the dark for 15min at room temperature; after 15min incubation, add 100µl stop solution to the plate and do not empty the contents of the plates; Transfer the plate to the microplate reader, determine the absorbance at 450nm in 15min after adding the stop solution.

8.2.12 Purification and transfer of B cells

Transfer dissected mouse spleen to a 15ml tube containing 5ml MACS buffer; smash spleen in a cell strainer, and transfer single-cell suspension to the 15ml tube, spin down at 300g for 10min, 4°C; then remove the supernatant in the tube, add 100µl CD45R(B220) MicroBeads and 220µl l MACS buffer, incubate at 4°C fridge for 10min; wash cells by adding 9ml MACS buffer, then spin down at 300g for 10min, 4°C; and remove the supernatant and add 1ml MACS buffer to re-suspend cells, rinse column with 3ml MACS buffer; then transfer 1ml MACS buffer with cells to run through the column; after buffer run through the column, remove column from the separator and place it on a collection tube; add 3ml MACS buffer to the column, flush out fraction with the magnetically labeled cells immediately. Finally, spin down cells at 300g for 10min, 4°C, then remove the supernatant and add 3ml PBS to re-suspend B cells, count cells then transfer 15million B cells to each mouse.

8.2.13 Cell depletion

NK cells were depleted with i.v. injection of 400µl anti-NK1.1 (clone PK136) at day -3 and day -1 before viral infection. CD8 and CD4 cell were depleted with i.v. injection of 400µl anti-CD8 (clone YTS169.4) or 400µl antiCD4 (clone YTS 191) at day -3 and day -1 before viral infection. For depletion of macrophages, mice were treated with 200µl clodronate-liposomes at day -1 before viral infection. Control mice were treated with 200µl empty control-liposomes at day -1 before viral infection.

8.2.14 RT-PCR analysis

Prepare master mix solution for RT-PCR reaction as follow:

2x Master Mix: 5μl/sample

40x Inhibitor Mix: 0.25μl/sample

DEPC-dH2O: 2.25μl/sample

Taqman primer: 0.5μl/sample

Then set up and run RT-PCR program as follow:

50°C 10min

95°C 3min

95°C 15seconds

60°C 1min

The number of reaction cycle: 45repeats

Since the RT-PCR program is finished, the expression levels of the target genes are normalized to *Gapdh* (glyceraldehyde-3-phosphate dehydrogenase) expression levels (ΔCt), gene expression values are then calculated based on the ΔΔCt method relative to naive wild-type (Mackay, Woodcock et al.) controls, RQ (relative quantities) are determined by the following equation: $RQ=2^{-\Delta\Delta Ct}$.

8.2.15 NK cell purification and Culture

Single cell suspended splenocytes were collected according to the manufacturer's instructions with the DX5 MACS kit. Positively sorted NK cells were stimulated with 1000U/ml IL-2.

8.2.16 *In vitro* Flow-cytometry-based NK killing assay

Negatively sorted $CD8^+$ T cells were stimulated with 5ug/ml Concanavalin A for 24h, and dead cells were removed by gradient centrifugation. Live activated $CD8^+$ T cells

were co-cultured with IL-2-derived NK cells and B cells at indicated effector-to-target ratio in the presence of 2U/ml IL-2 for 4h. Dead $CD8^+$ T cells were identified by 7-amino actinomycin D (7-AAD) staining. NK specific killing was calculated by (% liver T cells $_{\text{(without co-incubation with NK cells)}}$-% liver T cells $_{\text{(co-incubation with NK cells)}}$)/ (%live T cells $_{\text{(without co-incubation with NK cells)}}$)

8.2.17 Statistical analysis

Data are expressed as mean ± SEM (standard errors of the mean). For analysis of detect statistically significant differences between two groups, Student's t test is used. Significant differences between several groups are detected by one-way ANOVA with Bonferroni or Dunnett post hoc tests or as mentioned specifically in the figure legends. The P value is less than 0.05, which is considered as statistically significant.

9 Results

9.1 Part I: Deficiency of the B cell-activating factor receptor results in limited CD169^{+} macrophage function during viral infection

Haifeng C. Xu[*], Jun Huang[*], Vishal Khairnar, Vikas Duhan, Aleksandra A. Pandyra, Melanie Grusdat, Prashant Shinde, David R. McIlwain, Sathish Maney, Jennifer Gommerman, Max Löhning, Pamela S. Ohashi, Tak W. Mak, Kathrin Piper, Heiko Sic, Matthaios Speletas, Hermann Eibel, Carl F. Ware, Alexel V. Tumanov, Andrey A. Kruglov, Sergel A. Nedospasov, Dieter Häussinger, Mike Recher, Karl S. Lang, Philipp A. Lang (2015). "Deficiency of the B cell-activating factor receptor results in limited CD169^{+} macrophage function during viral infection." J Virol **89**(9): 4748-4759

Declaration and statement of contribution

I, Jun Huang, declare that the content of this dissertation is original. I have cited appropriate texts and figures wherever is necessary. No part of this dissertation is submitted somewhere else for consideration of a degree. This part of results in the dissertation is published on Journal of Virology on 2015 as I cited in the beginning.

I was involved in execution of the experiments, analysis, writing and preparation part of the manuscript which consists round 20% of the published manuscript.

The figures of this part result which I prepared and analyzed are as follow: FIG 1C-D; FIG2B-E; FIG 2B, 2D-E; FIG 4; FIG 5A-D; FIG 6A; FIG 7A; B and E and Graphical fig 1-4 in the introduction part.

Contribution in the other published manuscripts is mentioned in the list of the publications.

Jun Huang

Date:
Place: Düsseldorf, Germany

9.1.1 BAFFR is important for preventing from viral infection

BAFF receptor deficiency in mice leaded to severe B cell lymphopenia but did not have a main impact on the numbers of T cell, neutrophil and dendritic cell (FIG 1A and B). In addition, *Baffr*$^{-/-}$ animals also showed reduced neutralizing antibody production after VSV infection (FIG 1C). While WT mice could overcome VSV infection, *Baffr*$^{-/-}$ mice exhibited a reduced survival rate which is consistent with the phenotype of delayed neutralizing antibody production (FIG 1D). Furthermore, the presence of VSV could be detected when neuronal tissue was collected from the sick animals at day7 to day 10 after infection (data not shown). Above all, these data indicated that after VSV infection, the deficiency of BAFFR resulted in fatal disease development.

FIG 1

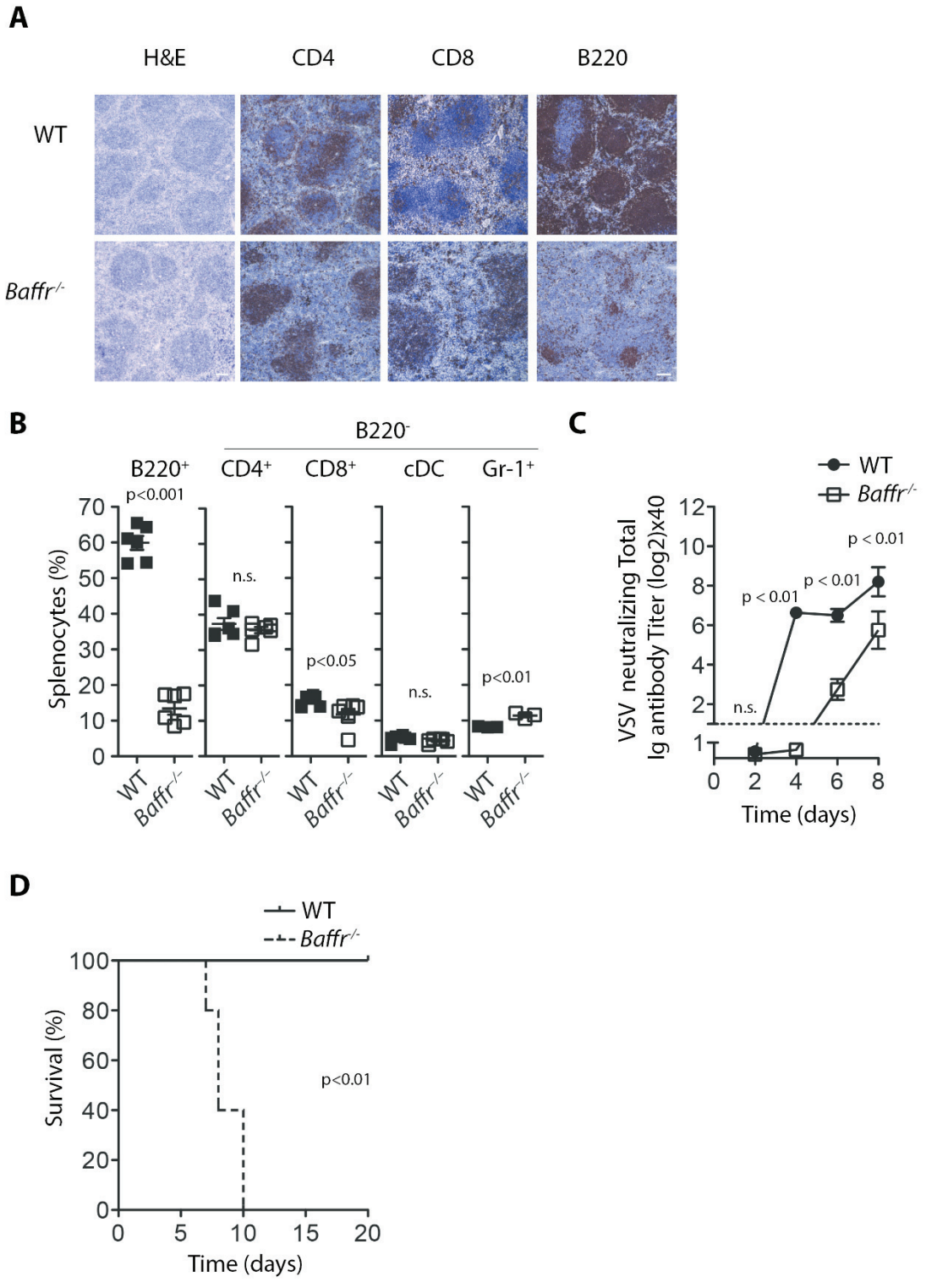

A
H&E
CD4
CD8
B220
WT
Baffr-/-
B
B220-
B220+
CD4+
CD8+
cDC
Gr-1+
p<0.001
n.s.
p<0.05
n.s.
p<0.01
Splenocytes (%)
WT
Baffr-/-
C
WT
Baffr-/-
p < 0.01
p < 0.01
p < 0.01
n.s.
VSV neutralizing Total Ig antibody Titer (log2)x40
Time (days)
D
WT
Baffr-/-
p<0.01
Survival (%)
Time (days)

FIG 1 BAFFR is important for antiviral infection. (Xu, Huang et al. 2015)

(A) Sections from snap-frozen spleen tissues of WT and *Baffr*$^{-/-}$ animals were stained with H&E (scale bar=100μm) and stained with anti-CD4, anti-CD8, or anti-B220 antibodies (scale bar=50μm) (n=5). (B) Single-cell suspensions of splenocytes harvested from WT and *Baffr*$^{-/-}$ animals were monitored by specific monoclonal antibodies for the indicated immune cell populations. (n=6, n.s.=not significant). (C and D) WT and BAFFR-deficient animals were infected with 10^5pfu of VSV. (C) antiviral neutralizing antibodies in serum were monitored at indicated time points by PRNT assay (n[initial]=8). (D) Survival rate of WT and *Baffr*$^{-/-}$ animals was analyzed over the indicated time points (n[initial]=5). The error bars show SEM; n.s.=not significant.

9.1.2 Enforced viral replication is mediated by BAFFR after viral infection

Virus titers could be detected in the spleen of WT mice at 8h and 24h after VSV infection, but virus was below the detection limit in *Baffr*$^{-/-}$ animals (FIG 2A), however, the VSV replication was detectable in the central neutron system at later time point of infection. Nevertheless, the production of neutralizing antibodies was not the only factor to control VSV infection, innate type I interferon production also played an essential role. *Baffr*$^{-/-}$ animals exhibited decreased type I interferon production in the serum, but WT mice had high type I interferon levels in the early time points after VSV infection, which is consistent with reduced viral replications in the spleen of *Baffr*$^{-/-}$ animals (FIG 2B). Additionally, compared with corresponding controls, the interferon regulated genes, such as Isg15, Mx1 and Ifit2, was reduced in the brain of *Baffr*$^{-/-}$ mice following VSV infection at 24h (FIG 2C). However, after injection of poly I:C, the Toll-like receptor 3 agonist, competent level of type I interferon was detected in *Baffr*$^{-/-}$ mice (FIG 2D). Moreover, the expression levels of interferon regulated genes in the brain from BAFFR deficient mice and WT controls after treatment with poly I:C were similar (FIG 2E), which indicated that the ability to induced type I interferon was normal in *Baffr*$^{-/-}$ mice. Furthermore, similar IRG expression levels were also observed in the brain tissue of *Baffr*$^{-/-}$ and WT mice following treatment of recombinant IFN-α (FIG 2E). In sum, decreased production of type I interferon in *Baffr*$^{-/-}$ animals after VSV infection was likely explained by another mechanism than defective pathogen recognition receptor signaling.

FIG 2

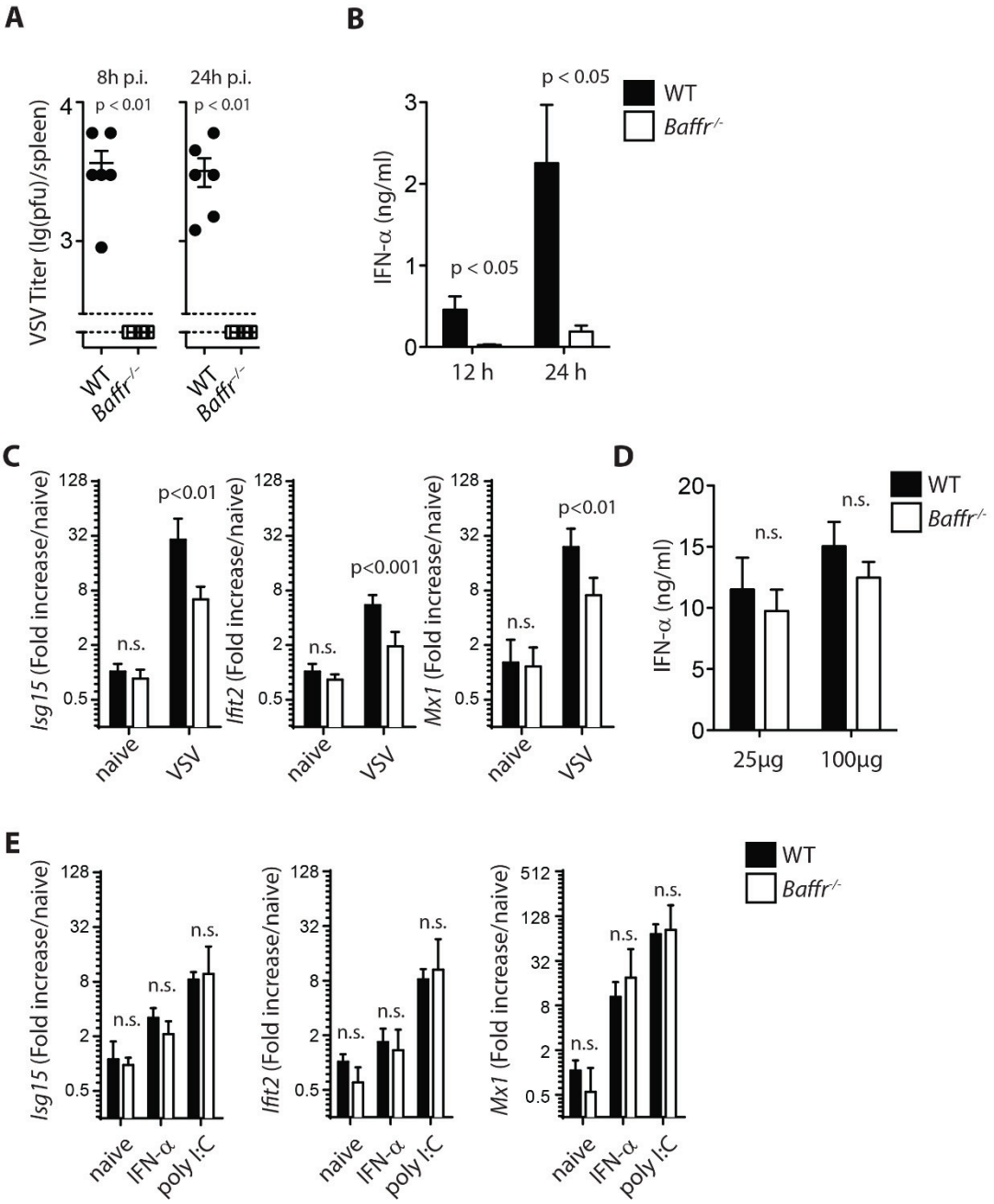

A
8h p.i.
24h p.i.
p < 0.01
p < 0.01
VSV Titer (lg(pfu)/spleen)
WT
Baffr-/-
B
p < 0.05
p < 0.05
IFN-α (ng/ml)
12 h
24 h
C
p<0.01
p<0.001
p<0.01
n.s.
Isg15 (Fold increase/naive)
Ifit2 (Fold increase/naive)
Mx1 (Fold increase/naive)
naive
VSV
D
n.s.
n.s.
IFN-α (ng/ml)
25µg
100µg
E
n.s.
Isg15 (Fold increase/naive)
Ifit2 (Fold increase/naive)
Mx1 (Fold increase/naive)
naive
IFN-α
poly I:C

FIG 2 Enforced viral replication was mediated by BAFFR after viral infection (Xu, Huang et al. 2015)

(A to C) WT and BAFFR deficient animals were infected with 10^5pfu of VSV. (A) Virus titers at 8h(left) and 24h(right) following infection in spleen were monitored. (n=6). The detection limit was indicated by the dashed line. (B) IFN-α in the serum was measured at 12h and 24h after infection with 105pfu of VSV from WT and *Baffr*$^{-/-}$ animals (n=6). (C) The mRNA expression levels of *Isg15*, *Mx1* and *Ifit2* in brain tissue were analyzed from naïve and infected animals at 24h. (n=5). (D) IFN-α in the serum from WT and *Baffr*$^{-/-}$ animals were measured after 25μg and 100μg poly I:C injection. (n=6). (E) The mRNA expression levels of *Isg15*, *Mx1* and *Ifit2* in brain tissue were measured from WT and *Baffr*$^{-/-}$ animals at naïve conditions and at 6h after treatment with 10000 units of mouse recombinant IFN-α or 100μg poly I:C. (n=6). The error bar indicated SEM; n.s.=not significant.

9.1.3 BAFFR signaling is important for maintenance of metallophilic macrophage in the spleen

Early viral replication was dependent on $CD169^+$ metallophilic macrophages in the spleen and is induced by Usp18-mediated resistance to type I interferon in these cells. $CD169^+$ cells were contact with bloodstream directly and could remove virus particles and apoptotic cells from circulation in the spleen of mice. The deficiency of $CD169^+$ macrophages blocks virus replication in the early time point after infection, which results in limited antigen amplification and decreased virus induced immune responses. Since we noticed that *Baffr*$^{-/-}$ mice showed reduced viral titers early after infection, the potential mechanisms by which BAFFR signaling might be related to enforced virus replication were investigated. In the bone marrow, Myeloid progenitor populations, such as the GMP (Lin^-, $Sca1^-$, $IL\text{-}7R^-$, $CD117^+$, $CD34^+$ and $CD16/32^+$) or the CMP (Lin^-, $Sca1^-$, $IL\text{-}7R^-$, $CD117^+$, $CD34^+$ and $CD16/32^-$) were not different between *Baffr*$^{-/-}$ and WT animals (FIG 3A). But $CD169^+$ cells, which were essential following early viral replication, were highly decreased in the spleen of *Baffr*$^{-/-}$ mice (FIG 3B), while red-pulp macrophages exhibited similar frequencies between two groups (FIG 3C). These data illustrated that BAFFR was required for maintenance of $CD169^+$ cells.

Furthermore, the presence of $CD169^+$ cells after infection was investigated. The numbers of $CD169^+$ cells were reduced in *Baffr*$^{-/-}$ mice, while there was still a residual population in the naïve condition (FIG 3D and E). However, compared to WT controls, $CD169^+$ cells sharply disappeared in *Baffr*$^{-/-}$ animals shortly following VSV infection (FIG 3D). And VSV levels (detected by the VSV-specific monoclonal antibody Vi10) were decreased in the spleen of *Baffr*$^{-/-}$ mice after VSV infection, but the virus could be detectable in the spleen of WT controls (FIG 3E). Moreover, whether type I interferon affected $CD169^+$ cell survival directly was also investigated. The numbers and distributions of $CD169^+$ cells in spleen of *Ifnar*$1^{-/-}$ mice and WT controls were similar (FIG

4). Above all, these data illustrated that the deficiency of BAFFR expression was related to a reduced presence of splenic $CD169^+$ cells after virus infection, and the number of $CD169^+$ cells was decreased in the early time point following VSV infection.

FIG 3

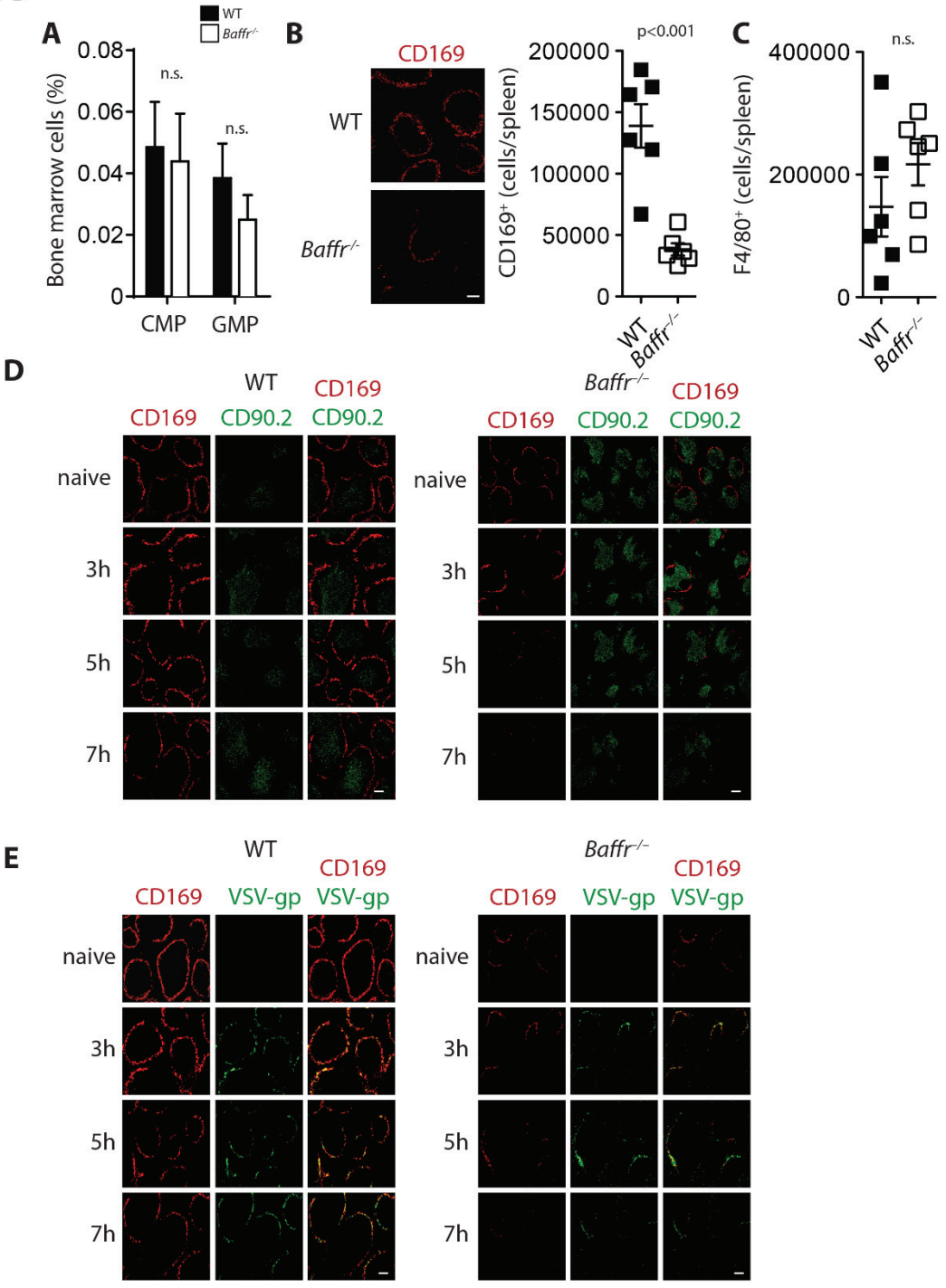

A
WT
Baffr-/-
Bone marrow cells (%)
0.08
0.06
0.04
0.02
0
n.s.
n.s.
CMP
GMP
B
CD169
WT
Baffr-/-
CD169+ (cells/spleen)
200000
150000
100000
50000
0
p<0.001
WT
Baffr-/-
C
F4/80+ (cells/spleen)
400000
200000
0
n.s.
WT
Baffr-/-
D
WT
CD169
CD169
CD90.2
CD169
CD90.2
Baffr-/-
CD169
CD90.2
CD169
CD90.2
naive
3h
5h
7h
E
WT
CD169
CD169
VSV-gp
CD169
VSV-gp
Baffr-/-
CD169
VSV-gp
CD169
VSV-gp
naive
3h
5h
7h

FIG 3 BAFFR signaling is important for the maintenance of metallophilic macrophage and viral replication in the spleen (Xu, Huang et al. 2015)

(A) CMP and GMP from the bone marrow in WT and *Baffr*$^{-/-}$ animals were monitored by flow cytometry. (n=6, n.s.=not significant). (B) (left) Sections of snap-frozen spleen tissues from WT and *Baffr*$^{-/-}$ animals were stained with anti-CD169 (clone MOMA-1) and analyzed by fluorescence microscopy. (n=6, scale bar=100μm). (right) CD169^{+} cells were monitored in spleen from WT and *Baffr*$^{-/-}$ animals by flow cytometry (n=6). (C) F4/80^{+} cells in the spleen from WT and *Baffr*$^{-/-}$ animals were monitored by flow cytometry (n=6). (D) Sections of snap-frozen spleen from WT and *Baffr*$^{-/-}$ animals were stained with anti-CD169 and anti-CD90.2 antibodies at naïve condition and 3h, 5h and 7h following VSV infection. (n=6, scale bar=100μm). (E) Sections of snap-frozen spleen from WT and *Baffr*$^{-/-}$ animals were stained with anti-CD169 and anti-VSV-G protein (clone Vi10) at naïve condition and at 3h, 5h and 7h after VSV infection. (n=6, scale bar=100μm).

FIG 4

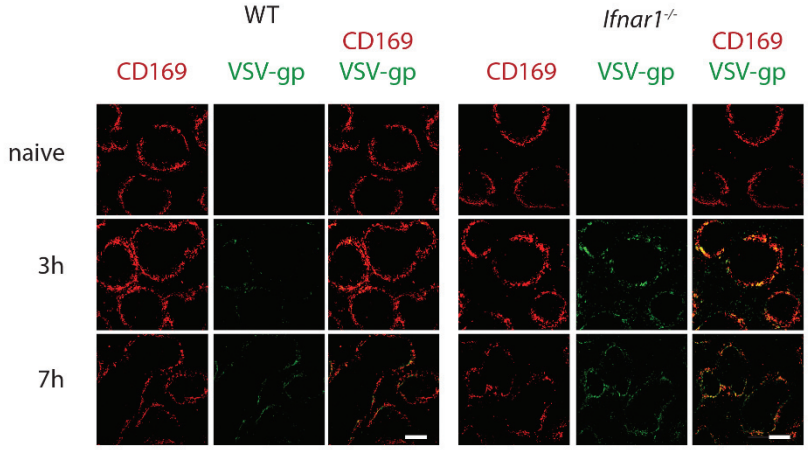


FIG 4 CD169^{+} survived after VSV infection in *Ifnar1*$^{-/-}$ animals (Xu, Huang et al. 2015) Sections of snap-frozen spleen from WT and *Ifnar*1$^{-/-}$ animals were stained with anti-CD169 and anti-VSV-G protein (clone Vi10) antibodies at naïve condition and at 3h, 7h after VSV infection. (n=6, scale bar=100μm)

9.1.4 Lack of BAFFR will cause diminished B cell-mediated maintenance of CD169$^+$ cells

Since we observed that the BAFFR signaling is important for maintenance of metallophilic macrophage in the spleen, then we furthermore addressed the mechanism whether the reduction of CD169$^+$ cells in *Baffr*$^{-/-}$ animals occurred due to severe B cell lymphopenia. First, B cells that were harvested from WT controls were transferred into *Baffr*$^{-/-}$ animals, then CD169$^+$ cells were monitored in spleen after 40 days following cell transfer. And CD169$^+$ cells could be detected in spleen of *Baffr*$^{-/-}$ mice that were supplemented with B cells (FIG 5A). Moreover, the productions of type I interferon and neutralizing antibody were also increased in the WT B cells transfer group when compared with *Baffr*$^{-/-}$ animals (FIG 5B and 5C). And these mice that received WT B cells could overcome the VSV infection (FIG 5D). Taken together, these data suggested that B cells played a critical role in mediating maintenance of CD169$^+$ cells and consequently contributed to innate immunity following viral infection.

Additionally, Lymphotoxin signaling was important for CD169$^+$ cell development in spleen and lymph node. And lymphotoxins that derived from B cells were essential for maintenance of CD169$^+$ cells. Consistent with previous research achievements, we also observed that compared to corresponding controls, *Baffr*$^{-/-}$ mice showed reduced expression levels of lymphotoxin alpha and beta (FIG 6A). These data demonstrated that lower lymphotoxin expression in B cell deficient animals may be insufficient to maintenance normal levels of CD169$^+$ cells. To further investigate the role of lymphotoxin beta in enforced viral replication, *Ltb*$^{fl/fl}$ X *CD19-Cre*$^+$ mice were infected with VSV and compared with their corresponding controls. And *Ltb*$^{fl/fl}$ X *CD19-Cre*$^+$ mice showed reduced CD169$^+$ cells compared to WT mice (FIG 6B). Moreover, decreased type I interferon production early following infection in *Ltb*$^{fl/fl}$ X *CD19-Cre*$^+$ mice were observed (FIG 6C), which is consistent with previous researches and out data from *Baffr*$^{-/-}$ animals. And we also observed lower production of neutralizing antibodies after low-

dose infection (FIG 6D). Taken together, these data suggested that deficiency of B cells or B cell-derived expression of Ltβ leaded to limited innate immune responses and delayed adaptive immune priming.

FIG 5

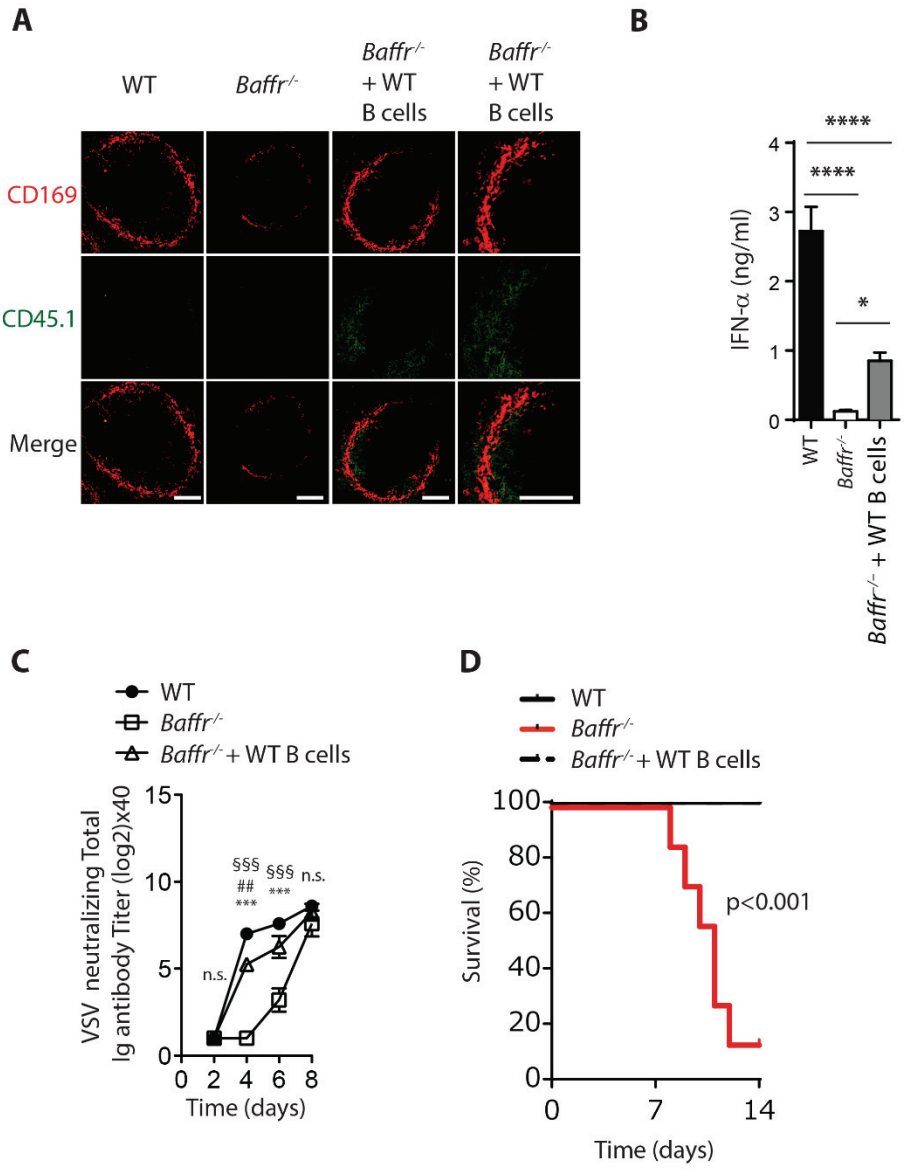

A
WT
Baffr-/-
Baffr-/- + WT B cells
Baffr-/- + WT B cells
CD169
CD45.1
Merge
B
IFN-α (ng/ml)
****
****
*
WT
Baffr-/-
Baffr-/- + WT B cells
C
WT
Baffr-/-
Baffr-/- + WT B cells
VSV neutralizing Total Ig antibody Titer (log2)x40
§§§ ## ***
§§§ ***
n.s.
n.s.
Time (days)
D
WT
Baffr-/-
Baffr-/- + WT B cells
Survival (%)
p<0.001
Time (days)

FIG 5 B cells mediated the maintenance of $CD169^+$ cells (Xu, Huang et al. 2015)

(A) Purified WT B cells were transferred into *Baffr*$^{-/-}$ animals, after 40 days, sections of snap-frozen spleen from the indicated groups were stained with anti-$CD169^+$ and anti-CD45.1 antibodies. (n=5, scale bar=100μm). (B to D) Purified WT B cells were transferred into *Baffr*$^{-/-}$ animals, after 40 days, mice were infected with 10^5pfu VSV. (B) The concentration of IFN-α was measured at 24h after infection in the indicated groups. (*, $p<0.05$; ****, $p<0.0001$; the Holm-Sidak test was used for post hoc testing). (C) Total neutralizing Ig antibodies were measured at day2, day4, day6 and day8 after VSV infection. (n=4-5, WT and *Baffr*$^{-/-}$ ***, $p<0.001$; WT and *Baffr*$^{-/-}$ with transferred B cells ##, $p<0.01$; *Baffr*$^{-/-}$ and *Baffr*$^{-/-}$ with transferred B cells §§§, $p<0.001$). (D) The survival rate was monitored among the indicated groups after 10^5pfu VSV infection (n=7-8). The error bar showed SEM; n.s=not significant.

FIG 6

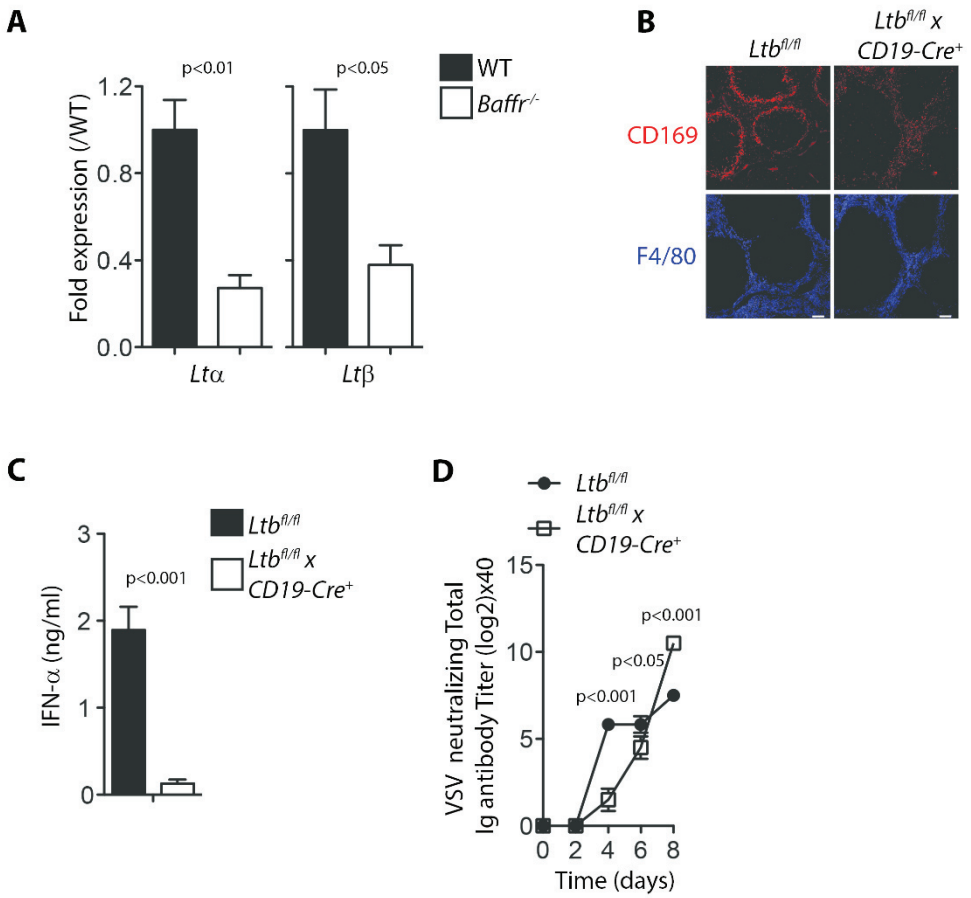


FIG 6 B cells mediated the maintenance of CD169^{+} cells (Xu, Huang et al. 2015)

(A) The mRNA expression levels of Lymphotoxin alpha (left) and beta (right) in spleen from WT and *Baffr*$^{-/-}$ animals were measured. (n=5-7). (B) Sections of snap-frozen spleen from *Ltb*$^{fl/fl}$ X *CD19-Cre*$^{+}$ and control mice were stained with anti-CD169 and anti-F4/80 antibodies (n=3). (C) The concentration of IFN-α was analyzed at 24h after 105pfu VSV infection in the serum from the indicated groups. (n=4-6). (D) The neutralizing antibody in the serum was monitored at naïve condition and at the indicated time points after VSV infection. (n=4-6). The error bar showed SEM; n.s.=not significant.

9.1.5 After LCMV infection BAFFR deficiency leads to limited innate immune activation

To further investigate the essence of BAFFR following viral infection and the induction of antiviral immunity, *Baffr*$^{-/-}$ mice were infected with LCMV. Similar as we observed after VSV infection, *Baffr*$^{-/-}$ mice also exhibited decreased LCMV titer in spleen at 72h following infection when compared with WT controls (FIG 7A). Moreover, the levels of type I interferon were also sharply reduced in the serum of *Baffr*$^{-/-}$ mice (FIG 7B). And when we measured tetramer-positive LCMV-specific T cells, *Baffr*$^{-/-}$ animals showed highly decreased specific T cells (FIG 7C). Furthermore, reduced IFN-γ production after in vitro re-stimulation with LCMV peptides was also observed in both CD8^{+} and CD4^{+} T cells from *Baffr*$^{-/-}$ mice (FIG 7D). Finally, virus titers were detected at day20 following infection, virus was eliminated from all organs tested in WT animals, while in *Baffr*$^{-/-}$ animals, high virus was persistent in all of organs which were tested (FIG 7E). In sum, these data indicated that the absence of BAFFR signaling resulted in impaired induction of innate and adaptive immune responses following LCMV infection.

FIG 7

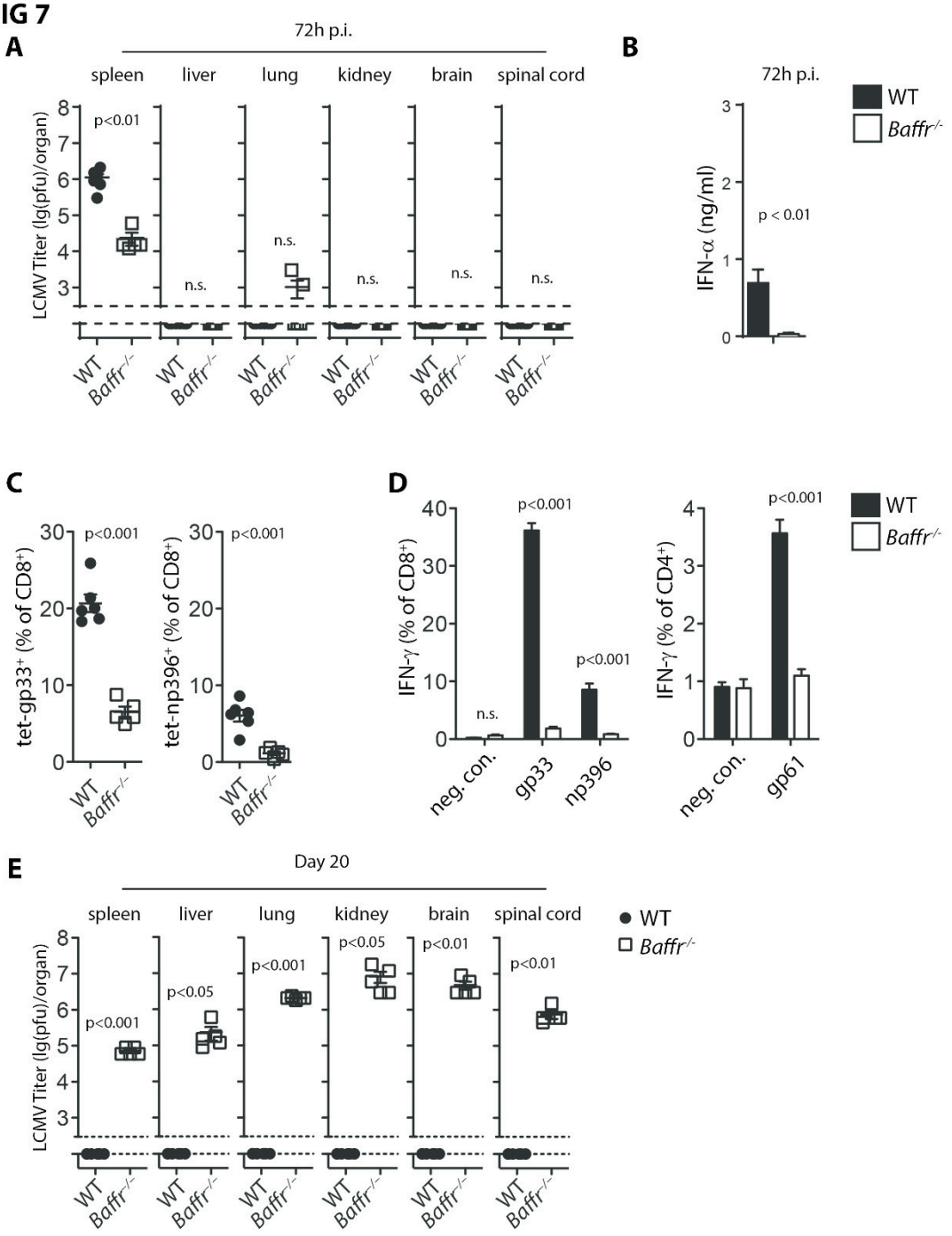

A
72h p.i.
spleen
liver
lung
kidney
brain
spinal cord
LCMV Titer (lg(pfu)/organ)
p<0.01
n.s.
n.s.
n.s.
n.s.
n.s.
WT
Baffr-/-
B
72h p.i.
IFN-α (ng/ml)
p < 0.01
WT
Baffr-/-
C
tet-gp33+ (% of CD8+)
p<0.001
tet-np396+ (% of CD8+)
p<0.001
D
IFN-γ (% of CD8+)
p<0.001
n.s.
p<0.001
neg. con.
gp33
np396
IFN-γ (% of CD4+)
p<0.001
neg. con.
gp61
E
Day 20
spleen
liver
lung
kidney
brain
spinal cord
p<0.001
p<0.05
p<0.001
p<0.05
p<0.01
p<0.01

FIG 7 Deficient innate and adaptive immunity in *Baffr*$^{-/-}$ animals after LCMV infection (Xu, Huang et al. 2015)

WT and *Baffr*$^{-/-}$ animals were infected with 200pfu LCMV-Docile strain. (A) Virus titers were analyzed at 72h after infection in spleen, liver, lung, kidney, spinal cord and brain. (n=5-6). (B) The concentration of IFN-α in the serum was measured at 72h after infection. (n=5-6). (C) Virus-specific $CD8^+$ T cells were measured by tetramer staining in the spleen after 20 days. (n=5-6). (D) The production of INF-γ in the T cells was analyzed by intracellular-cytokine staining and flow cytometry analysis after in vitro re-stimulation with the MHC-I peptides gp33 and np396 (left) and the MHC-II peptide gp61 (right). (n=5-6). (E) Virus titers were monitored at 20 days after infection in spleen, liver, lung, kidney, spinal cord and brain. (n=5-6). The error bar showed SEM, n.s.=not significant, the detection limit was indicated by the dashed line.

9.2 Part II: 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 (2017). "Lymphocytes Negatively Regulate NK Cell Activity via Qa-1b following Viral Infection." Cell Rep 21(9): 2528-2540

Declaration and statement of contribution

I, Jun Huang, declare that the content of this dissertation is original. I have cited appropriate texts and figures wherever is necessary. No part of this dissertation is submitted somewhere else for consideration of a degree. This part of results in the dissertation is published on Cell Reports on 2017 as I cited in the beginning.

I was involved in execution of the experiments, analysis, writing and preparation part of the manuscript which consists round 20% of the published manuscript.

The figures of this part result which I prepared and analyzed are as follow: FIG 8, FIG 9A-C; FIG 10; FIG11; FIG 12; FIG 13; FIG 14; FIG 15; FIG 16; FIG 17; FIG 21, FIG 22; FIG 23; FIG 25; FIG 26; FIG 27; FIG 29A; FIG 30A-D; FIG 31; FIG 32B-D; FIG 33; FIG 34; FIG 35.

Contribution of the other published manuscripts is mentioned in the list of the publications.

Jun Huang

Date:
Place: Düsseldorf, Germany

9.2.1 NK cell activity rapidly reduces after infection with a large dose of virus

Because the significant role of ILCs in the early time points after viral infection, firstly we characterized different ILC subsets early after infection in WT mice (FIG 8) to investigate whether LCMV infection impacts ILC populations. The presence of NK cells and other ILC subsets remained stable after infection with lower dose of LCMV, however we observed a rapid decrease in NK cells, ILC1s, ILC2s and LTis after infection with higher doses of LCMV 2 days post infection (FIG 9A). This observation was not only restricted to spleen tissue, but we also observed a reduced number of ILC1s after infection in liver tissue and blood (FIG 9B-C). As expect, LCMV titers were also highly increased in the organs of mice infected with higher LCMV doses (FIG 10A-C). Next, we wondered whether these differences activity of NK cells also affect their regulatory effects against T cells. Then we transferred negatively sorted $CD8^+$ T cells from mouse expressing a transgenic T cell receptor recognizing the LCMV peptide gp33 as a transgene (P14) (Pircher, Burki et al. 1989) into WT mice followed by low and high dose LCMV infection. Interestingly, we observed reduced presence of transferred cells early during infection, which was related to reduced viability in high dose infected mice (FIG 9D). However, at day 4 after infection, enhanced presence and viability of the transferred T cells prompted us to speculate whether NK cell mediated regulation of anti-viral T cells is inhibited during the infection.

FIG 8

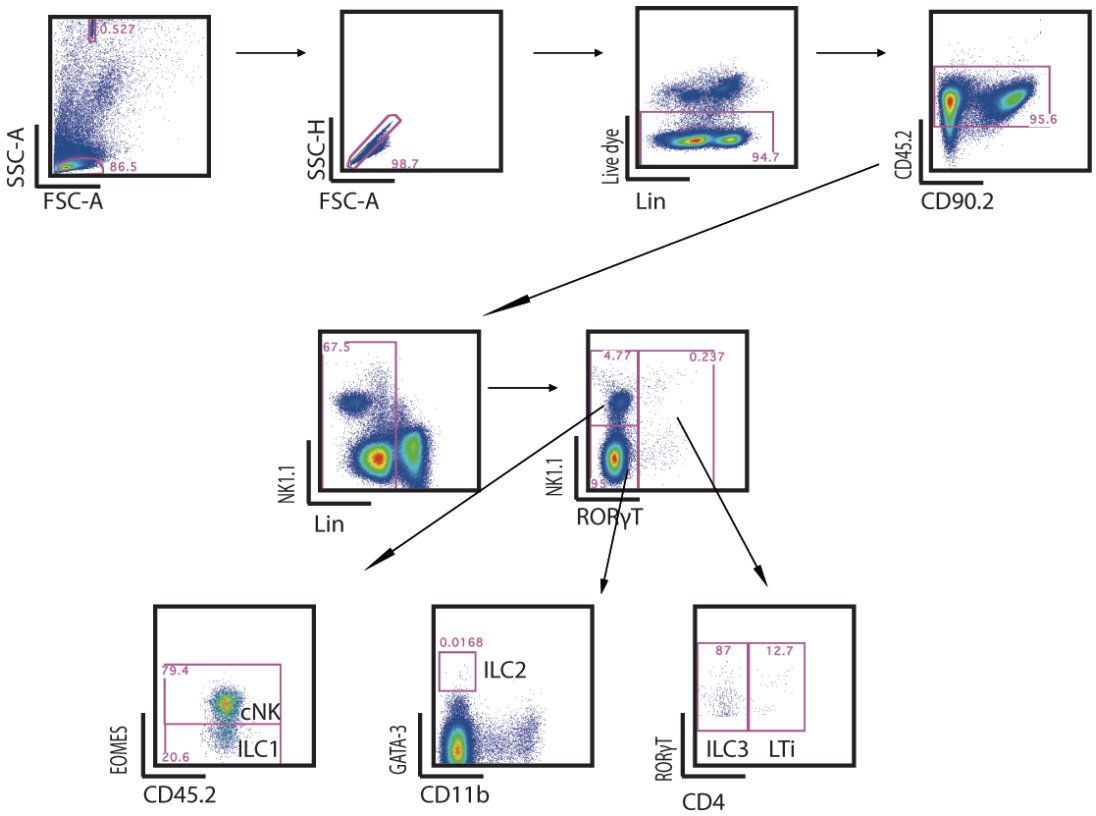


FIG 8 Characterization of different ILC subsets early after infection in WT mice (Xu, Huang et al. 2017)

Representative flow cytometry plots (n=6) and gating strategy of spleen ILCs in C57BL/6 mice are presented. Dead cells and Lin^+ (CD3, CD5, CD8, CD19, Ly-6G, TCRb and FcεR1) cells were excluded, viable $CD45.2^+CD90.2^+$ Lin^- cells were defined by conventional NK cells ($NK1.1^+RORγT^-Eomes^+$), ILC1s ($NK1.1^+RORγT^-Eomes^-$), ILC2s ($NK1.1^-RORγT^-CD11b^-GATA\text{-}3^+$), ILC3s ($RORγT^+CD4^-$), and LTis ($RORγT^+CD4^+$).

FIG 9

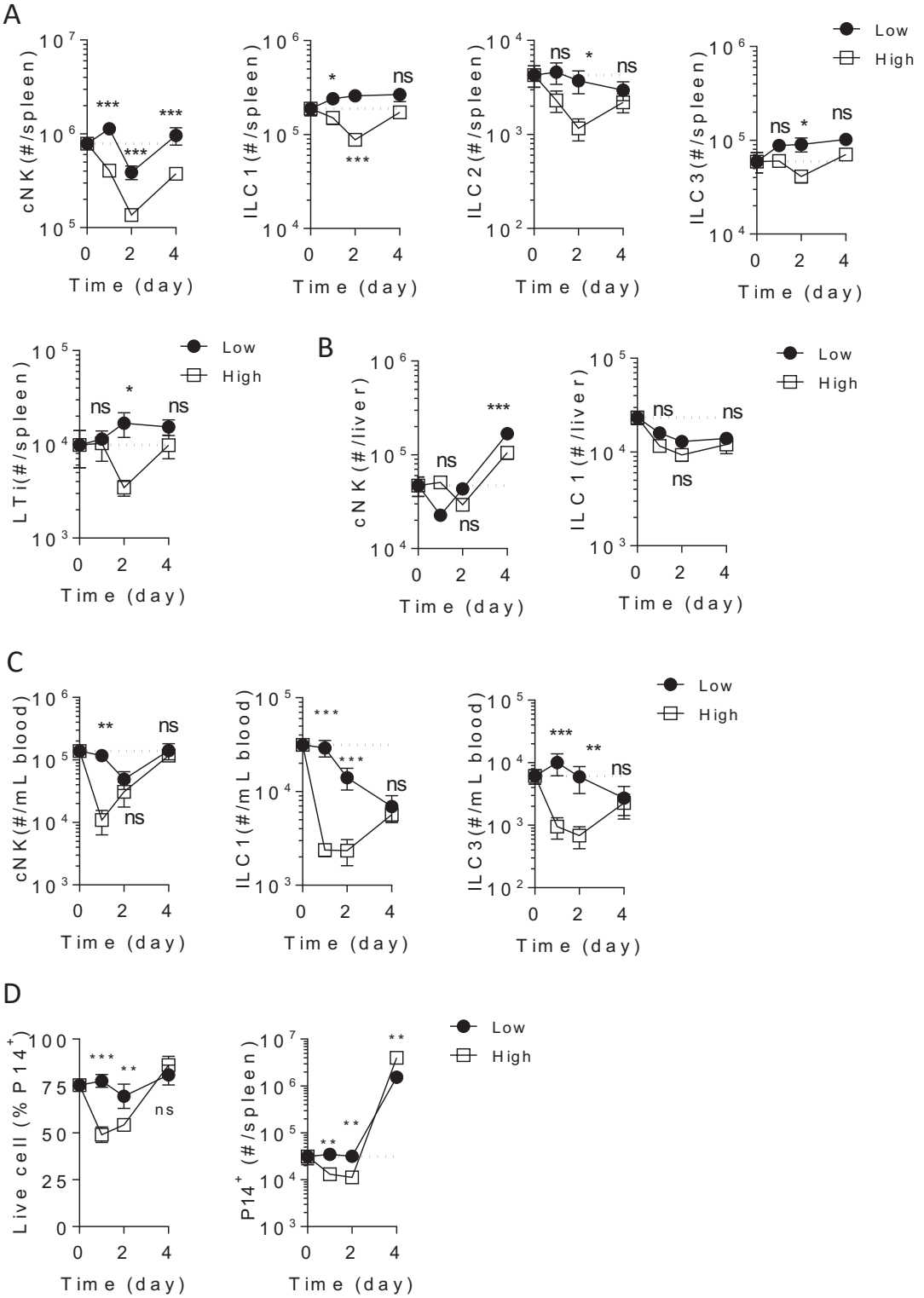

A
cNK (#/spleen)
ILC1 (#/spleen)
ILC2 (#/spleen)
ILC3 (#/spleen)
LTi (#/spleen)
B
cNK (#/liver)
ILC1 (#/liver)
C
cNK (#/mL blood)
ILC1 (#/mL blood)
ILC3 (#/mL blood)
D
Live cell (% P14+)
P14+ (#/spleen)
Time (day)
Low
High
ns
*
**
***

FIG 9 ILCs Rapidly reduce after High-dose LCMV infection (Xu, Huang et al. 2017)

(A-D) C57BL/C mice were infected with low-dose (200pfu) or high-dose ($2x10^6$pfu) LCMV WE. (A-C) ILC subsets in the spleen (A), liver (B), and blood (C) were monitored at indicated time points (n=6). (D) 10^6 negatively sorted $CD8^+$ T cells from $P14^+CD45.1^+$ mice were transferred into $CD45.2^+$ WT hosts followed by no infection or infection with low- or high-dose LCMV WE. Transferred $P14^+$ cell number and viability were measured at the indicated time points (n=4). Error bars show SEM; *P< 0.05, **P< 0.01, ***P< 0.001; ns, not statistically significant between the indicated groups.

FIG 10

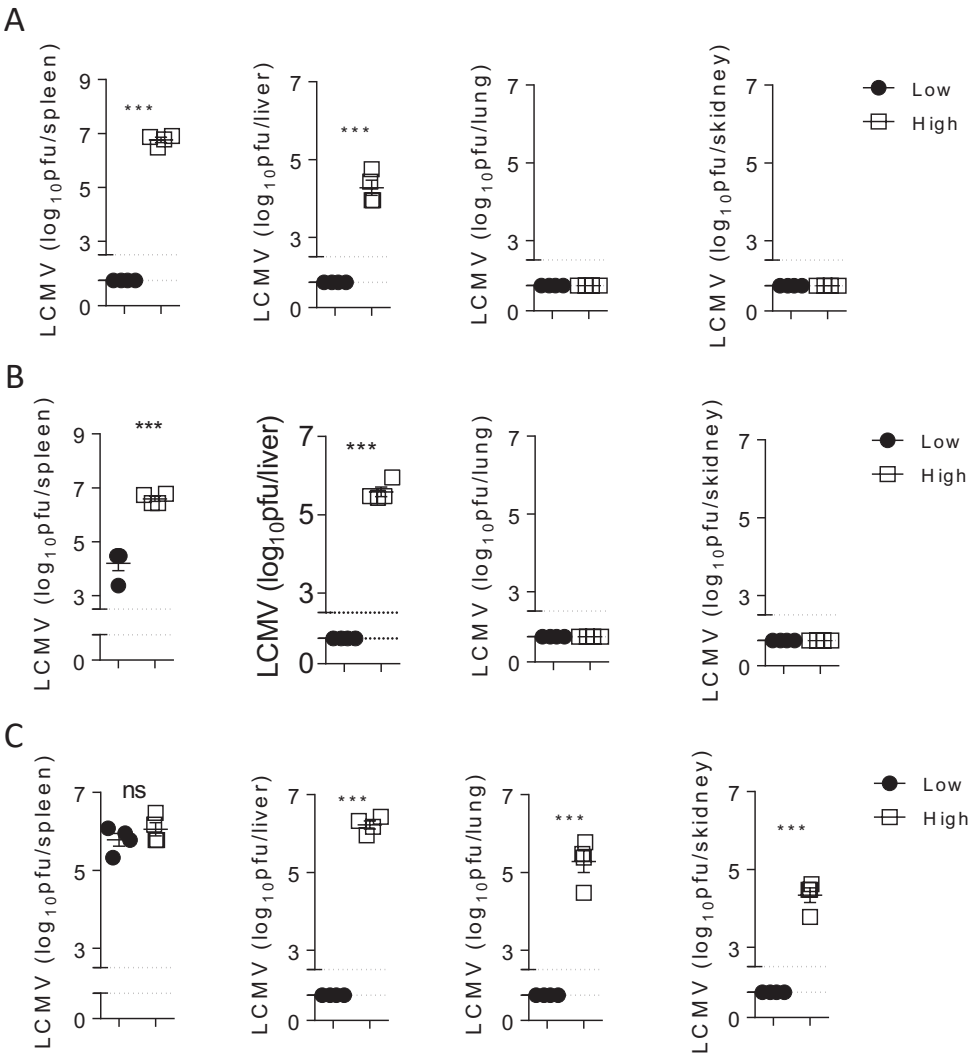


FIG 10 High dose infected animals display enhanced virus titers (Xu, Huang et al. 2017)

C56BL/6 were infected with low dose or high dose LCMV WE. (A-C) Virus titers were measured from spleen, liver, lung, and kidney tissue (A) one day post infection (n=4) (B) two days post infection (n=4) (C) four days post infection (n=4). Error bars show SEM, ***$p < 0.001$, ns indicates statistically not significant between the indicated groups.

9.2.2 Qa-1b triggers inhibition of NK cells and ILC1s after LCMV infection

To further investigate the underlying mechanism of NK cell mediated regulation of anti-viral T cells, firstly we checked gene expression of ligands for activating and inhibitory receptors of NK cells in whole spleen tissue one day post infection. In high dose LCMV infected mice, genes encoding for activating ligands of NK cell receptors were rather upregulated when compared with low dose LCMV infected mice (FIG 11A). But some ligands, such as *Cd23*, also displayed lower expression levels when compared with low dose LCMV infected mice (FIG 11A). However, when we measured gene expression of ligands for inhibitory receptors of NK cells, we noticed increased RNA level of *H2-t23* after high dose LCMV infection (FIG 11A). Then we also found that enhanced expression of Qa-1b overlapped with B cell markers in spleen tissue sections in infected mice (FIG 11B). Similar results were also observed in the flow cytometry analysis (FIG 12B) and H2-t23 expression was also significantly increased in sorted B cells from high dose LCMV infected mice (FIG 12A). But when we investigate the expression level of Qa-1b in B cell deficient $JhT^{-/-}$ or B and T cell deficient $Rag1^{-/-}$ mice, we found reduced level of *H2-t23* transcripts and Qa-1b protein in spleen tissue after infection (FIG 13A-B). Clodronate treatment did not have any impact on Qa-1b expression on an mRNA or protein level (FIG 13A-B). However, *H2-t23* gene and Qa-1b protein expression in the spleen was similar in control and infected mice after depletion of $CD4^+$ and $CD8^+$ T cells (FIG 14A-B and FIG 15). Taken together, these results indicate that Qa-1b is mainly expressed on B cells.

Notably, Qa-1b expression in spleen tissue was dependent on type I interferon, and we observed that lack of the IFNAR abolished the expression of Qa-1b after LCMV infection (FIG 16A-B). Then we wondered whether the expression of Qa-1b on B cells might be an important checkpoint for NK cell-mediated regulation of anti-viral T cells. To measure this, we firstly infected control ($H2\text{-}t23^{+/+}$ or $H2\text{-}t23^{+/-}$) and $H2\text{-}t23^{-/-}$ mice (henceforth referred to as $Qa\text{-}1b^{-/-}$ mice) with LCMV. When we measured NK cells and

ILCs, we observed decreased number of NK cell and other ILC subsets in spleen and liver between control and Qa-1b deficient mice at day 1 and day 2 after high dose of LCMV infection, and there was also no difference between WT and *Qa-1b*$^{-/-}$ mice (FIG 17A-B). Furthermore, the expression of surface molecules between control and *Qa-1b*$^{-/-}$ animals was also similar in NK cell and other ILCs (FIG 19). Consistently, granzyme B and perforin expression was comparable in control and *Qa-1b*$^{-/-}$ mice (FIG 20A). In addition, we mainly detected positive staining with an anti-NKG2A/C/E (clone: 20D5) antibody in NK cell and ILC1 (FIG 18A and FIG 20B), while cells from NKG2A deficient mice have no positive signal (FIG 20C). These results show that NKG2A is predominantly expressed on NK cells and ILC1s. Next, we investigate whether NK cell mediated regulation of anti-viral T cells could be modulated by Qa-1b. Firstly we transferred P14^{+}CD8^{+} T cells into control and Qa-1b knockout mice. We found reduced numbers of transferred LCMV-specific T cells in Qa-1b deficient mice at day 2 after LCMV infection (FIG 18B). Similarly, the transferred LCMV specific T cells also displayed reduced viability when compared with control mice (FIG 18C). Moreover, when we co-cultured concanavalin A activated CD8^{+} T cells with NK cells, we observed that *Qa-1b*$^{-/-}$ CD8^{+} T cells were more susceptible to NK cell mediated killing (FIG 18D). The Qa-1b bind peptide Qdm can increase Qa-1b expression on B cells (FIG 18E). Notably, NK cell mediated killing of T cells could be partially rescued by adding Qdm-stimulated B cells (FIG 18F). Collectively, these results suggest that NK cells show enhanced regulatory functions against T cells in the absence of Qa-1b.

FIG 11

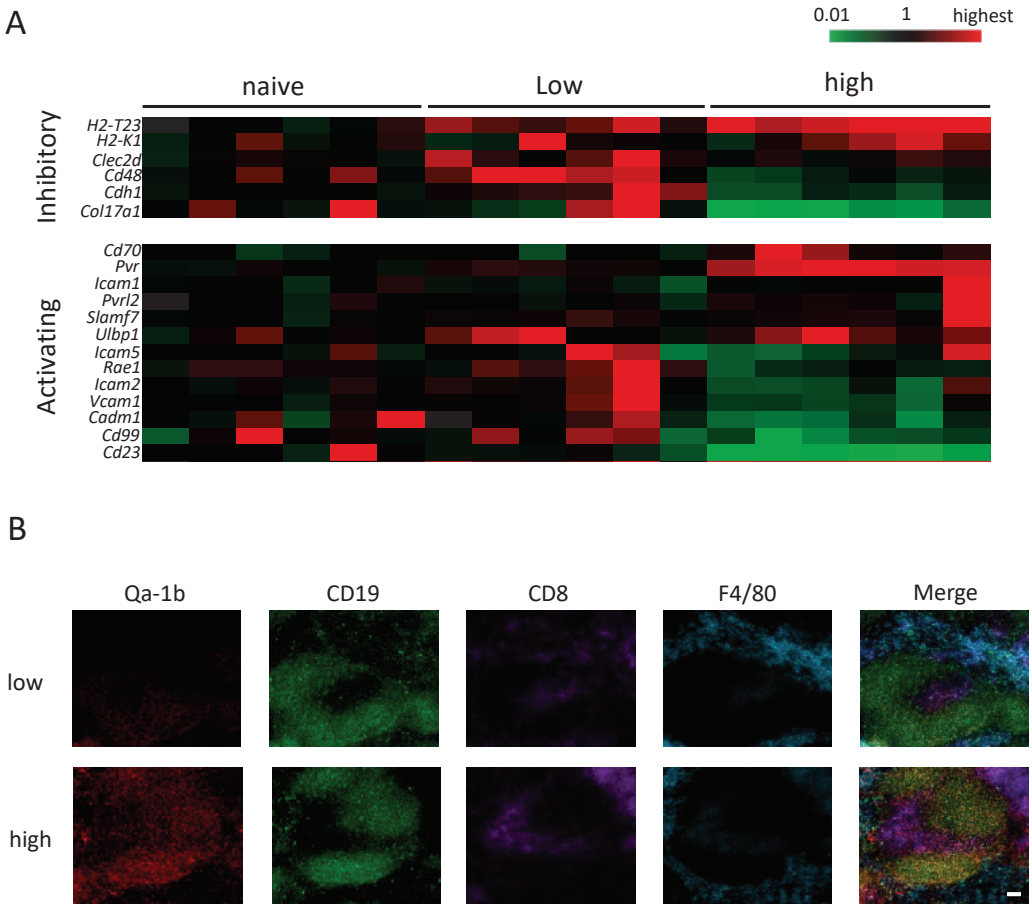


FIG 11 High dose of LCMV infection can stimulate the expression of Qa-1b (Xu, Huang et al. 2017)

(A-B) C57BL/6 mice were infected with low- or high-dose LCMV WE one day post-infection. (A) The expression levels of genes for NK inhibitory or activating ligands were measured in whole spleen tissue (n=6). (B) Sections of snap frozen spleen tissue were monitored for the expression of Qa-1b, CD19, CD8 and F4/80 (n=6; scale bar=50μm).

FIG 12

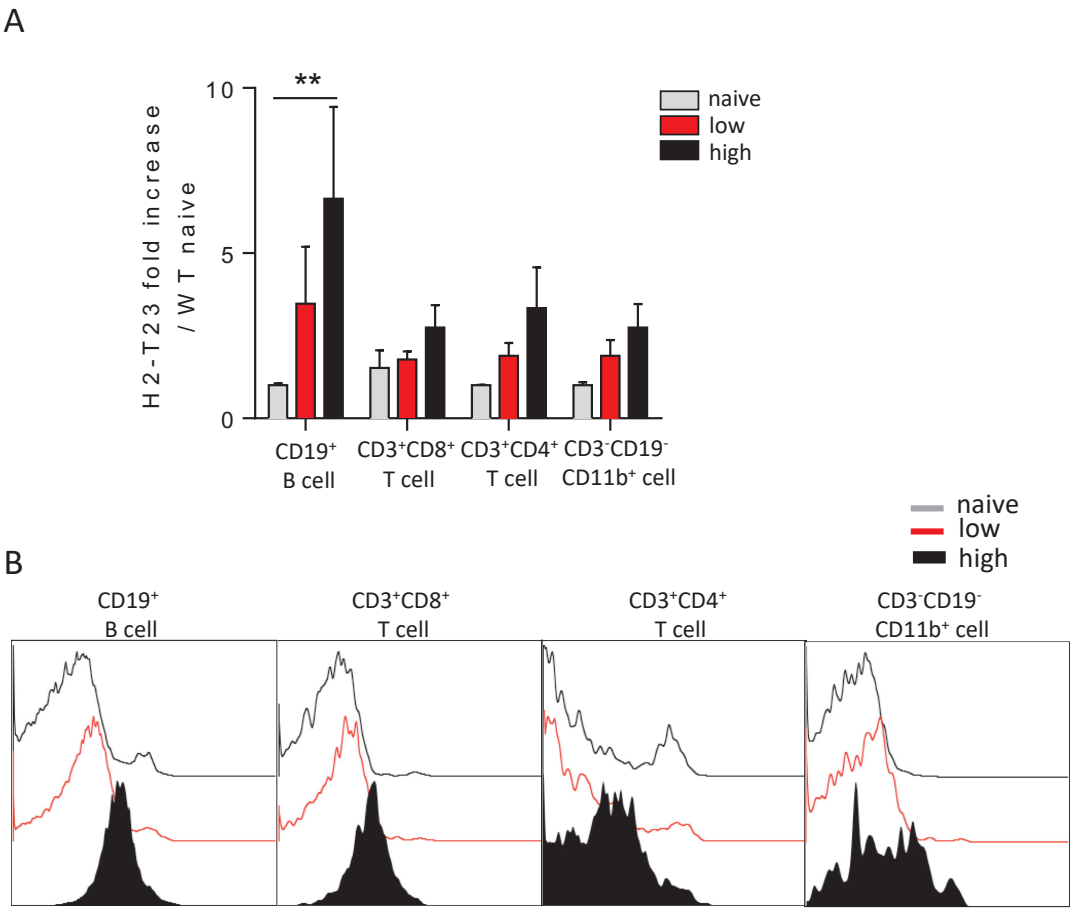


FIG 12 The expression of Qa-1b is dominantly on B cells after LCMV infection (Xu, Huang et al. 2017)

(A) C57BL/6 were infected with low or high dose LCMV WE one day after infection, the expression level of *H2-t23* in sorted $CD3^-CD19^+$ B cells, $CD3^+CD8^+$ T cells, $CD3^+CD4^+$ T cells and $CD3^-CD19^-CD11b^+$ cells from spleen tissue was determined (n=4). (B) The expression of Qa-1b was analyzed on $CD3^-CD19^+$ B cells, $CD3^+CD8^+$ T cells, $CD3^+CD4^+$ T cells and $CD3^-CD19^-CD11b^+$ cells; one of four representative histograms is shown. Error bars show SEM, $**p < 0.01$, ns indicates statistically not significant between the indicated groups.

FIG 13

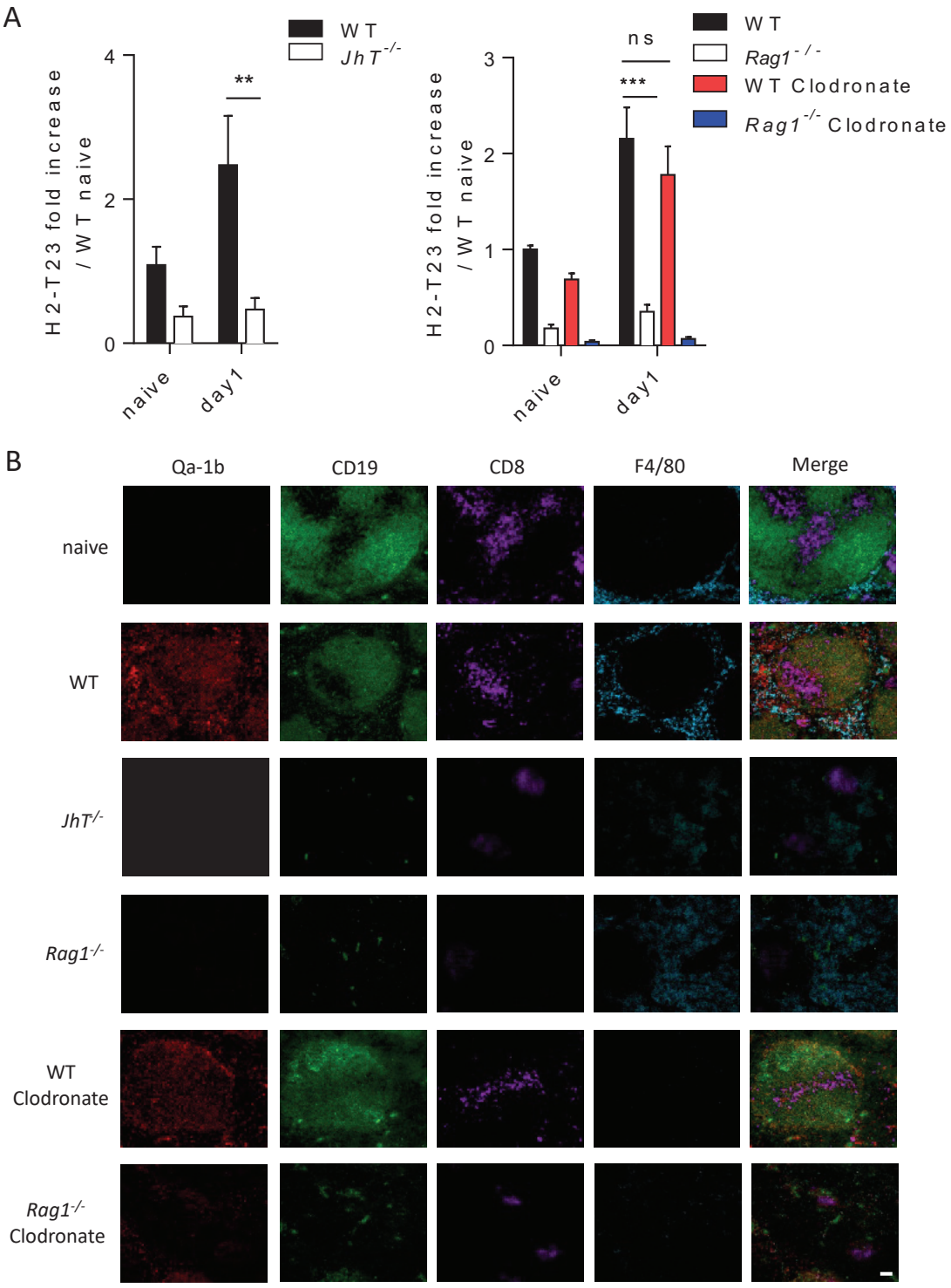

A
WT
JhT-/-
H2-T23 fold increase / WT naive
4
2
0
**
naive
day1
ns
***
3
2
1
0
WT
Rag1-/-
WT Clodronate
Rag1-/- Clodronate
B
Qa-1b
CD19
CD8
F4/80
Merge
naive
WT
JhT-/-
Rag1-/-
WT Clodronate
Rag1-/- Clodronate

FIG 13 The expression of Qa-1b is dominantly on B cells after LCMV infection (Xu, Huang et al. 2017)

(A-B) WT, *JhT*$^{-/-}$, *Rag1*$^{-/-}$, clodronate-treated WT or *Rag1*$^{-/-}$ mice were infected with high-dose LCMV WE one day following infection (A) The expression level of *H2-t23* in spleen from WT or *JhT*$^{-/-}$ mice or WT, *Rag1*$^{-/-}$, clodronate treated WT or *Rag1*$^{-/-}$ mice were measured (n=3-4). (B) Sections of snap frozen spleen tissue were analyzed for the expression of Qa-1b, CD19, CD8 and F4/80 (n=3-4; scale bar=50 μm). Error bars show SEM, $**p < 0.01$, $***p < 0.001$, ns indicates statistically not significant between the indicated groups.

FIG 14

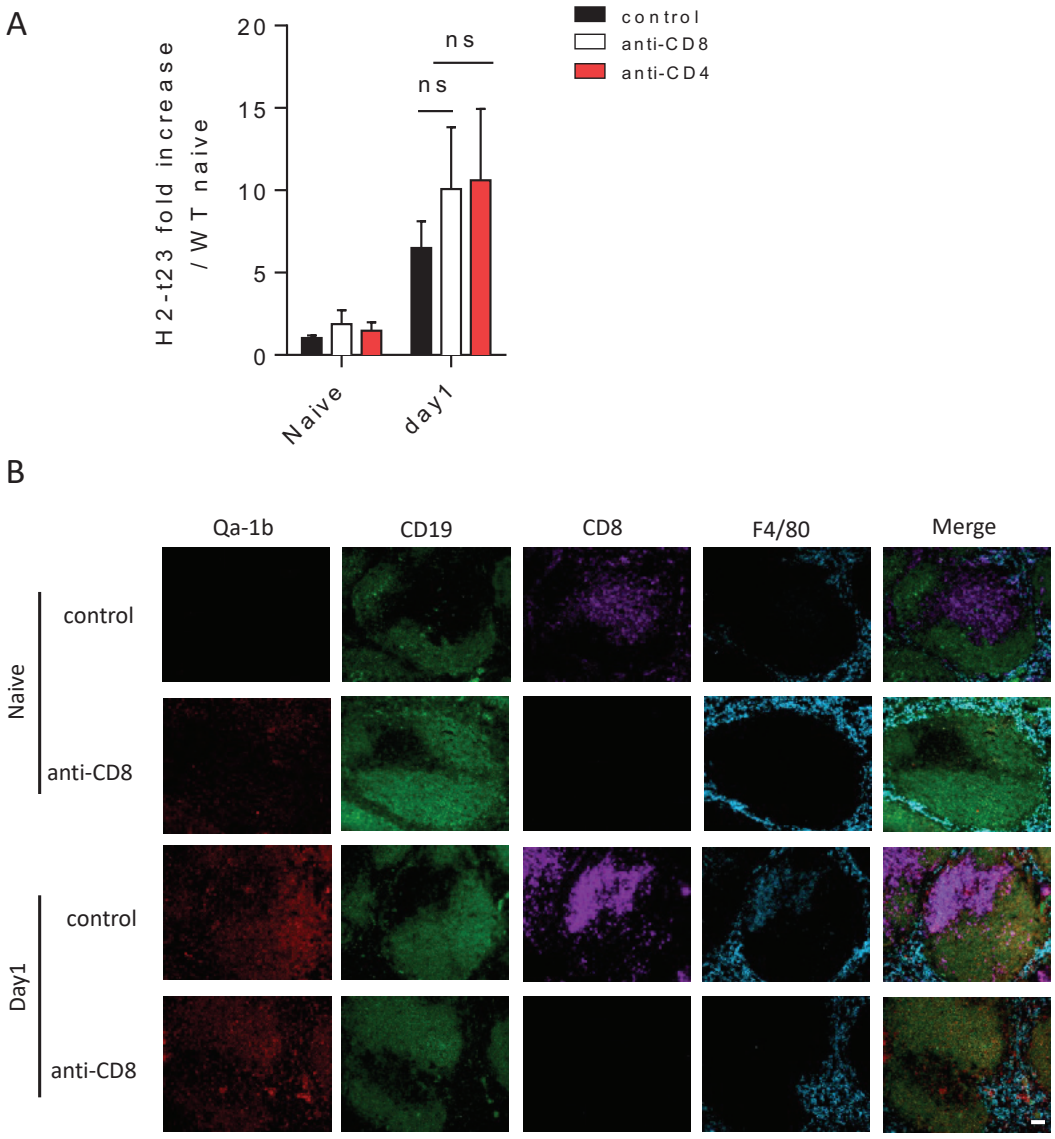


FIG 14 $CD4^+$ or $CD8^+$ T-cell depletion does not impact the expression of Qa-1b after LCMV infection (Xu, Huang et al. 2017)

$CD8^+$ cell-depleted or non-depleted C56BL/6, $CD4^+$ cell-depleted or non-depleted C56BL/6 mice were infected with high dose of LCMV WE. (A) The expression level of *H2-t23* was measured in spleen tissue one day after infection (n=4). (B) Spleen tissue was analyzed for the expression of Qa-1b, CD19, CD8 and F4/80 (n=4, scale bar=50μm). Error bars show SEM, ns indicates statistically not significant between the indicated groups.

FIG 15

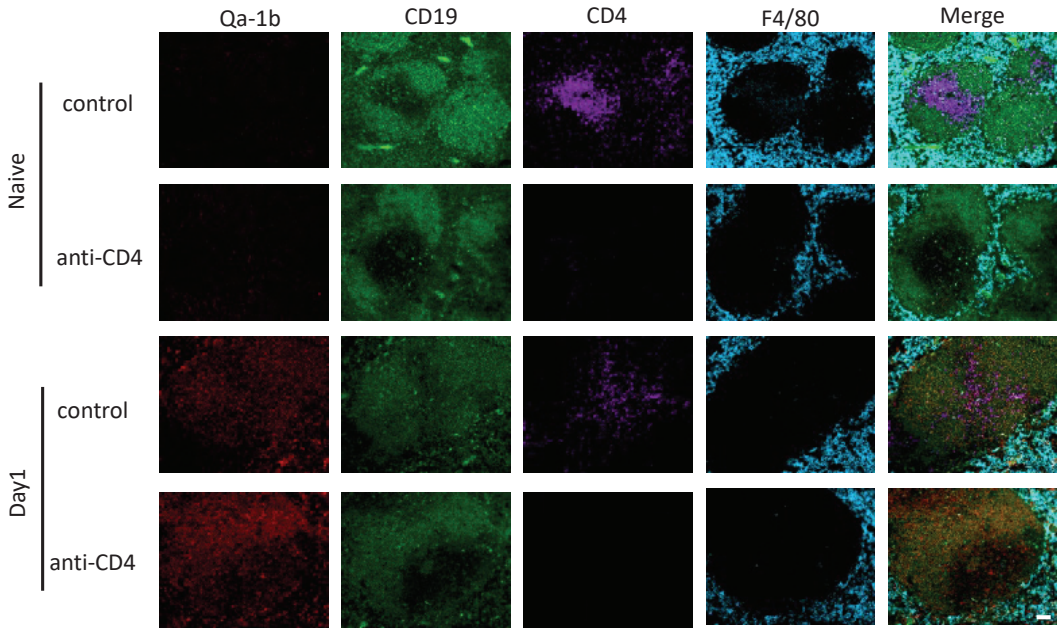


FIG 15 $CD4^+$ T cell depletion does not impact the expression of Qa-1b after LCMV infection (Xu, Huang et al. 2017)

$CD4^+$ cell-depleted or non-depleted C56BL/6 mice were infected with high dose of LCMV WE. one day after infection., spleen tissue was analyzed for the expression of Qa-1b, CD19, CD8 and F4/80 (n=4; scale bar=50μm)

FIG 16

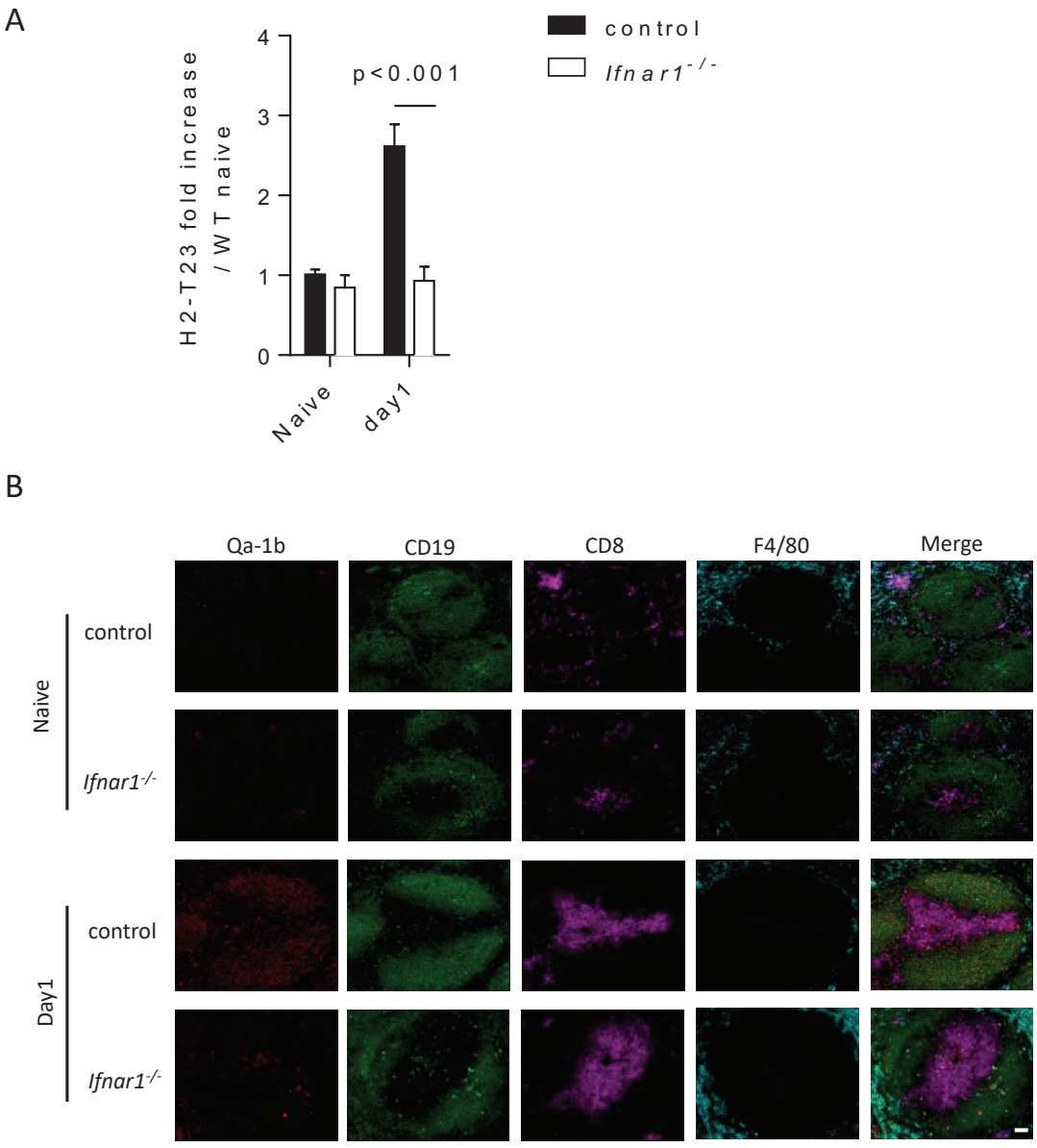


FIG 16 The expression of Qa-1b is mediated by type I interferon on B cells after LCMV infection (Xu, Huang et al. 2017)

(A-B) Control (C56BL/6) or *Ifnar1*$^{-/-}$ animals were infected with high dose of LCMV WE. one day after infection, spleen tissue was analyzed for (A) the gene expression of *H2-t23* gene and (B) the expression level of Qa-1b, CD19, CD8 and F4/80 (n=6; scale bar=50μm). Error bars show SEM, ∗∗∗p < 0.001, ns indicates statistically not significant between the indicated groups.

FIG 17

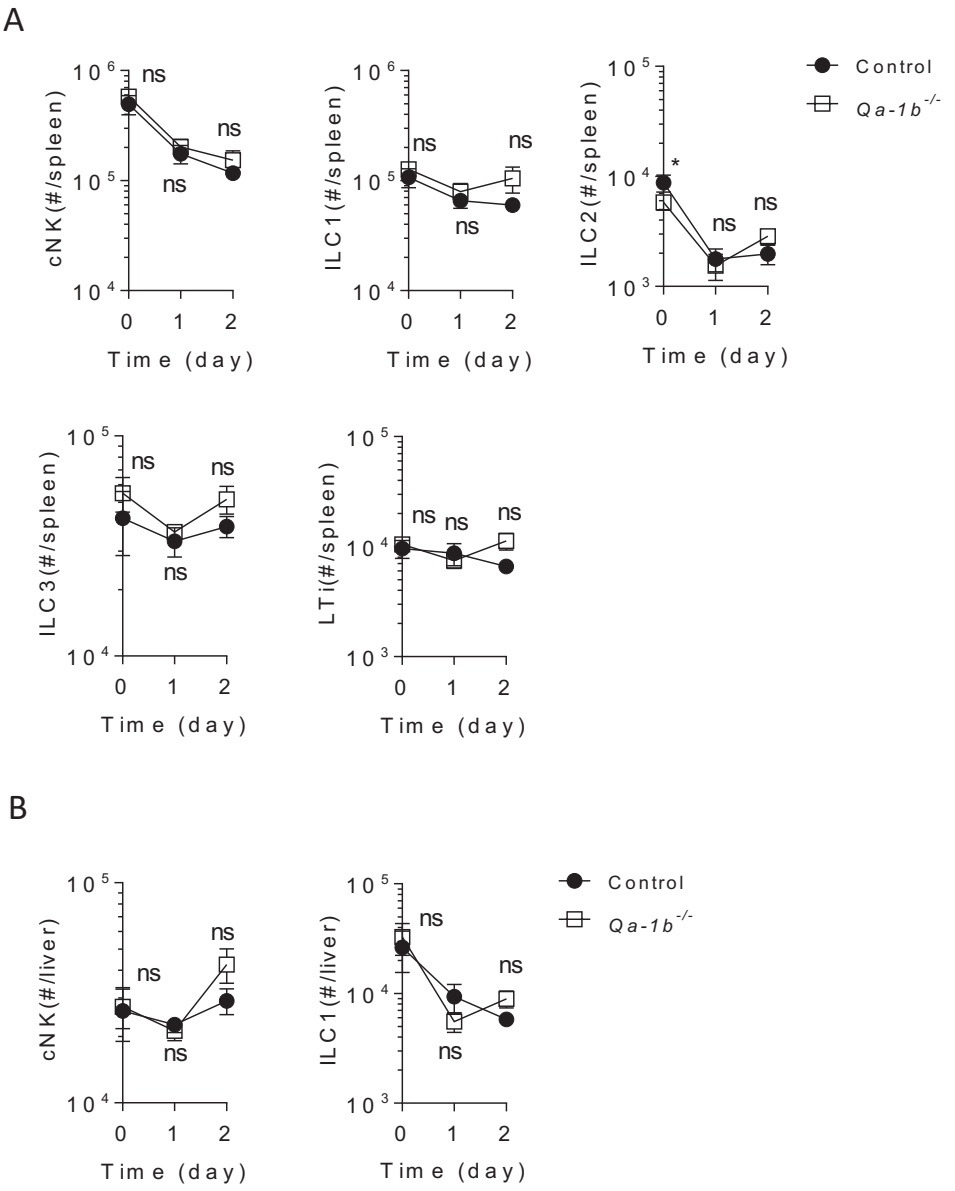


FIG 17 No difference of ILC subsites between Control and *Qa-1b*$^{-/-}$ mice following LCMV infection (Xu, Huang et al. 2017)

(A-B) Control mice (*Qa-1b*$^{+/+}$ or *Qa-1b*$^{+/-}$) and *Qa-1b*$^{-/-}$ mice were infected with high dose of LCMV WE, and the splenic ILC subsets (A) and liver ILC subsets (B) were monitored (n=6). Error bars show SEM, $*p < 0.05$, ns indicates statistically not significant between the indicated groups.

FIG 18

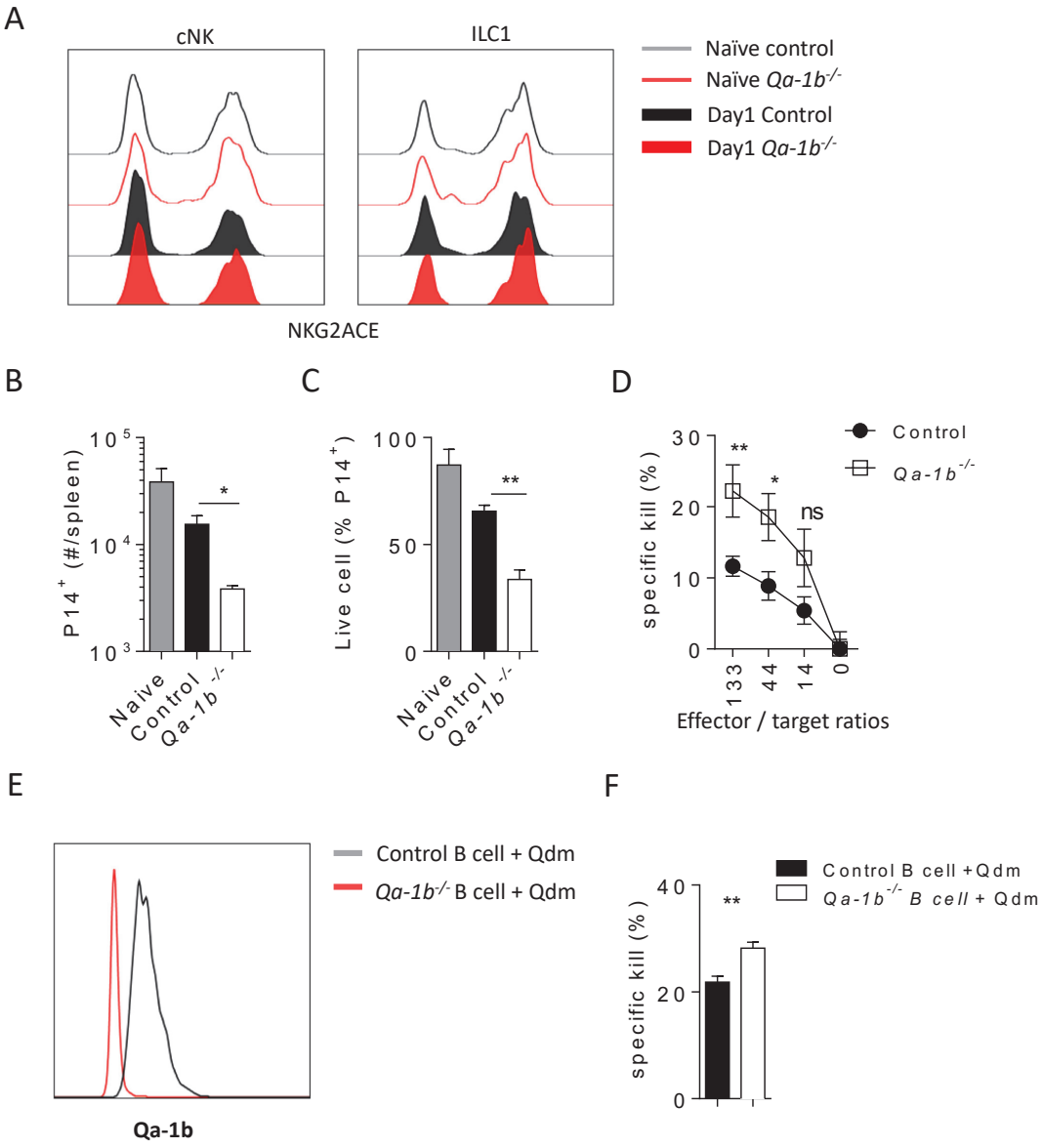

A
cNK
ILC1
Naïve control
Naïve Qa-1b-/-
Day1 Control
Day1 Qa-1b-/-
NKG2ACE
B
P14+ (#/spleen)
Naive
Control
Qa-1b-/-
C
Live cell (% P14+)
Naive
Control
Qa-1b-/-
D
Control
Qa-1b-/-
specific kill (%)
ns
Effector / target ratios
E
Control B cell + Qdm
Qa-1b-/- B cell + Qdm
Qa-1b
F
Control B cell +Qdm
Qa-1b-/- B cell + Qdm
specific kill (%)

FIG 18 NK cell-mediated regulation of T cells is inhibited by the expression of Qa-1b on B cells (Xu, Huang et al. 2017)

(A) The expression of NKG2A on NK and ILC1 cells were measured by staining with anti-NKG2A/C/E antibody (clone: 20D5) (n=4). (B-C) 10^6 negatively sorted $CD8^+$ T cells from $P14^+CD45.1^+$ mice were transferred into $CD45.2^+$ control or $CD45.2^+$ *Qa-1b*$^{-/-}$ hosts following high dose LCMV WE infection. (B) The numbers of transferred $P14^+$ cells were measured two days post infection (n=3-4). (C) $P14^+$ viability was determined by Annexin V and 7AAD two days after infection (n=3-4). (D) Negatively sorted $CD8^+$ T cells from control and *Qa-1b*$^{-/-}$ mice were stimulated with Concanavalin A, and 24h later 1000 activated $CD45.2^+CD8^+$ T cells were co-incubated with IL-2 derived $CD45.1^+$ WT NK cells at the indicated effector-to-target ration. Following a four-hour incubation, the viability of activated $CD45.2^+CD8^+$ T cells was measured by 7AAD staining (n=6). (E) Positive sorted $CD19^+$ B cells from control and *Qa-1b*$^{-/-}$ were incubated with 100μg/ml Qdm peptide (AMAPRTLLL), and the expression of Qa-1b on B cells was monitored 24h later, one of six representative histograms is shown. (F) A total of 1000 Concanavalin-A activated cells of negatively sorted $CD8^+$ T cells form *Qa-1b*$^{-/-}$ mice were co-incubated with IL-2-derived $CD45.1^+$ WT NK cells at an effector-to-target ration of 50:1 in the presence of 30000 B cells from (E). Following a four-hour incubation, the viability of activated $CD45.2^+CD8^+$ T cells was measured by 7AAD staining (n=6). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, ns indicates statistically not significant between the indicated groups.

FIG 19

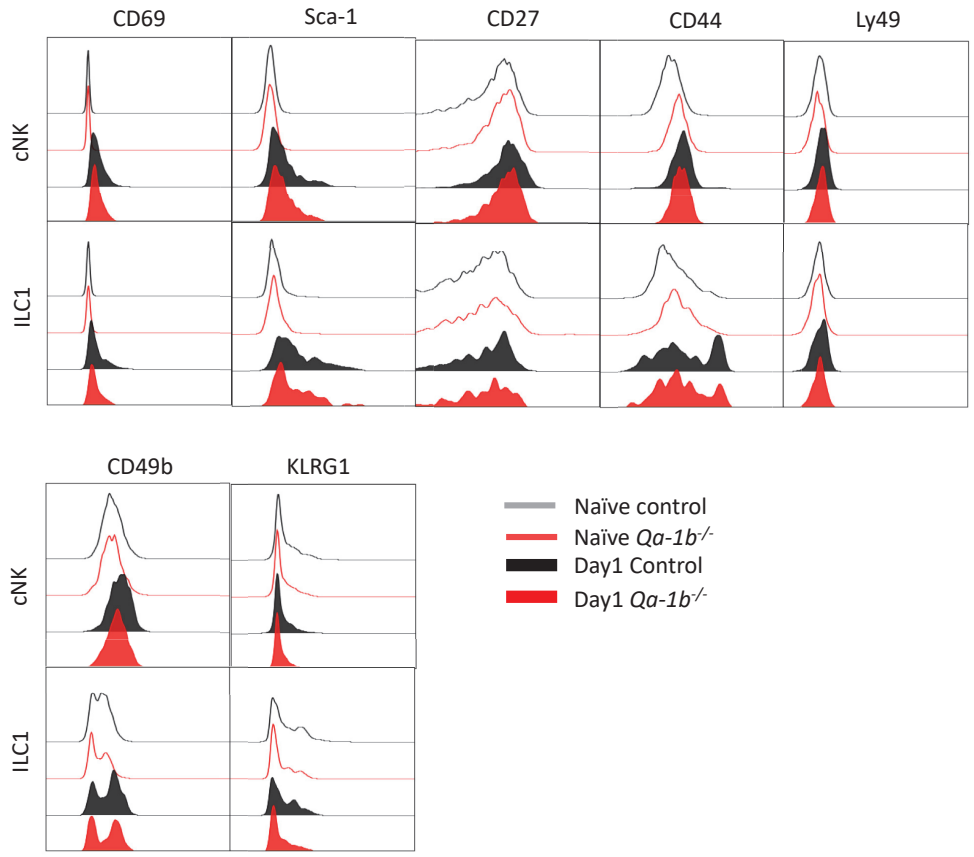
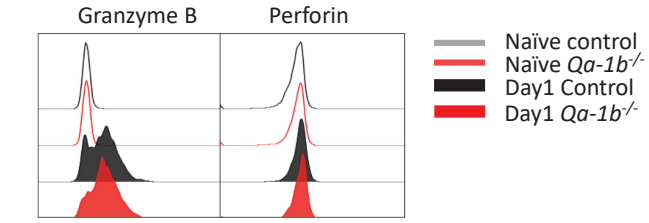


FIG 19 The expression of surface molecules is not affected by Qa-1b (Xu, Huang et al. 2017)

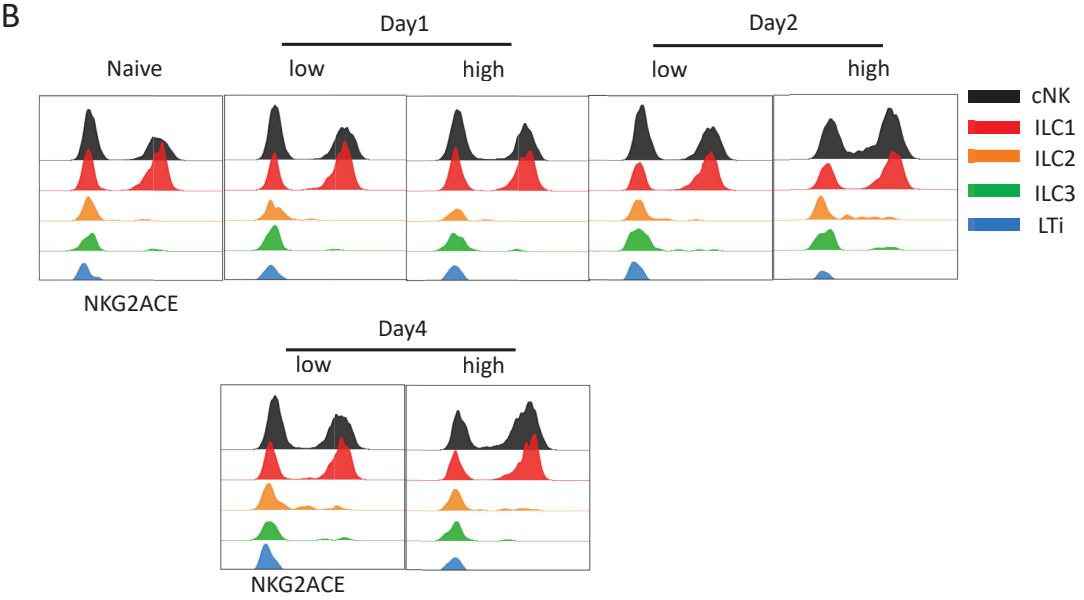
Control or *Qa-1b*$^{-/-}$ mice were infected with high dose of LCMV WE. The expression of surface molecules on NK or ILC1 is shown as indicated in spleen tissue one day post infection. One representative FACS blot of n=4-5 is shown.

FIG 20

A


Granzyme B
Perforin
Naïve control
Naïve Qa-1b-/-
Day1 Control
Day1 Qa-1b-/-

B


Day1
Day2
Naive
low
high
low
high
cNK
ILC1
ILC2
ILC3
LTi
NKG2ACE
Day4
low
high
NKG2ACE

C

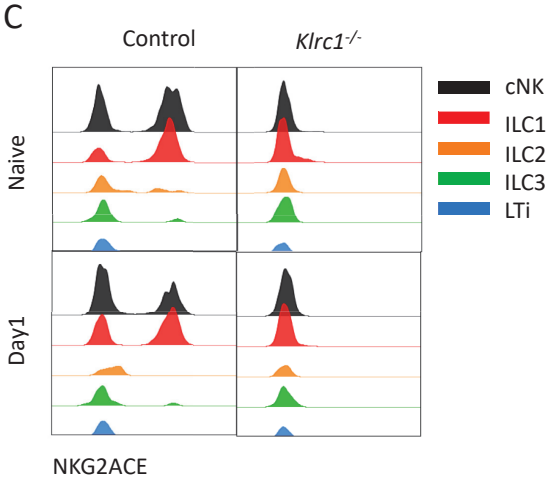

Control
Klrc1-/-
Naive
Day1
cNK
ILC1
ILC2
ILC3
LTi
NKG2ACE

FIG 20 The expression of Granzyme B, perforin and NKG2A is not affected by Qa-1b (Xu, Huang et al. 2017)

(A) The expression of Granzyme B and perforin was determined on splenic cNK cells one day after infection. One representative FACS blot of n=4-5 is shown. (B) C57BL/6 were infected with low dose or high dose of LCMV WE. The expression of NKG2 was determined on indicated ILC subsets (n=4). (C) Control or NKG2A deficient animals were infected with high dose of LCMV WE. One day post infection. The expression of NKG2 were determined on indicated ILC subsets

9.2.3 Deficiency of Qa-1b leads to impaired T cell immunity and enhanced pathology

Because NK cells can target anti-viral T cells to result in immunopathology and chronic viral infections (Waggoner, Cornberg et al. 2011, Lang, Lang et al. 2012, Cook and Whitmire 2013). Hence, we measured anti-viral T cell response in control and Qa-1b deficient mice following LCMV infection. Reduced numbers of LCMV-specific T cells were observed in blood of *Qa-1b*$^{-/-}$ mice after LCMV infection (FIG 21A). Furthermore, similar results were also found in spleen and liver tissue after LCMV infection (FIG 21B). Because NK cells can target antigen presenting cells such as dendritic cells, and lead to decreased T cell response in the deficiency of Qa-1b (Crome, Lang et al. 2013). However, we did not find any differences in the numbers of dendritic cells between control and Qa-1b knockout mice at day 1 and day 2 post LCMV infection (FIG 23B), even though the expression of co-stimulatory molecules on dendritic cells was increased after LCMV infection in control and *Qa-1b*$^{-/-}$ mice (FIG 23C). Additionally, the level of type I interferon was also no differences between two groups in the early time points (FIG 23A). Consistently, LCMV titers in spleen, liver, lung and kidney were also comparable between control and *Qa-1b*$^{-/-}$ mice in the early time points (FIG 23D-E).

Next, we observed LCMV-specific CD8^{+} T cells exhibited enhanced expression of PD-1, TIM-3 and 2B4 in *Qa-1b*$^{-/-}$ mice compared with wildtype mice at day 12 after LCMV infection, indicating an exhausted state (FIG 21C), however, the numbers of the effector, effector memory and central memory T cells were not different between control and *Qa-1b*$^{-/-}$ mice (FIG 24). This indicated that Qa-1b did affect the function of anti-viral T cells. Consistently, cytokine production of CD8^{+} T cells was reduced after re-stimulation with LCMV-specific T cells epitopes (FIG 22A). Furthermore, we also found that the number of LCMV-specific CD8^{+} T cells in the spleen of *Qa-1b*$^{-/-}$ mice was highly reduced at day 4 and day 8 after LCMV infection (FIG 25A-C), although

the expression of surface molecules suggesting an exhausted state was similar in control and *Qa-1b*$^{-/-}$ mice (FIG 25D). We also observed increased LCMV virus in the spleen of Qa-1b deficient mice at day 8 following infection (FIG 26A), but at day 12 after infection, the virus titers in spleen, liver, lung and kidney were all significantly increased in the *Qa-1b*$^{-/-}$ mice (FIG 22B). These findings were further supported with the data of LCMV conventional staining in the liver at day 12 after infection (FIG 22C). Additionally, enhanced activity of alanine aminotransferase (ALT) was detected in the blood stream of Qa-1b deficient mice, suggesting more liver damage in *Qa-1b*$^{-/-}$ mice (FIG 22D). Similarly, the expression level of α-smooth muscle actin (α-SMA) was higher in the liver tissue of Qa-1b deficient mice than in the liver tissue of wildtype mice after LCMV infection at day 12 (FIG 22E).

Since we found increased Qa-1b expression on B cells after LCMV infection, we reconstituted lethally irradiated C57BL/6 mice with mixed bone marrow from *Qa-1b*$^{-/-}$, B cell-deficient *JhT*$^{-/-}$, B cell and T cell-deficient *Rag1*$^{-/-}$, and CD8^{+} T cell-deficient *Cd8*$^{-/-}$ mice. We observed similar anti-viral CD8^{+} T cell response in mice reconstituted with *Qa-1b*$^{-/-}$: *Cd8*$^{-/-}$ bone marrow when compared with the corresponding controls (FIG 26B). However, reduced anti-viral CD8^{+} T cell response was observed in *Qa-1b*$^{-/-}$: *Rag1*$^{-/-}$ and *Qa-1b*$^{-/-}$: *JhT*$^{-/-}$ hosts when compared with the corresponding controls (FIG 27A and 27C). Consistently, elevated viral titers were also detected in tissues either from *Qa-1b*$^{-/-}$: *Rag1*$^{-/-}$ or *Qa-1b*$^{-/-}$: *JhT*$^{-/-}$ hosts when compared with their corresponding controls (FIG 27B and 27D).

These data indicated that the expression of Qa-1b on B cells promotes anti-viral CD8^{+} T cell immunity.

FIG 21

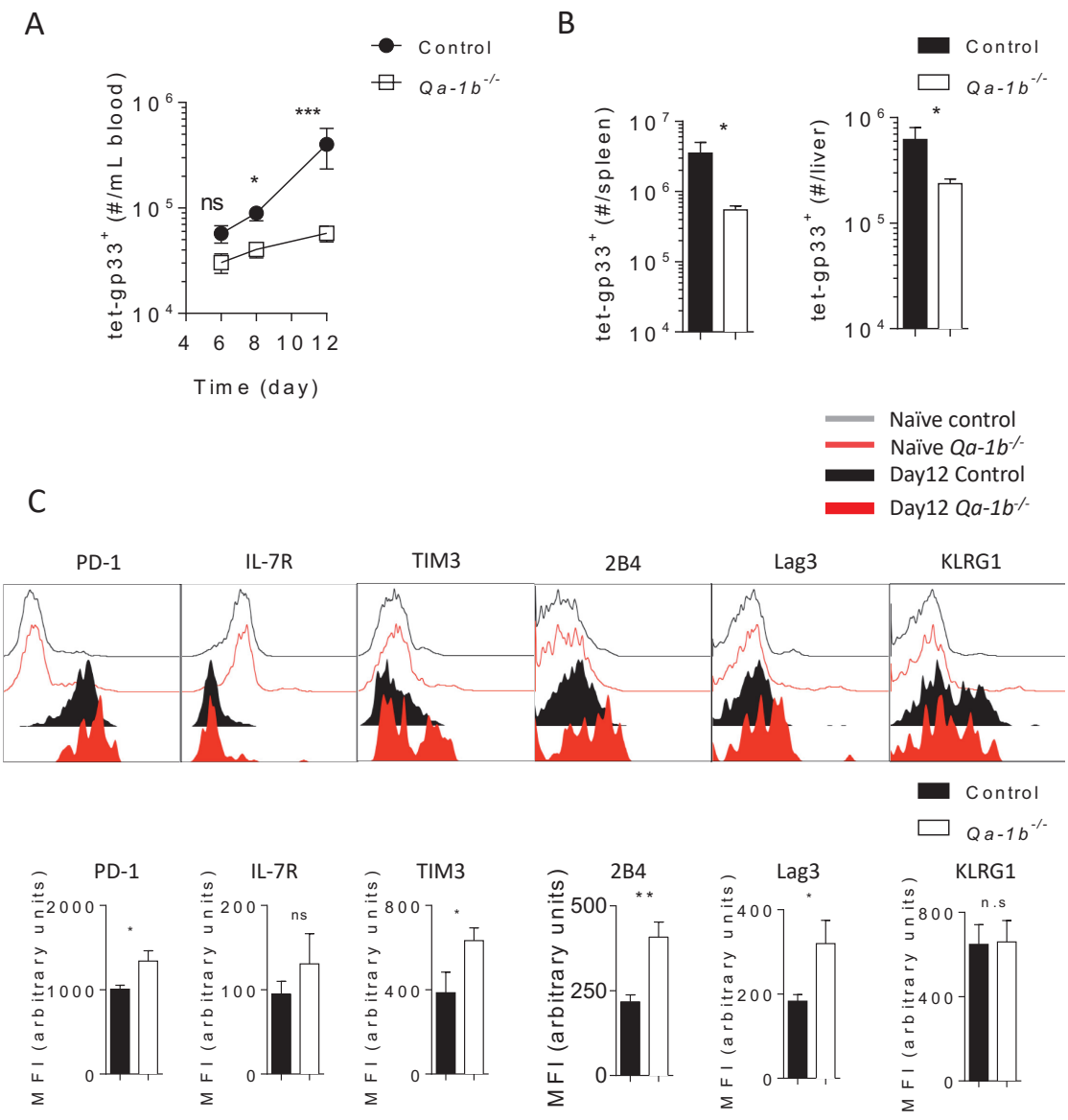


FIG 21 The deficiency of Qa-1b impairs T cell response after LCMV infection (Xu, Huang et al. 2017)

Control and *Qa-1b*$^{-/-}$ mice were infected with high dose of LCMV WE. (A) Gp33-specific tetramer was measured in the blood at the indicated time points after infection (n=7-9). (B) Gp33-specific tetramer in the spleen (left) and liver (right) were shown after 12 days infection. (C) (Up) Representative histograms of day 12 splenic gp33-specific tetramer or naïve splenic CD8^{+} T cells were shown. (Bottom) Mean fluorescence intensity (MFI) of indicated molecules was quantified from day 12 splenic

gp33-specific tetramer (n=7-9).

FIG 22

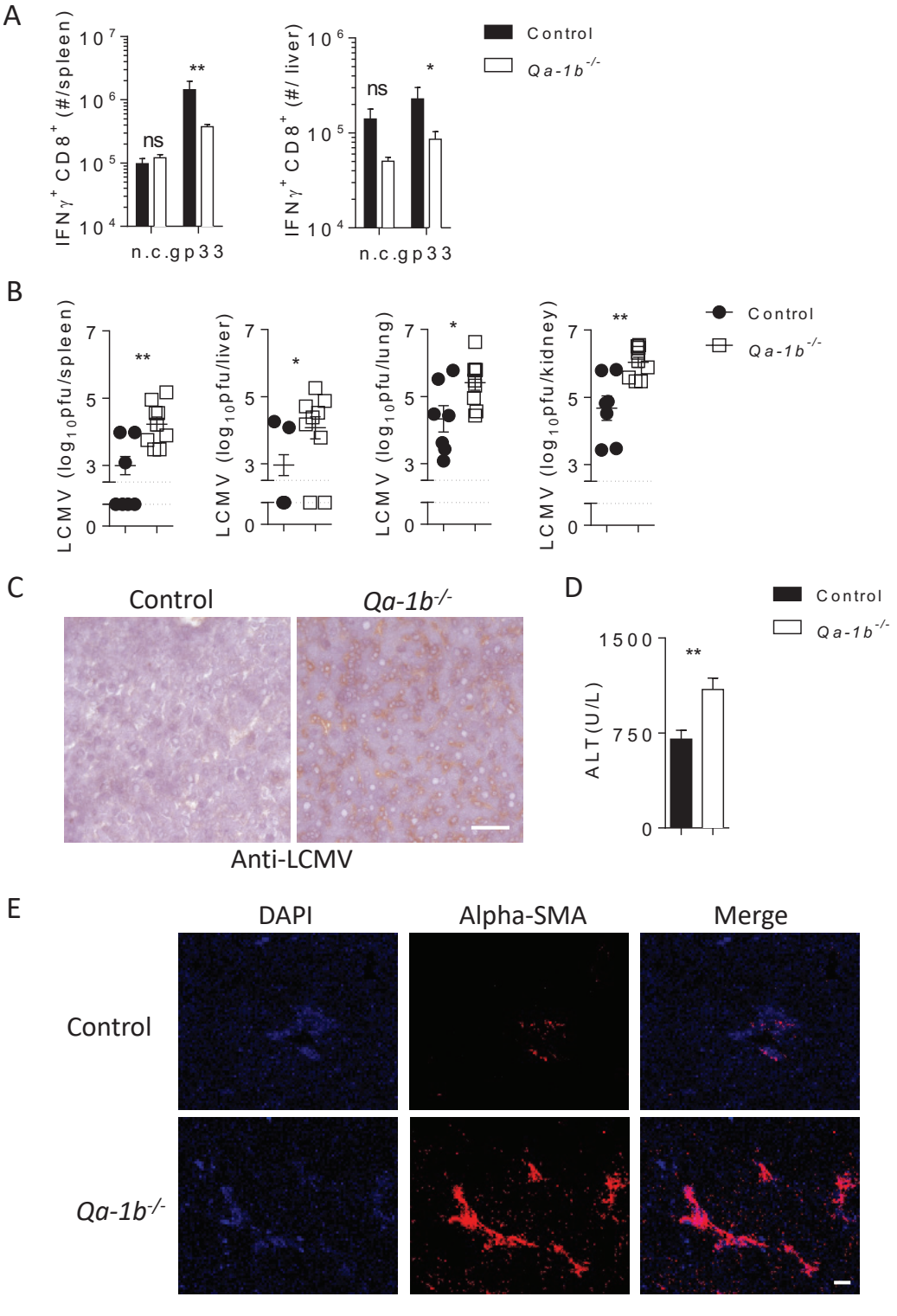

A
IFNγ+ CD8+ (#/spleen)
IFNγ+ CD8+ (#/ liver)
ns
**
*
n.c.gp33
Control
Qa-1b-/-
B
LCMV (log10pfu/spleen)
LCMV (log10pfu/liver)
LCMV (log10pfu/lung)
LCMV (log10pfu/kidney)
C
Control
Qa-1b-/-
Anti-LCMV
D
ALT(U/L)
1500
750
0
E
DAPI
Alpha-SMA
Merge
Control
Qa-1b-/-

FIG 22 The deficiency of Qa-1b displays reduced IFN-γ production, enhanced virus titer and increased immunopathology after LCMV infection (Xu, Huang et al. 2017)

(A) At day 12 after infection, splenocytes (left panel) or liver cells (right panel) were re-stimulated with LCMV-specific epitope gp33 to measure the production of IFN-γ (n=7-9). (B) Virus titers were measured in spleen, liver, lung and kidney at day 12 post infection (n=7-9). (C) Section of snap frozen liver tissue from control and *Qa-1b*$^{-/-}$ mice at day 12 post infection were analyzed for the expression of LCMV nucleoprotein by immunohistochemistry; one representative image of n=7-9 was shown (scale bar=50μm). (D) The concentration of ALT in the serum of control and *Qa-1b*$^{-/-}$ mice was determined at day 8 post infection (n=7-9). (E) Sections of snap frozen liver tissue from control and *Qa-1b*$^{-/-}$ mice at day 12 post infection were stained with α-SMA antibody, one representative image of n=7-9 was shown, scale bar=50μm). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, ns indicates statistically not significant between the indicated groups.

FIG 23

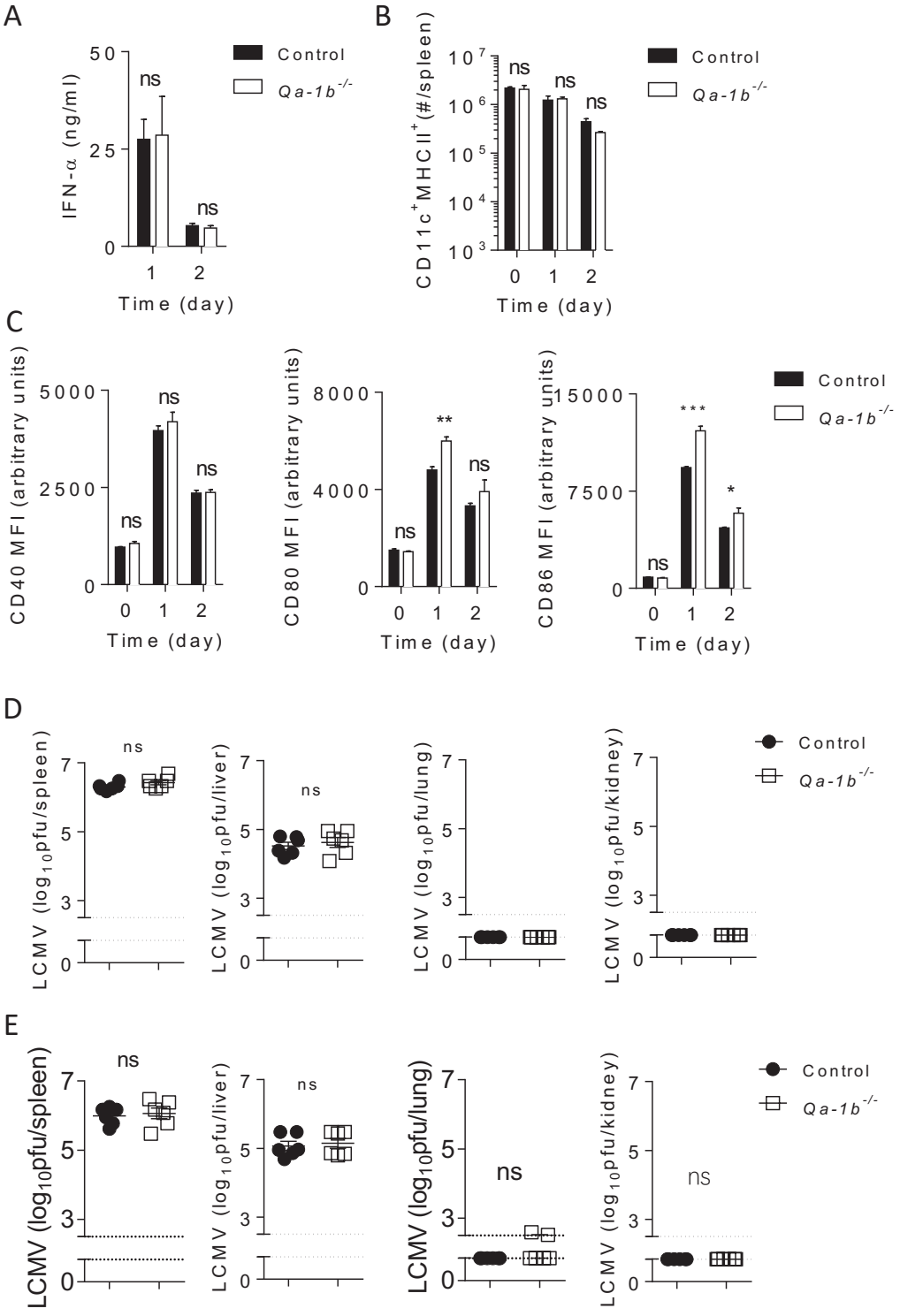

A
IFN-α (ng/ml)
50
25
0
ns
ns
1
2
Time (day)
Control
Qa-1b-/-
B
CD11c+MHCII+ (#/spleen)
10 7
10 6
10 5
10 4
10 3
ns
ns
ns
0
1
2
Time (day)
Control
Qa-1b-/-
C
CD40 MFI (arbitrary units)
5000
2500
0
ns
ns
ns
Time (day)
CD80 MFI (arbitrary units)
8000
4000
0
ns
**
ns
Time (day)
CD86 MFI (arbitrary units)
15000
7500
0
ns
***
*
Time (day)
Control
Qa-1b-/-
D
LCMV (log10pfu/spleen)
LCMV (log10pfu/liver)
LCMV (log10pfu/lung)
LCMV (log10pfu/kidney)
7
5
3
0
ns
ns
Control
Qa-1b-/-
E
LCMV (log10pfu/spleen)
LCMV (log10pfu/liver)
LCMV (log10pfu/lung)
LCMV (log10pfu/kidney)
7
5
3
0
ns
ns
ns
ns
Control
Qa-1b-/-

FIG 23 Normal IFN-I response triggers similar early virus replication between Control and *Qa-1b*$^{-/-}$ mice (Xu, Huang et al. 2017)

(A-E) Control or *Qa-1b*$^{-/-}$ mice were infected with high dose of LCMV WE. (A) The concentrations of IFN-α from sera of infected mice were measured at indicated time points (n=3). (B) The 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) The expression level 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) one day post infection (n=6) (E) two days post infection (n=6). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns indicates statistically not significant between the indicated groups.

FIG 24

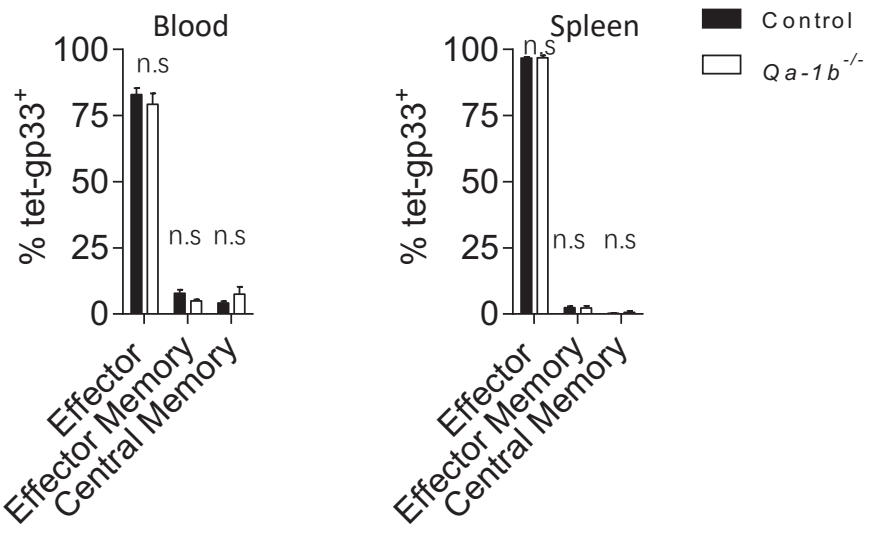


FIG 24 Comparable effector, effector memory and central memory T cells between Control and *Qa-1b*$^{-/-}$ mice (Xu, Huang et al. 2017)

Control and *Qa-1b*$^{-/-}$ mice were infected with high dose of LCMV WE. 12 days post infection, the proportions of effector ($CD62L^{-}IL7R^{-}$), effector memory ($CD62L^{-}IL7R^{+}$), central memory ($CD62L^{+}IL7R^{+}$) of blood and splenic gp33-specific tetramer were shown (n=7-9). Error bars show SEM, ns indicates statistically not significant between the indicated groups.

FIG 25

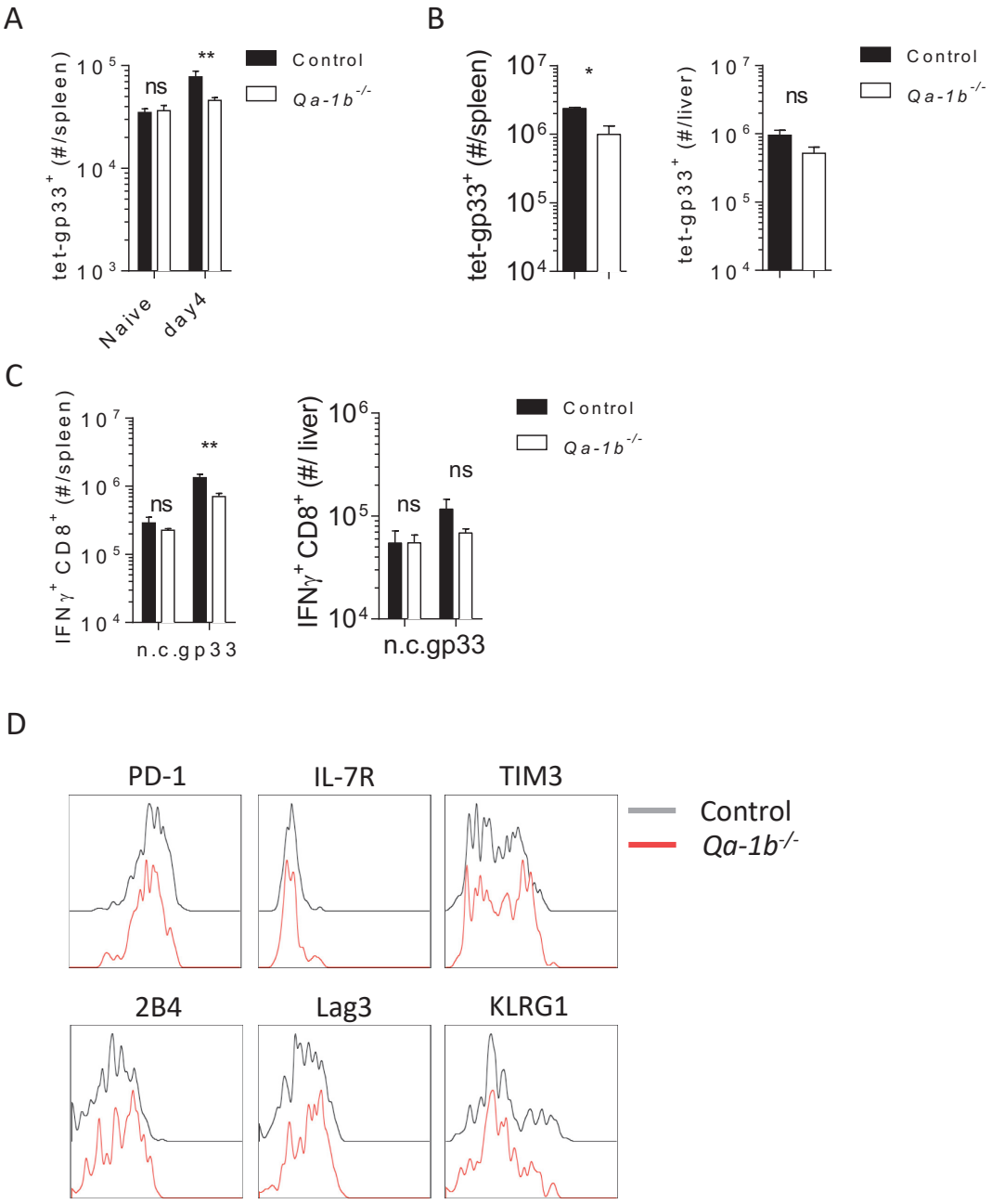

A
B
C
D
Control
Qa-1b-/-
ns
**
*
Naive
day4
tet-gp33+ (#/spleen)
tet-gp33+ (#/liver)
IFNγ+ CD8+ (#/spleen)
IFNγ+ CD8+ (#/ liver)
n.c.gp33
PD-1
IL-7R
TIM3
2B4
Lag3
KLRG1

FIG 25 The absence of Qa-1b inhibits T cell immunity at day 4 and day 8 (Xu, Huang et al. 2017)

(A-D) Control and *Qa-1b*$^{-/-}$ mice were infected with high dose of LCMV WE. (A) Gp33-specific tetramer was measured in the spleen 4 days after infection (n=4). (B) Gp33-specific tetramer was measured in the spleen (left) and liver tissue (right) 8 days post infection (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 with the measurement of IFN-γ production (n=3). (D) One representative histogram of the expression of surface molecules at day 8 splenic gp33-spefific tetramer was shown (n=3). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, ns indicates statistically not significant between the indicated groups.

FIG 26

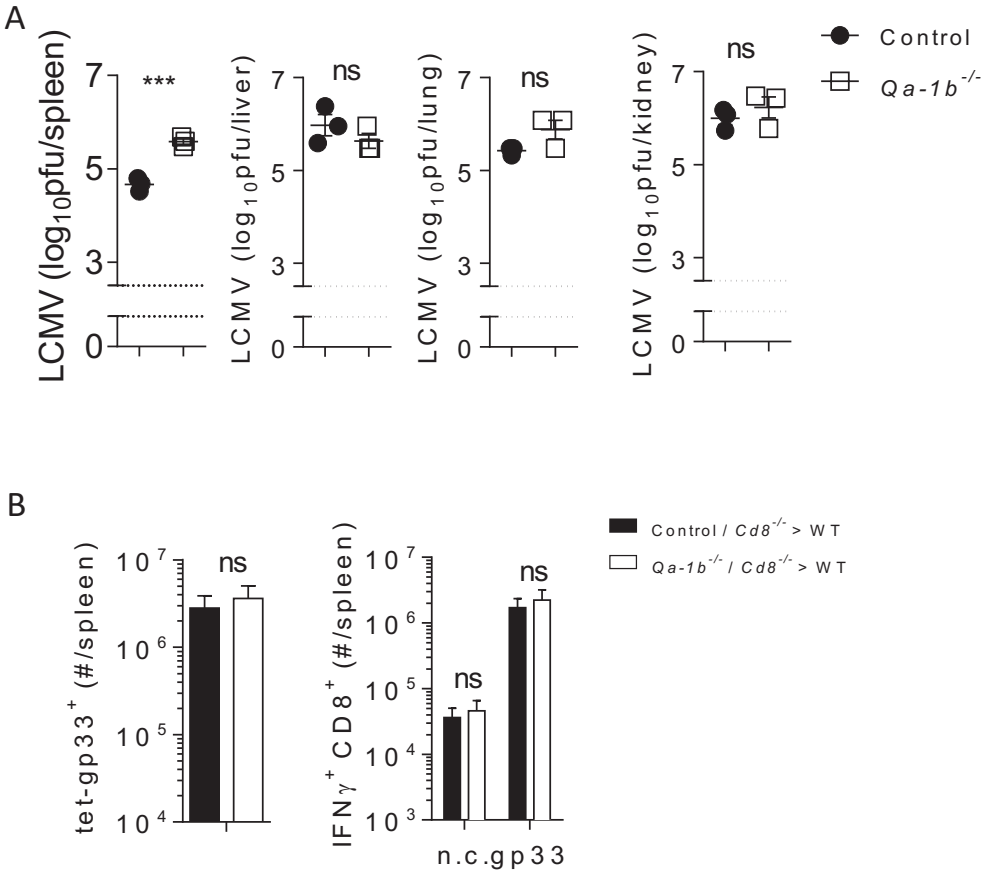


FIG 26 Increased virus titers in *Qa-1b*$^{-/-}$ mice but similar T cell response in control or *Qa-1b*$^{-/-}$ with *Cd8*$^{-/-}$ mix chimeras' mice after LCMV infection (Xu, Huang et al. 2017)

(A) Virus titers were determined in spleen, liver, lung, and kidney tissue 8 days post infection. (n=3). (B) Bone marrow cells from control or *Qa-1b*$^{-/-}$ were mix at 1:1 ratio with bone marrow cells from *Cd8*$^{-/-}$ were transferred into lethally irradiated WT mice. One month later, these mix chimeric mice were infected with $2x10^5$ pfu LCMV WE, 12 days after infection (left) Gp33-specific tetramer in the spleen, (right) splenocytes cells were re-stimulated with the LCMV-specific epitope gp33, followed by staining of IFN-γ (n=4-5). Error bars show SEM, ∗∗∗$p < 0.001$, ns indicates statistically not significant between the indicated groups.

FIG 27

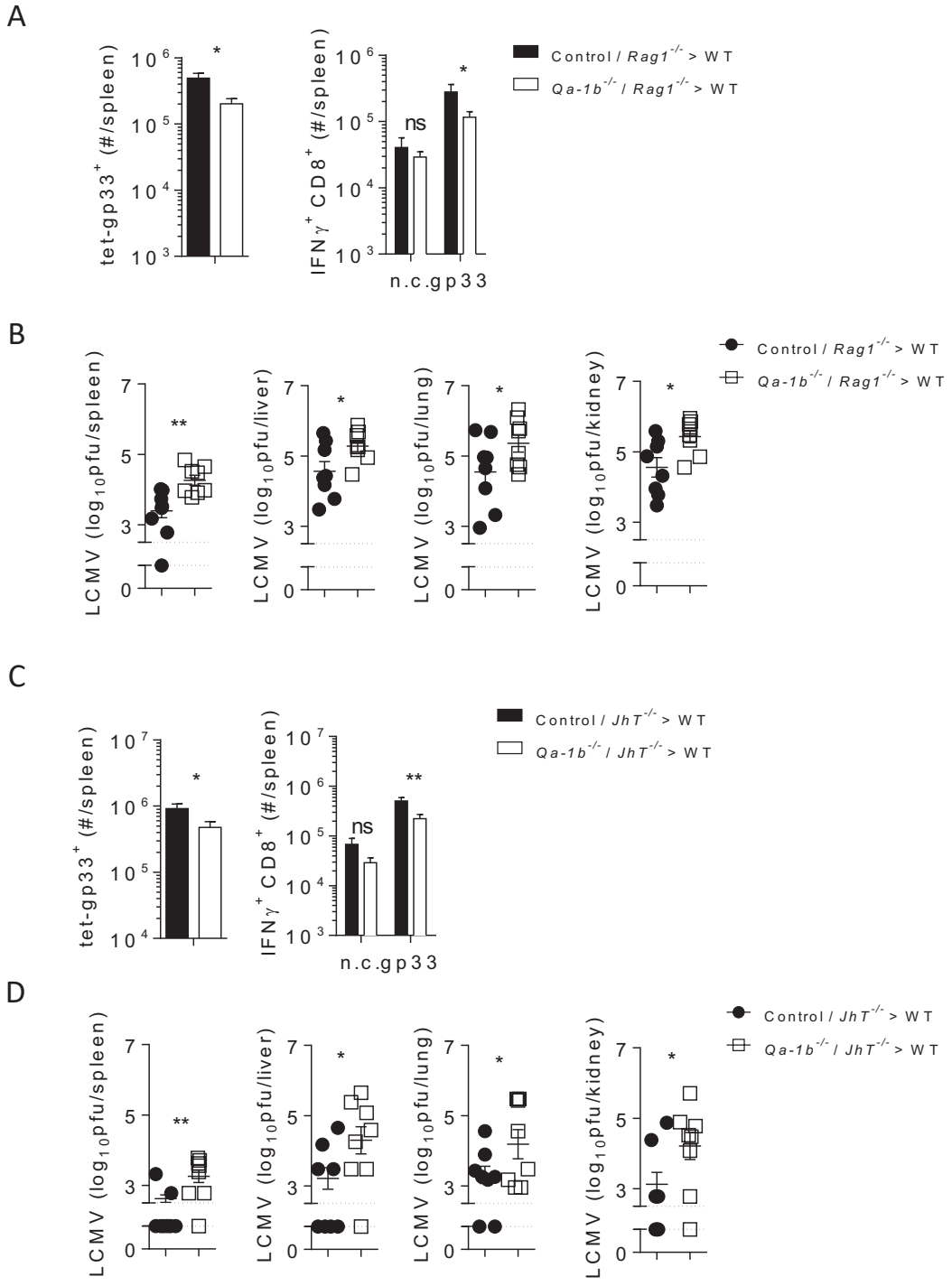

A
tet-gp33+ (#/spleen)
IFNγ+ CD8+ (#/spleen)
ns
n.c.gp33
Control / Rag1-/- > WT
Qa-1b-/- / Rag1-/- > WT
B
LCMV (log10pfu/spleen)
LCMV (log10pfu/liver)
LCMV (log10pfu/lung)
LCMV (log10pfu/kidney)
Control / Rag1-/- > WT
Qa-1b-/- / Rag1-/- > WT
C
tet-gp33+ (#/spleen)
IFNγ+ CD8+ (#/spleen)
ns
n.c.gp33
Control / JhT-/- > WT
Qa-1b-/- / JhT-/- > WT
D
LCMV (log10pfu/spleen)
LCMV (log10pfu/liver)
LCMV (log10pfu/lung)
LCMV (log10pfu/kidney)
Control / JhT-/- > WT
Qa-1b-/- / JhT-/- > WT

FIG 27 The deficiency of Qa-1b impacts T cell response in the experiment of mix chimeras (Xu, Huang et al. 2017)

(A-D) Bone marrow cells from control or *Qa-1b*$^{-/-}$ mice were mixed at a 1:1 ration with bone marrow cells from *Rag1*$^{-/-}$ or *JhT*$^{-/-}$ mice and were transferred into lethally irradiated WT mice. One month later, these mixed chimeric mice were infected with $2x10^5$ pfu LCMV WE, and splenocytes harvested 12 days after infection. (A) Gp33-specific tetramer$^+$CD8$^+$ T cells in the spleen were measured in *Qa-1b*$^{-/-}$: *Rag1*$^{-/-}$ and WT: *Rag1*$^{-/-}$ reconstituted chimeras (n=8, left). IFN-γ$^+$CD8$^+$ T cells were determined in splenocytes from these mice following re-stimulation with the LCMV epitope gp33 (n=8, right). (B) Virus titers were measured from spleen, liver, lung and kidney tissues in WT: *Rag1*$^{-/-}$ and *Qa-1b*$^{-/-}$: *Rag1*$^{-/-}$reconstituted chimeras (n=8). (C) Gp33-specific tetramer$^+$CD8$^+$ T cells in the spleen were measured in WT: *JhT*$^{-/-}$ and *Qa-1b*$^{-/-}$: *JhT*$^{-/-}$ reconstituted chimeras (n=8, left). IFN-γ$^+$CD8$^+$ T cells were determined in splenocytes from these mice following re-stimulation with the LCMV epitope gp33 (n=8, right). (D) Virus titers were measured from spleen, liver, lung and kidney tissues in WT: *JhT*$^{-/-}$ and *Qa-1b*$^{-/-}$: *JhT*$^{-/-}$ reconstituted chimeras (n=8). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns indicates statistically not significant between the indicated groups.

9.2.4 Deficiency of Qa-1b results in impaired T cell immunity during chronic infection

Next, we also wondered whether Qa-1b could impact the T cell immunity during chronic LCMV infection. Then we infected control and Qa-1b deficient mice with the chronic LCMV strain Docile and observed similarly decreased $CD8^+$ T cell frequencies in *Qa-1b*$^{-/-}$ mice when compared with control mice (FIG 28A). This result was related with enhanced expression of PD-1 and reduced expression of IL-7 receptor (IL-7R), suggesting that LCMV-specific $CD8^+$ T cells were also in an exhausted state in the deficiency of Qa-1b (FIG 28B). Furthermore, the production of IFN-γ was also reduced by $CD8^+$ T cells at day 75 after LCMV Docile infection (FIG 28C). NK cells can not only target to anti-viral $CD8^+$ T cells, but also virus-specific $CD4^+$ T cells following viral infection (Waggoner, Cornberg et al. 2011). Interestingly, LCMV-specific $CD4^+$ T cells were also decreased in *Qa-1b*$^{-/-}$ mice after viral infection when compared with control mice (FIG 28D-E). Finally, we also found prolonged LCMV in the sera of Qa-1b deficient mice when compared with control mice (FIG 28F). LCMV was persistent in the spleen, liver, lung, kidney, spinal cord and brain of *Qa-1b*$^{-/-}$ mice at day 75 post infection (FIG 29A-B). These results illustrate that LCMV-specific T cell response is reduced in *Qa-1b*$^{-/-}$ mice during LCMV chronic infection.

FIG 28

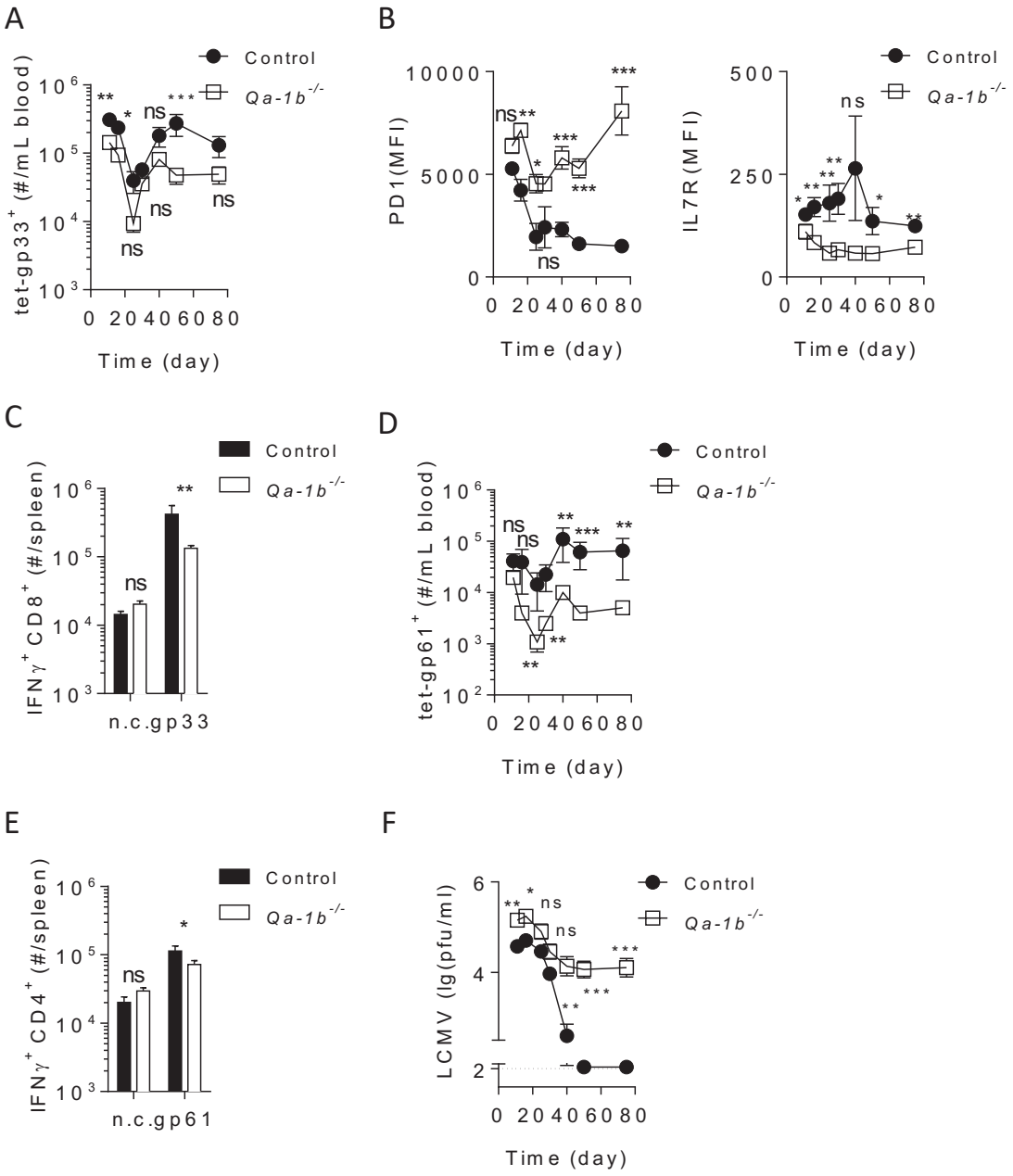

A
Control
Qa-1b-/-
tet-gp33+ (#/mL blood)
Time (day)
B
PD1(MFI)
IL7R(MFI)
Time (day)
C
Control
Qa-1b-/-
IFNγ+ CD8+ (#/spleen)
n.c.gp33
D
tet-gp61+ (#/mL blood)
Time (day)
E
IFNγ+ CD4+ (#/spleen)
n.c.gp61
F
LCMV (lg(pfu/ml)
Time (day)

FIG 28 The deficiency of Qa-1b results in prolonged LCMV infection (Xu, Huang et al. 2017)

Control and *Qa-1b*$^{-/-}$ were infected with 2x10^4 pfu LCMV Docile. (A) Gp33-specific tetramer was measured in the blood at the indicated time points following infection (n=4-6). (B) The expression levels of PD-1 (left) and IL-7R (right) on blood gp33-specific tetramer at the indicated time after infection were shown (n=4-6). (C) At day 75 post infection, splenocytes were re-stimulated with LCMV epitope gp33 to measure the production of IFN-γ (n=4-6). (D) Gp61-specific CD4$^+$ tetramer response in the blood at the indicated time points after infection was monitored (n=4-6). (E) At day 75 post infection, splenocytes were re-stimulated with LCMV epitope gp61 to measure of the production of IFN-γ (n=4-6). (F) LCMV titers were measured in the blood at indicated time points following infection (n=4-6). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns indicates statistically not significant between the indicated groups.

FIG 29

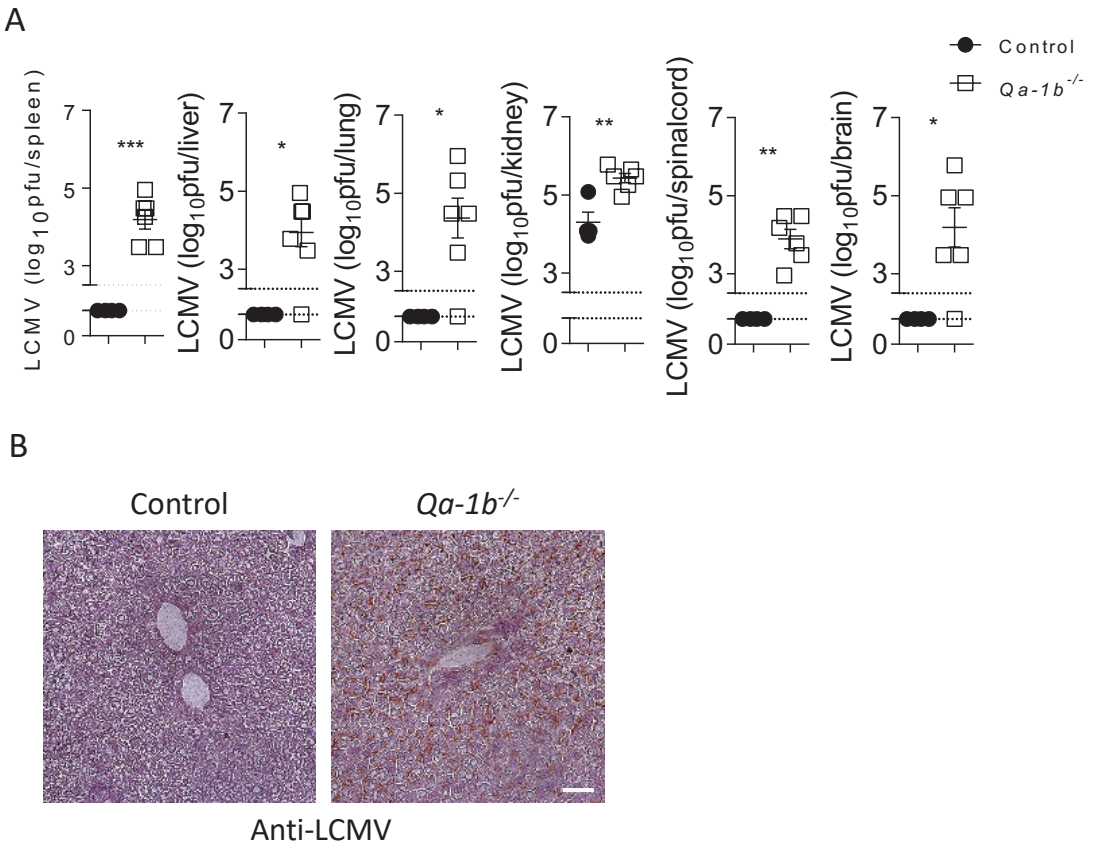


FIG 29 The deficiency of Qa-1b results in virus persistent in *Qa-1b*$^{-/-}$ mice after chronic infection (Xu, Huang et al. 2017)

(A) LCMV titers were measured in the spleen, liver, lung, kidney, spinal cord and brain tissues 75 days post infection (n=4-6). (B) Sections of snap frozen liver tissue from WT and *Qa-1b*$^{-/-}$ mice 75 days post infection were analyzed for the expression of LCMV nucleoprotein by immunohistochemistry, one representative image was shown (n=4-6, scale bar=50μm). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns indicates statistically not significant between the indicated groups.

9.2.5 Qa-1b and its receptor NKG2A promote anti-viral T cell response and alleviate virus-mediated pathology

We further investigate whether the absence of Qa-1b binding receptor NKG2A can recapitulate the effects of Qa-1b deficiency after LCMV infection. The number of LCMV-specific $CD8^+$ T cells was decreased in NKG2A deficient mice (FIG 30A), Furthermore, the production of IFN-γ was also reduced in NKG2A deficient *Klrc1*$^{-/-}$ mice when compared with wildtype controls (FIG 30B). LCMV titers were also enhanced in *Klrc1*$^{-/-}$ mice after LCMV infection (FIG 30C-D). However, the effects we found in NKG2A deficient mice were not as prominent as those incurred by Qa-1b deficiency. This may be explained by a T cell-intrinsic role of NKG2A (Rapaport, Schriewer et al. 2015). Moreover, LCMV-specific T cells displayed positive staining for anti-NKG2A/C/E (clone 20D5) in control and *Qa-1b*$^{-/-}$ mice after LCMV infection, while this staining was absent in NKG2A deficient mice, indicating that LCMV-specific T cells express NKG2A (FIG 30E-F). To study the role of NKG2A on LCMV-specific T cells, we reconstituted lethally irradiated C57BL/6 mice with mixed bone marrow from *Cd8*$^{-/-}$ and *Klrc1*$^{-/-}$ mice. After LCMV infection, we found enhanced $CD8^+$ T cell response at day 12 in the hosts receiving the *Cd8*$^{-/-}$: *Klrc1*$^{-/-}$ bone marrow when compared with host receiving the *Cd8*$^{-/-}$: WT bone marrow (FIG 31A). ALT activity was enhanced in NKG2A deficient mice following LCMV infection (FIG 31B). Finally, NKG2A deficient mice also showed the increased expression of α-SMA, suggesting a higher degree of fibrosis after LCMV infection (FIG 31C). In summary, these data suggest that Qa-1b and its receptor NKG2A promote anti-viral T cell immunity and alleviate virus-mediated pathology during LCMV infection.

FIG 30

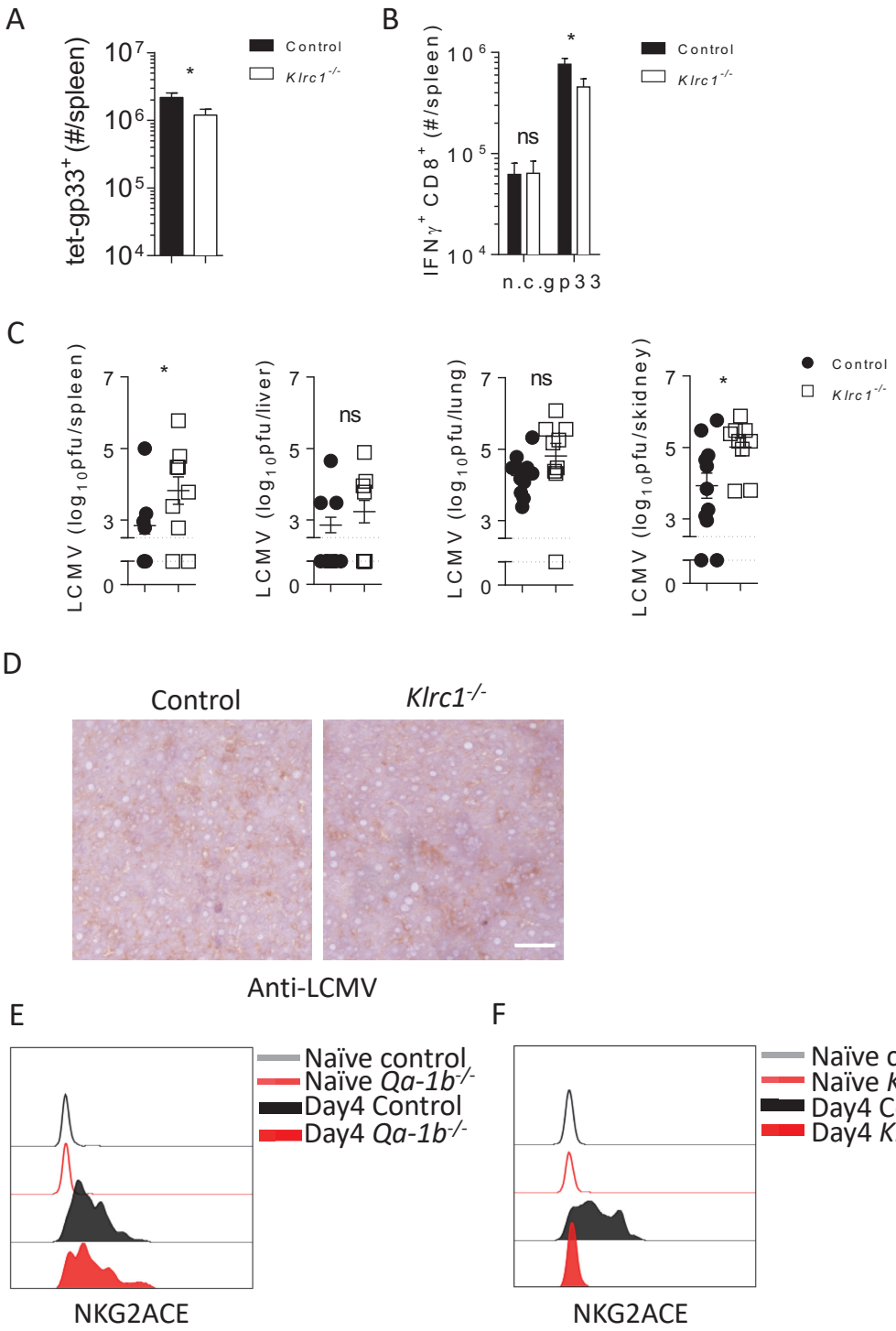
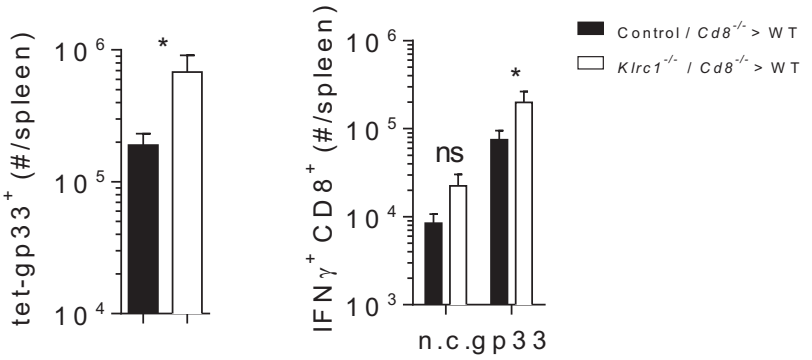

A
tet-gp33+ (#/spleen)
Control
Klrc1-/-
B
IFNγ+ CD8+ (#/spleen)
ns
n.c.gp33
C
LCMV (log10pfu/spleen)
LCMV (log10pfu/liver)
LCMV (log10pfu/lung)
LCMV (log10pfu/skidney)
D
Control
Klrc1-/-
Anti-LCMV
E
Naïve control
Naïve Qa-1b-/-
Day4 Control
Day4 Qa-1b-/-
NKG2ACE
F
Naïve control
Naïve Klrc1-/-
Day4 Control
Day4 Klrc1-/-
NKG2ACE

FIG 30 The absence of NKG2A limits anti-viral T cell response and results in increased virus replication after LCMV infection (Xu, Huang et al. 2017)

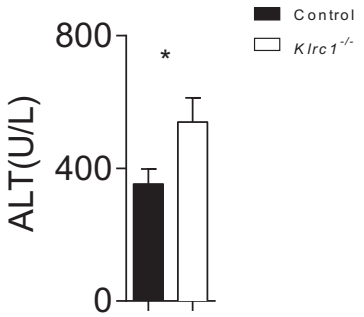
Control (C57BL/6) and *Klrc1*$^{-/-}$ mice were infected with high dose of LCMV WE. (A) Splenic gp33-specific tetramer was determined 12 days after infection (n=9-11). (B) At day 12 post infection, splenocytes were re-stimulated with LCMV-specific epitope gp33 to measure the production of IFN-γ (n=9-11). (C) Virus titers were measured in the spleen, liver, lung and kidney at day 12 after infection (n=9-11). (D) Sections of snap frozen liver tissue form control and *Klrc1*$^{-/-}$ mice 12 days post infection were monitored for the expression of LCMV nucleoprotein by immunochemistry, one representative image was shown (n=7-8, scale bar=50μm). (E) Control and *Qa-1b*$^{-/-}$ 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). (F) Control and *Klrc1*$^{-/-}$ mice were infected with high dose of LCMV WE, 4 days post infection, expression of NKG2A/C/E was determined on splenic gp33-specific tetramer (n=4). Error bars show SEM, $*p < 0.05$, ns indicates statistically not significant between the indicated groups.

FIG 31

A


tet-gp33+ (#/spleen)
10 6
10 5
10 4
*
IFNγ+ CD8+ (#/spleen)
10 6
10 5
10 4
10 3
ns
*
n.c.gp33
Control / Cd8-/- > WT
Klrc1-/- / Cd8-/- > WT

B


ALT(U/L)
800
400
0
*
Control
Klrc1-/-

C

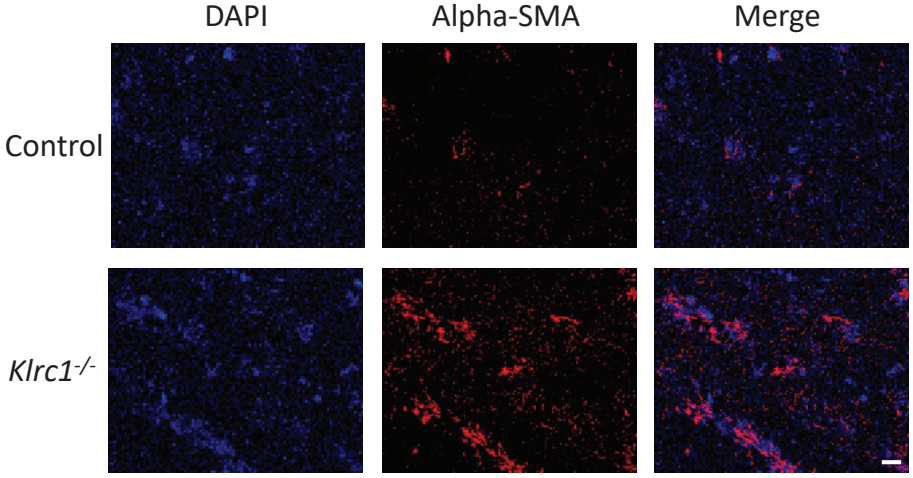

DAPI
Alpha-SMA
Merge
Control
Klrc1-/-

FIG 31 The absence of NKG2A impacts anti-viral T cell response in the settings of mix chimeras and results in enhanced immunopathology after LCMV infection (Xu, Huang et al. 2017)

(A) Bone marrow cells from control or *Klrc1*$^{-/-}$ were mix at 1:1 ratio with bone marrow cells from *Cd8*$^{-/-}$ were transferred into lethally irradiated WT mice. One month later, these mix chimeric animals were infected with $2x10^5$pfu LCMV WE, 12 days after infection (left) Gp33-specific tetramer in the spleen, (right) splenocytes cells were re-stimulated with the LCMV-specific epitope gp33, followed by staining for IFN-γ (n=4-5). (B) The activity of ALT in the serum of control and *Klrc1*$^{-/-}$ mice was monitored at day 8 after infection (n=11-12). (C) Sections of snap frozen liver tissue from control and *Klrc1*$^{-/-}$ mice 12 days post infection were stained with α-SMA antibody, one representative image was shown (n=7-8, scale bar=50μm). Error bars show SEM, $*p < 0.05$, ns indicates statistically not significant between the indicated groups.

9.2.6 The depletion of NK cells partially rescues defective T cell response and prevents pathology in Qa-1b deficient mice

Enhanced activity of NK cells might limit $CD8^+$ T cell response and prolong viral replication and pathology. NK cells can be depleted by the anti-NK1.1 antibody (clone: PK136) (FIG 35A). Then we investigate whether depletion of NK cells can improve T cell response in *Qa-1b*$^{-/-}$ mice. Firstly, we transferred negatively sorted LCMV-specific T cells into NK cell-depleted and NK cell-competent *Qa-1b*$^{-/-}$ and wildtype mice following LCMV infection. We observed that depletion of NK cells could rescue the increased susceptibility of $P14^+CD8^+$ T cells in Qa-1b deficient mice (FIG 32A). Furthermore, depletion of NK cells leads to the enhanced presence of LCMV-specific endogenous $CD8^+$ T cells in Qa-1b deficient mice (FIG 32B). Similarly, we also found increased response of LCMV-specific $CD8^+$ T cell in the spleen of *Qa-1b*$^{-/-}$ mice after NK cell depletion (FIG 32C). The production of IFN-γ following re-stimulation with LCMV-specific epitope gp33 was increased in the absence of NK cells in *Qa-1b*$^{-/-}$ mice (FIG 32C). Consistent with previously published data, the response of T cells was enhanced in cell-depleted control mice when compared with NK cell-competent control mice (Cook and Whitmire, 2013; Lang et al., 2012; Waggoner et al., 2012). However, we did not observe a complete rescue in the spleens of *Qa-1b*$^{-/-}$ mice by NK cell depletion, suggesting that there might be other NK cell-independent factors contributing to diminished LCMV-specific T cell response in the spleen tissue of *Qa-1b*$^{-/-}$ mice (FIG 32C). However, the expression of surface molecules (PD-1, TIM-3, 2B4 and Lag-3) were reduced in the *Qa-1b*$^{-/-}$ mice following NK cell depletion (FIG 32D and FIG 35B). Moreover, viral titers in the spleen, liver, lung and kidney of *Qa-1b*$^{-/-}$ mice were highly reduced after treatment with anti-NK1.1 antibody (FIG 33A). We also did not find LCMV-positive cells in snap frozen liver tissue in control and *Qa-1b*$^{-/-}$ mice with the treatment of NK depletion antibody (FIG 33B). The concentration of ALT was reduced in both control and *Qa-1b*$^{-/-}$ mice after NK depletion (FIG 34A). Finally, fluorescent staining also showed decreased the expression of α-SMA in the *Qa-1b*$^{-/-}$

mice after the treatment with anti-NK1.1 antibody when compared with NK cell-competent *Qa-1b*$^{-/-}$ mice (FIG 34B). Above all, these results demonstrate that NK cell depletion can partially restore defective T cell response and accordingly prevent pathology in *Qa-1b*$^{-/-}$ mice.

FIG 32

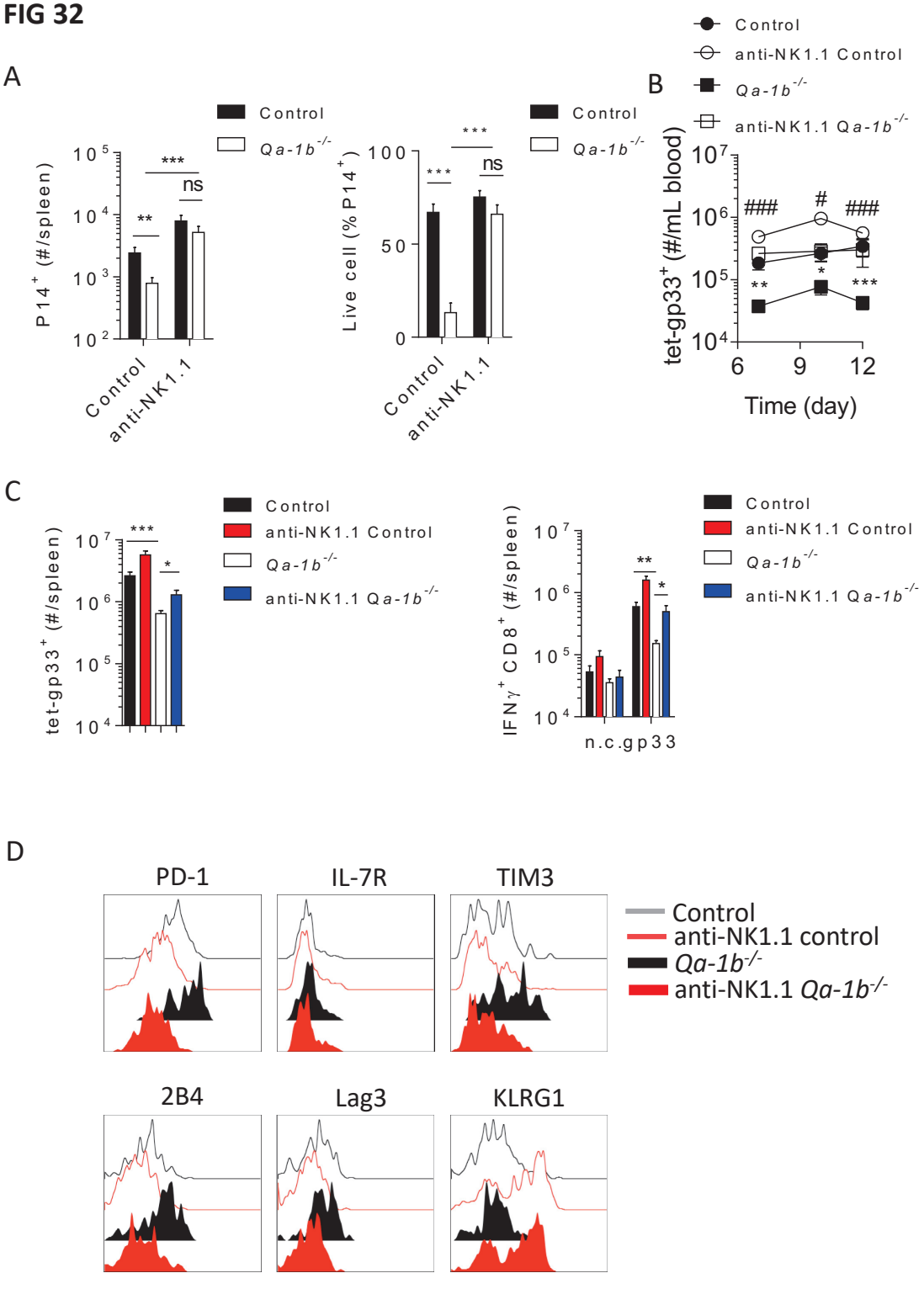
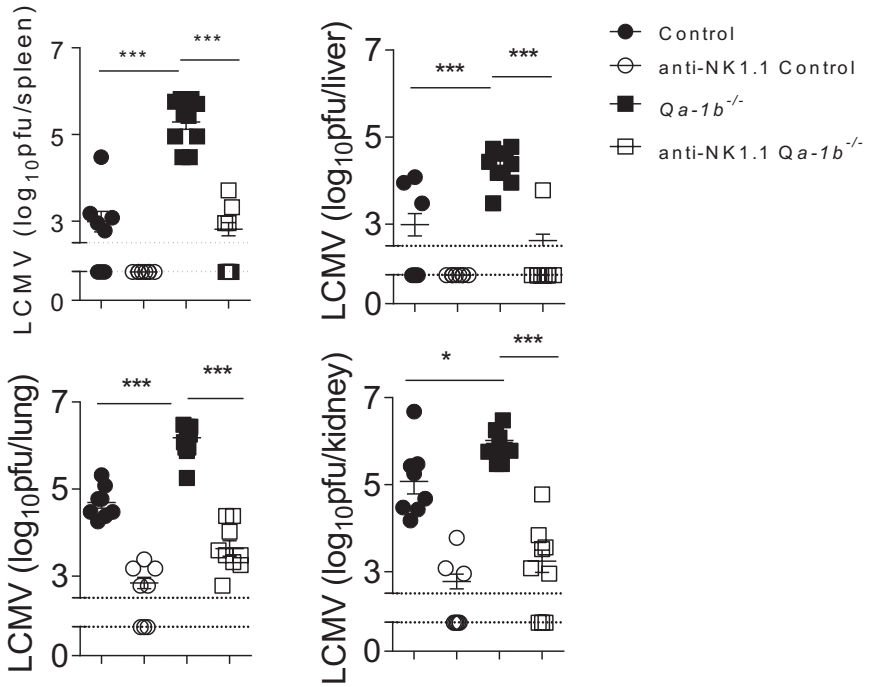

A
Control
Qa-1b-/-
P14+ (#/spleen)
Live cell (% P14+)
Control
anti-NK1.1
ns
***
**
B
Control
anti-NK1.1 Control
Qa-1b-/-
anti-NK1.1 Qa-1b-/-
tet-gp33+ (#/mL blood)
Time (day)
###
#
C
tet-gp33+ (#/spleen)
IFNγ+ CD8+ (#/spleen)
n.c.gp33
D
PD-1
IL-7R
TIM3
2B4
Lag3
KLRG1
Control
anti-NK1.1 control
Qa-1b-/-
anti-NK1.1 Qa-1b-/-

FIG 32 The depletion of NK cell partially restores defective T cell immunity in Qa-1b deficient mice (Xu, Huang et al. 2017)

NK cell-depleted. non-depleted control and *Qa-1b*$^{-/-}$ mice were infected with high dose of LCMV WE, (A) A total of 10^6 negatively sorted CD8$^+$ T cells from P14$^+$CD45.1$^+$ mice were transferred into NK-cell depleted or non-depleted CD45.2$^+$ control or CD45.2$^+$ *Qa-1b*$^{-/-}$ hosts followed by infection with high dose of LCMV WE. The number of transferred P14$^+$ cells was measured two days after infection (n=4-5, left). P14$^+$ cell viability was measured by Annexin V and 7AAD two days after infection (n=4-5, right) (B) Gp33-specific tetramer were measured in the blood at the indicated time points after infection (n=8-10, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ between control and *Qa-1b*$^{-/-}$ group, $\#p < 0.05$, $\#\#\#p < 0.001$ between *Qa-1b*$^{-/-}$ and NK cell-depleted *Qa-1b*$^{-/-}$ mice). (C) Gp33-specific CD8$^+$ T cells in the spleen were determined 12 days post infection (n=8-10, left). At day 12 post infection, splenocytes were re-stimulated with the LCMV-specific epitope gp33 to measure the production of IFN-γ (n=8-10, right). (D) Representative histograms of the indicated surface molecules on splenic gp33-specific CD8$^+$ T cells were shown (n=8-10). Error bars show SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns indicates statistically not significant between the indicated groups.

FIG 33

A


LCMV (log10pfu/spleen)
LCMV (log10pfu/liver)
LCMV (log10pfu/lung)
LCMV (log10pfu/kidney)
***
*
0
3
5
7
Control
anti-NK1.1 Control
Qa-1b-/-
anti-NK1.1 Qa-1b-/-

B

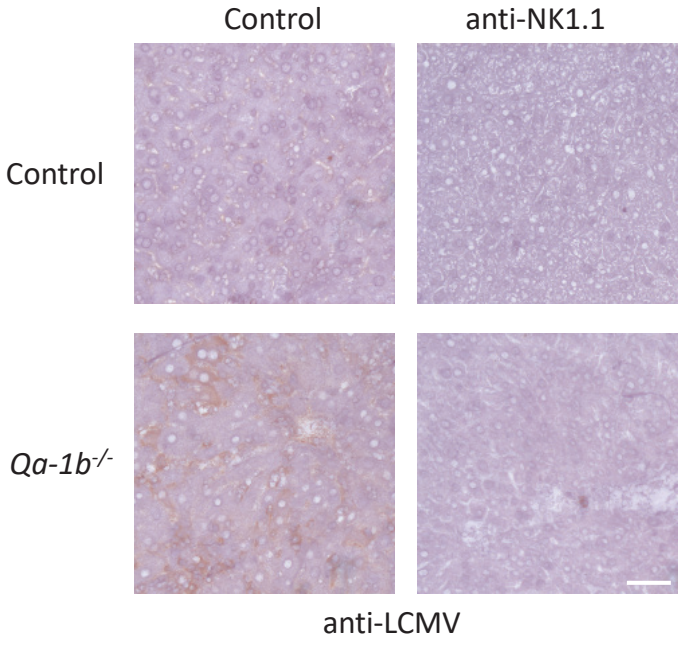

Control
anti-NK1.1
Control
Qa-1b-/-
anti-LCMV

FIG 33 The depletion of NK cell could reduce virus titers in Qa-1b deficient mice (Xu, Huang et al. 2017)

(A) Virus titers were determined in the spleen, liver, lung, and kidney tissue 12 days after infection. (n=8-10). (B) Sections of snap frozen liver tissue from NK cell-depleted or non-depleted control and *Qa-1b*$^{-/-}$ or NK-depleted *Qa-1b*$^{-/-}$ mice 12 days post infection were analyzed for the expression of LCMV nucleoprotein by immunohistochemistry. One representative picture was shown (n=8-10, scale bar=50μm).

FIG 34

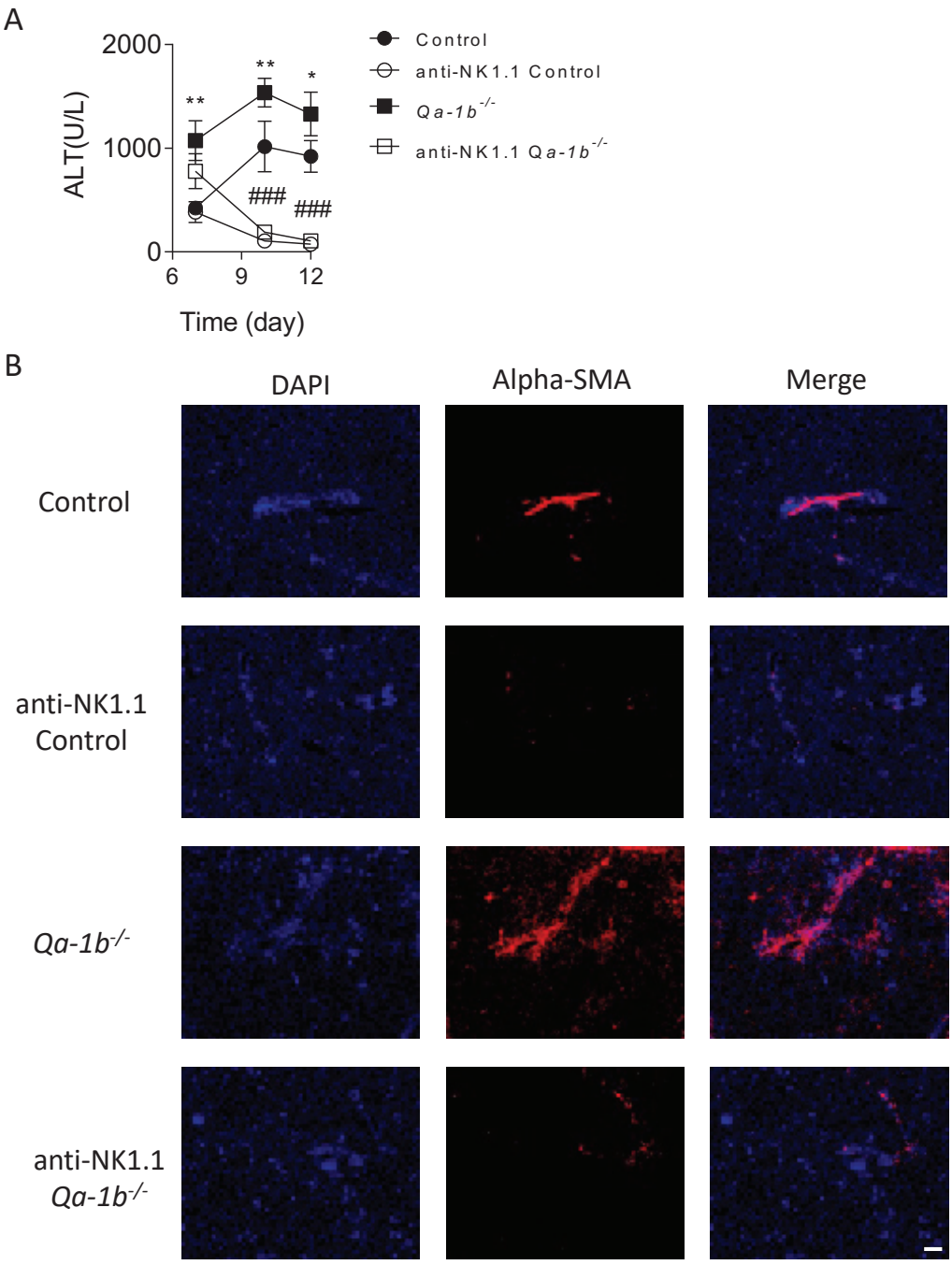
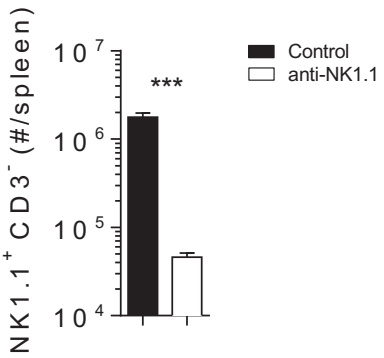

A
2000
1000
0
ALT(U/L)
6
9
12
Time (day)
**
**
*
###
###
Control
anti-NK1.1 Control
Qa-1b-/-
anti-NK1.1 Qa-1b-/-
B
DAPI
Alpha-SMA
Merge
Control
anti-NK1.1 Control
Qa-1b-/-
anti-NK1.1 Qa-1b-/-

FIG 34 The depletion of NK cell could rescue the phenotype of immunopathology in Qa-1b deficient mice (Xu, Huang et al. 2017)

(A) The activity of ALT in the serum of NK depleted or non-depleted control and *Qa-1b*$^{-/-}$ or NK-depleted *Qa-1b*$^{-/-}$ mice was determined at indicated time points (n=8-10, $*p < 0.05$, $**p < 0.01$ between control and *Qa-1b*$^{-/-}$ group, ###$p < 0.001$ between *Qa-1b*$^{-/-}$ and NK cell-depleted *Qa-1b*$^{-/-}$ mice). (B) Sections of snap frozen liver tissue were stained with α-SMA antibody. One representative image was shown (n=8-10, scale bar=50μm).

FIG 35

A


NK1.1+ CD3- (#/spleen)
10 7
10 6
10 5
10 4
***
Control
anti-NK1.1

B

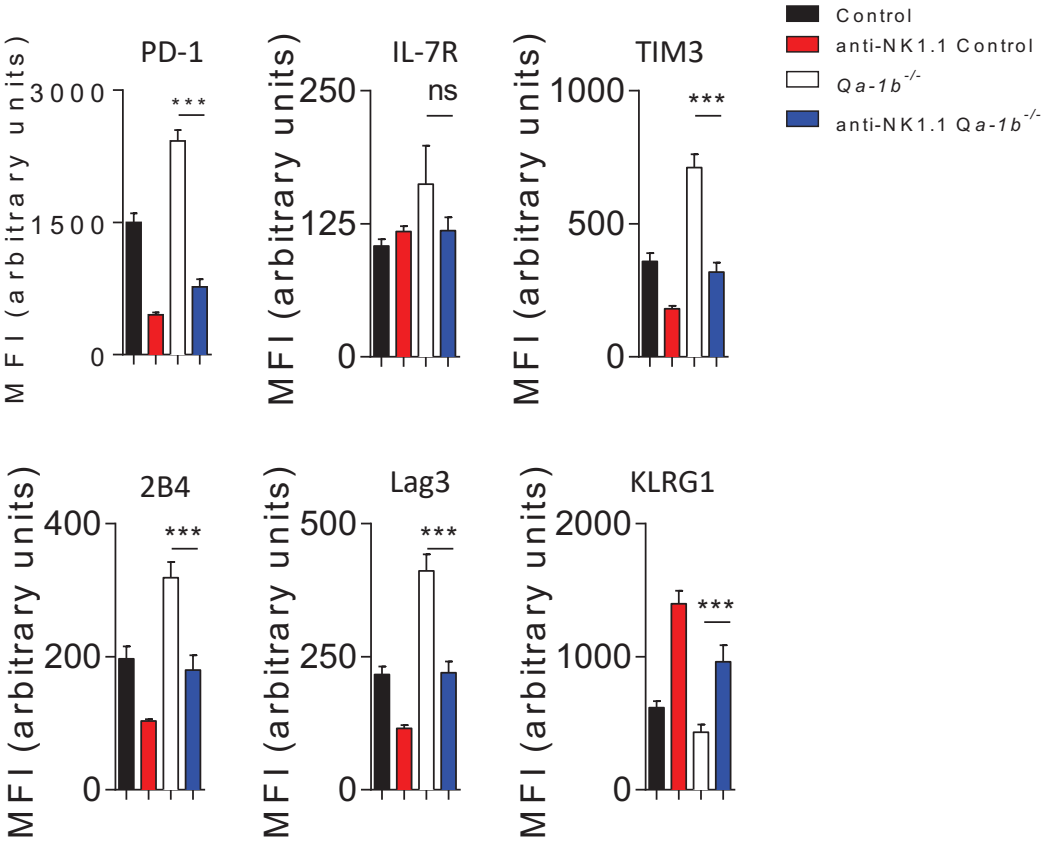

Control
anti-NK1.1 Control
Qa-1b-/-
anti-NK1.1 Qa-1b-/-
PD-1
IL-7R
TIM3
2B4
Lag3
KLRG1
MFI (arbitrary units)
3000
1500
0
250
125
1000
500
400
200
2000
ns
***

FIG 35 NK cell depletion partially restores defective T cell immunity, related to FIG 32 (Xu, Huang et al. 2017)

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

10 Discussion

In the first part, we demonstrated an essential role for BAFFR in the maintenance of $CD169^+$ macrophages. *Baffr*$^{-/-}$ mice displayed limited innate immune responses and reduced adaptive immune priming, correlated with fatal disease outcomes. Mechanistically, impaired B-cell development in *Baffr*$^{-/-}$ mice led to limited lymphotoxin expression and reduced presence of $CD169^+$ cells.

As mentioned in the introduction, BAFF can be produced by many cell populations, for instance macrophages, dendritic cells and neutrophils (Mackay and Schneider 2009). One recent study demonstrated that BAFF-producing neutrophils are in the marginal zone of the spleen; these neutrophil B helper cells play important roles in the marginal zone B-cell activation and antibody production against pathogens (Puga, Cols et al. 2011). According to our results, BAFF production by neutrophil B helper cells may also affect $CD169^+$ cell survival and subsequently enforce antigen amplification and presentation through promoting B cell-mediated lymphotoxin production. Moreover, BAFF overexpression has been correlated with a number of autoimmune diseases, for example rheumatoid arthritis, lupus erythematosus and Sjögren syndrome (Mackay, Silveira et al. 2007, Cancro, D'Cruz et al. 2009, Mackay and Schneider 2009, McCarthy, Kujawa et al. 2011). Indeed, the concentration of circulating BAFF is increased in sera of patients with autoimmune diseases (Cheema, Roschke et al. 2001, Zhang, Roschke et al. 2001, Groom, Kalled et al. 2002). The generation of rheumatoid factors results from activation of autoreactive B cells that are normally present in healthy individuals but typically remain quiescent. IgG2a-chromatin immune complexes can activate these B cells through delivering simultaneous signals by both B-cell receptors and toll-like receptors (Leadbetter, Rifkin et al. 2002). BAFF, as a B-cell survival factor, may help this detrimental process.

Furthermore, not only in autoimmune diseases but also in some viral infectious diseases

such as HIV/SIV infection, an increased level of BAFF has been observed in chronically HIV-infected persons (Stohl, Cheema et al. 2002). In this study, enhanced BAFF levels were associated with levels of self-antibodies only in patients with more than 200 $CD4^+$ T cells per microliters. And in these cases, classical monocytes overexpressing mBAFF are identified as a major source of soluble BAFF. Another study also put forth evidence that elevated levels of serum BAFF exhibit a sustained increase from the acute phase of infection in rapid and normal progress in HIV-infected individuals (Fontaine, Chagnon-Choquet et al. 2011). In a cohort of untreated patients with primary HIV infection, circulating BAFF levels were also consistently enhanced at the diagnosis phase but rapidly decreased to baseline levels during two to three months of infection. Additionally, the expression of mBAFF is preferentially increased in $CD1c^+$ DC and $CD14^{lo}CD16^{hi}$ monocytes in patients with primary HIV infection, while in healthy persons mBAFF is mainly present in $CD14^+CD16^+$ intermediate monocytes (Borhis, Burelout et al. 2016). Moreover, researchers also find a transient increase in BAFF plasma levels within two weeks of infection in acutely SIV-infected macaques, and the level of BAFF is associated with total IgG levels, plasma viral titers and inversely with $CD4^+$ T-cell numbers (Chaoul, Burelout et al. 2012). Consequently, increased BAFF levels are a good predictor of infected disease progression early in the chronic phase (Poudrier, Soulas et al. 2015).

Since BAFF/BAFFR signaling is of demonstrated importance during the process of autoimmune diseases and infected diseases, numerous clinical therapeutic strategies have been developed according to this targeting. For example, anti-BAFF and anti-BAFFR monoclonal antibodies are currently being evaluated in patients with systemic lupus erythematosus. Belimumab, as a fully human IgG1 monoclonal antibody against these diseases, is the first new drug to be approved for the treatment of systemic lupus erythematosus in the past 50 years. The drug can effectively decrease the total number of peripheral B cells and the levels of immunoglobulin and improve disease activity through reduction in the frequency of lupus flares (Sun, Lin et al. 2008). In addition,

two more new BAFFR antagonists, BAFFR fusion protein-Briobacept and peptide fusion protein-A-623, have been developed in clinical trials (Levesque 2009). Furthermore, combined therapeutic strategy has been developed, such as incorporating the features of anti-CD20 and anti-BAFFR-Fc in one compound. Compared with animals treated with either anti-CD20 or anti-BAFFR-Fc alone, mice treated with this combined therapy display a considerable drop in the number of certain B-cell subsets and qualitatively distinct outcomes (Marathe, Iyer et al. 2012). In conclusion, further investigation should improve the ability to select and determine the therapy for specific patients. However, during viral infection increased BAFF levels may impact not only B-cell medicated autoimmunity, but also B cell-mediated effects on $CD169^+$ macrophages to enhance bystander activation. These $CD169^+$ macrophages are found in the marginal zone of the spleen and the subcapsular sinus and the medullar of lymph nodes. Because of the areas in which they are located, $CD169^+$ macrophages are critical in the regulation of immune activation. For instance, $CD169^+$ macrophages can mediate rapid and long-lasting interactions with invariant natural killer T cells (iNKTs) following administration of lipid antigens (Barral, Polzella et al. 2010). According to other study, it used liposomes decorated with glycan to determine that $CD169^+$ macrophages can also induce a similar enhanced activation of iNKT cells *in vivo* (Kawasaki, Vela et al. 2013).

Furthermore, $CD169^+$ macrophages are also associated with the maintenance of immunological tolerance. For example, apoptotic cells induce the expression of CCL22 in $CD169^+$ macrophages in the splenic marginal zone, leading to rapid follicular accumulation of Tregs and $CD103^{neg}$ DCs. Therefore, recruited Tregs could be activated through apoptotic cell antigens when presented by professional APCs or constitutively self-antigens presented to maintain tolerogenic stimulation (Ravishankar, Shinde et al. 2014). Another type of study on $CD169^+$ macrophages involving immunological tolerance found that $CD169^+$ macrophages from the spleen or lymph nodes are required for capturing exosomes, cell-derived vesicles (Saunderson, Dunn et al. 2014). However,

the mechanisms concerning the generation and maintenance of immunological tolerance have not been completely illuminated, but CD169$^+$ macrophages are still considered essential players.

Besides the key role in immunological tolerance, CD169$^+$ macrophages are also extremely important for anti-viral responses. Firstly, CD169$^+$ macrophages in the subcapsular sinus are the main type I interferon producers involved in the activation of NK cells (Ikezumi, Hurst et al. 2003). Moreover, the model of VSV infection also indicates that CD169$^+$ macrophages are significantly important for antiviral immune surveillance (Garcia, Lemaitre et al. 2012, Moseman, Iannacone et al. 2012). Marginal zone CD169$^+$ macrophages enable the capture of viruses and enforce virus replication in the presence of a type I interferon and overexpressed Usp18, which is a potent inhibitor of the type I interferon signaling pathway. The deficiency of either CD169$^+$ macrophages or *Usp18* results in impaired adaptive immunity to protect the body from VSV infection. This study indicates that enforced viral replication in CD169$^+$ macrophages is critical for the stimulation of an efficient adaptive immune response (Honke, Shaabani et al. 2011). Finally, another investigation also demonstrated enhanced CD169 expression on human CD14$^+$ monocytes in response to HIV-1 infection, which indicates that CD169 strongly binds HIV-1 and is involved in facilitating virus dissemination (Rempel, Calosing et al. 2008, Zou, Chastain et al. 2011).

According to the discussion of the role of BAFFR and CD169 in immune responses, the deficiency of BAFFR expression may affect innate immunity following infection. This is probably triggered by reduced production of lymphotoxin beta by B cells, and the lack of lymphotoxin beta also causes reduced presence of CD169$^+$ macrophages (Futterer, Mink et al. 1998, Tumanov, Kuprash et al. 2002, Moseman, Iannacone et al. 2012). Additionally, lymphotoxins are involved in regulating the production of innate type I interferons after viral infection (Benedict, Banks et al. 2001, Schneider, Loewendorf et al. 2008). This may partially be triggered by enforced viral replication

in CD169^{+} macrophages, which is also essential for stimulation of adaptive immune priming (Honke, Shaabani et al. 2011, Honke, Shaabani et al. 2013). Therefore, based on our data in this study, deletions in BAFFR may not only impair B-cell driven immunity, but also trigger defects in innate immunity. Further investigation may try to analyze the role of BAFFR deficiency in innate immunity of human patients.

In conclusion, our study has demonstrated that BAFFR deficiency leads to reduced enforced viral replication, limited type I interferon production and impaired adaptive immunity compared to wild-type animals. Consequently, BAFFR-deficient animals are predisposed to fatal VSV infections. It also indicates that the expression of BAFFR is significantly important for the activation of innate immune and antiviral responses.

In the second section of this thesis, we demonstrated the essential role of Qa-1b in inhibiting NK-cell activity after LCMV infection. Qa-1b-deficient animals display increased NK cell-mediated regulation of T-cell immunity and consequently enhanced viral replication and liver pathology. Depletion of NK cells could partially rescue T-cell responses and decrease liver pathology.

NK cells, as one of important subsets in the family of group 1 innate lymphocytes, have the ability to produce cytokines and kill the target cells. In their essential role in the innate immune system, NK cells can be activated by innate cytokines such as IL-2, IL-15, IL-18 and type I interferons (Gidlund, Orn et al. 1978, DeBlaker-Hohe, Yamauchi et al. 1995, Takeda, Tsutsui et al. 1998, Biron, Nguyen et al. 1999, Fehniger, Cai et al. 2007, Lucas, Schachterle et al. 2007, Kang, Liang et al. 2008). Furthermore, NK cells express different germ-line encoded activating and inhibitory receptors, which can balance the functions of NK cell during immune responses (Lanier 1998, Moretta, Bottino et al. 2001). Normally, healthy cells of the host express ligands for inhibitory receptors on NK cells to protect themselves from NK-mediated killing. Every nucleated cell expresses classical MHC-I molecules and can interact with the inhibitory receptors KIRs

in human and Ly49A, C or D in mice. In addition, the non-classical MHC-I molecule HLA-E in humans or Qa-1b in mice interacts with the inhibitory receptor CD94/NKG2A, leading to a suppressed state of NK cells (Pegram, Andrews et al. 2011, Vivier, Ugolini et al. 2012).

However, MHC-1 molecules on the surface of infected cells are downregulated, which can make them invisible to $CD8^+$ T cell; the reduced expression levels of MHC-I ligands for inhibitory receptors on NK cells sensitize these cells for NK cell-mediated killing (Diefenbach and Raulet 2001, Vivier, Ugolini et al. 2012). Moreover, DNA damage and tumorigenesis also can activate stress pathway and result in the upregulation of activating ligands and stimulating the cytotoxicity and cytokine production of NK cells (Vivier, Tomasello et al. 2008).

NK cells can impact T-cell functions in different states. For example, NK cells circulate in blood and enter into LN early after infections, and they can localize close to T cells in the LN to regulate T cells and then shape the ensuing size and quality of the T-cell responses (Fehniger, Cooper et al. 2003, Ferlazzo, Thomas et al. 2004, Martin-Fontecha, Thomsen et al. 2004). Additionally, through modulating DC function, NK cells can enhance T-cell responses. In humans, the crosstalk between NK cells and DCs can impact T cell differentiation. Specifically, NK cells can promote the differentiation of naïve $CD4^+$ T cells into IFNγ-producing Th1 T cells (Wehner, Lobel et al. 2009). NK cells also developed another mechanism to impact T-cell responses in the priming phase. NK cells kill the target cells which results in the release of antigens which can be taken up by DCs in order to present to T cells through MHC-I molecules, leading to an enhanced T-cell response. On the one hand, NK cells can improve the maturation and cross-interaction of DCs to stimulate T-cell immunity; on the other hand, NK cells also have negative impact on T cells during the priming state, which is mediated through either affecting T cells directly or indirectly though DC modulation. NK cells can kill mature APCs to lead to reduced antigen presentation and negatively impact T-cell immunity.

In mice, during LCMV infection, depletion of NK cells can enhance the capacity of APCs to stimulate $CD8^+$ T cells, probably because of the increased number of APCs (Cook and Whitmire 2013). Furthermore, *in vitro* and *in vivo* stimulated murine NK cells can acquire MHC-II proteins from interacting DCs to inhibit proliferation of $CD4^+$ T cells (Nakayama, Takeda et al. 2011).

In our study, Qa-1b, as an important ligand for NK cells, can interact with the inhibitory NK-cell receptor CD94/NKG2A as well as activate the NK-cell receptor CD94/NKG2C (Lanier, Corliss et al. 1998, Vance, Kraft et al. 1998). However, only NKG2A seems to be expressed in murine NK cells and activated T cells in contrast to NKG2C or NKG2E (Rapaport, Schriewer et al. 2015). But depletion of CD94 leads to increased susceptibility to ectromelia virus infection (Fang, Orr et al. 2011). NKG2A knockout animals are also susceptible to ectromelia virus infections, suggesting that the effects of CD94 deficiency are transmitted by CD94/NKG2A (Rapaport, Schriewer et al. 2015). In our research, hyperactive NK cells targeted virus-specific T cells after infection, which impaired anti-viral immunity. Notably, Qa-1b is expressed mainly on B cells following infection, indicating that B cells govern the equilibrium between regulatory NK-cell function and T-cell stimulation (Rapaport, Schriewer et al. 2015). Although NK-cell depletion could only partially restore anti-viral T-cell immunity during LCMV infection, NK-cell depletion rescued virus-mediated pathology, which suggests that NK cells are critical components in the effects observed with Qa-1b deficiency.

In humans, the role of NK cells during chronic viral infection and regulation of virus specific T cells remains poorly understood. Human chronic viral infections with hepatitis B or hepatitis C virus lead to induced cytotoxicity of NK cells (Oliviero, Varchetta et al. 2009, Ahlenstiel, Titerence et al. 2010, Rehermann 2013). This NK-cell activation may be triggered by type I interferon (Ahlenstiel, Titerence et al. 2010), which can be produced by pDCs or Kupffer cells after sensing HCV RNA (Takahashi, Asabe et al. 2010, Lau, Negash et al. 2013). During infection with hepatitis C virus,

expression of NK cell inhibitory receptors and their corresponding ligands correlates with elimination of HCV (Khakoo, Thio et al. 2004, Paladino, Flores et al. 2007, Knapp, Warshow et al. 2010). However, treatment with type I interferon results in immediate increases in alanine aminotransferase (ALT) levels caused by NK cell-mediated virus elimination, suggesting a protective role of NK cells during viral-induced hepatitis (Ahlenstiel, Edlich et al. 2011). Furthermore, the reaction of NK cells to interferon treatment can serve as an indicator of effectiveness in patients suffering from hepatitis C virus infection (Oliviero, Mele et al. 2013) and as a predictor of treatment outcome (He, Ji et al. 2006, Sarasin-Filipowicz, Oakeley et al. 2008). During HCV and HBV infection, the activating NK-cell receptors NKp30, NKp46, NKG2C, NKG2D, CD122, and the inhibitory receptor NKG2A are upregulated (Rehermann 2013). Interestingly, the HCV peptide aa35-44 stabilizes the NKG2A-binding HLA-E and consequently increases its expression and triggers inhibition of NK cells (Nattermann, Nischalke et al. 2005). There are two polymorphisms of HLA-E, which only differ in one amino acid, an arginine (HLA-E*0101, HLA-E^R) or a glycine (HLA-E*0103, HLA-E^G) in position 107 with increased surface expression of HLA-E^R polymorphism (Strong, Holmes et al. 2003). Interestingly, homozygous expression of HLA-E^R correlates with clearance of HCV genotype 2 and 3 (Schulte, Vogel et al. 2009). As suggested by our data, increased HLA-E expression might reduce NK-cell activity and promote virus specific T cell immunity.

Above all, this study identifies Qa-1b to be predominantly upregulated on B cells during infection with LCMV. Furthermore, lack of Qa-1b resulted in NK cell-mediated diminished anti-viral T cell immunity and increased pathology which was partially rescued with NK-cell depletion.

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