B cells modulate the homeostasis of splenic marginal zone antigen-presenting cells to promote anti-viral $CD8^+$ T cell responses

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Xinyuan Liu

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Abbreviations

Table 1: List of Abbreviations

Abbreviation	Name
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
APC	Antigen-presenting cells
CDC	Complement-dependent cytotoxicity
BM	Bone marrow
cDC	Conventional DC
CMV	Cytomegalovirus
CNS	Central nervous system
CSR	Class switch recombination
CTLs	Cytotoxic T cells
DT	Diphtheria toxin
DSS	Dextran sulfate sodium
ESAM	Endothelial cell specific adhesion marker
FCS	Fetal calf serum
GCs	Germinal centers
HCMV	Human cytomegalovirus
IFN –γ	Interferon–γ
Ig	Immunoglobulin
IL-6	Interleukin-6
IMS	Infected mouse serum
iNKT	Invariant natural killer T cells

Abbreviation	Name
I.P.	Intraperitoneal injection
KLRG1	Killer cell lectinlike receptor subfamily G member 1
LC	Langerhans cells
LN	Lymph nodes
LTβ	Lymphotoxin β
MACS	Magnetic-activated cell sorting
MCMV	Murine cytomegalovirus
MHC	Major histocompatibility complex
MZ	Marginal zone
NIK	NFκB inducing kinase
NK	Natural killer cells
NMS	Normal mouse serum
PAMPs	Pathogen-associated molecular patterns
PD-L1	Programmed death ligand 1
RP	Red pulp
PFU	Plaque-forming unit
scRNAseq	Single-cell RNA sequencing
SCS	Subcapsular sinus
SGV	Salivary gland-derived virus
SHM	Somatic hypermutation
TCR	T cell transgenic
TCV	Tissue culture virus
TFH	T follicular helper cells

Abbreviation	Name
Th	T helper cells
TLRs	Toll-like receptors
TNF –α	Tumor necrosis factor –α
t-SNE	t-distributed stochastic neighbor embedding
WP	White pulp

1 Introduction

1. 1 Cytomegalovirus

Cytomegalovirus (CMV) is a member of the Herpesviridae family has coevolved with humans for millions of years[1]. CMV are double-stranded DNA enveloped virus of about 200 kb in length that lead to lifelong infection in their hosts. The viral envelope is derived from the cell membrane, and at least eight different viral glycoproteins are embedded in the lipid bilayer (**Figure 1**)[2]. Mature virus particles are 150- 200nm in diameter[3]. CMV exhibits strict host specificity, such as human cytomegalovirus (HCMV) and murine cytomegalovirus (MCMV) infect humans and mice respectively[4]. Infection with HCMV is very common, with approximates to 60% of the population in developed countries and more than 90% of the population in many developing countries being seropositive for HCMV[5]. Like all herpesviruses, HCMV induces persistent infection, which can be divided into acute and latent phases, and which are respectively characterized by active replication and quiescence of the virus[6]. HCMV can only be transmitted during the active phase of the virus. HCMV infection can be spread from person to person through contact with the bodily fluids of an infected person, including blood, saliva, semen, breast milk, and urine. In other words, touching the eyes or nostrils, spreading it sexually, or passing it from mother to child through breast milk are the most common route of transmission of HCMV. In addition, HCMV is transmitted through blood transfusion and organ transplantation and can also transmit from mother to child through the placenta. The majority of HCMV-infected individuals do not develop any symptoms, but in individuals with an immature or dysfunctional immune system, such as transplant recipients[7], HIV-infected patients, cancer patients receiving cytoreductive therapy, and patients with immunosuppression, HCMV is still a major cause of morbidity and mortality[8]. Primary infection with HCMV or MCMV is controlled by the immune system, primarily cytotoxic T cells (CTLs) and natural killer (NK) cells [9-11]. After the primary infection is controlled, CMVs remain in reservoirs in a state of latency.

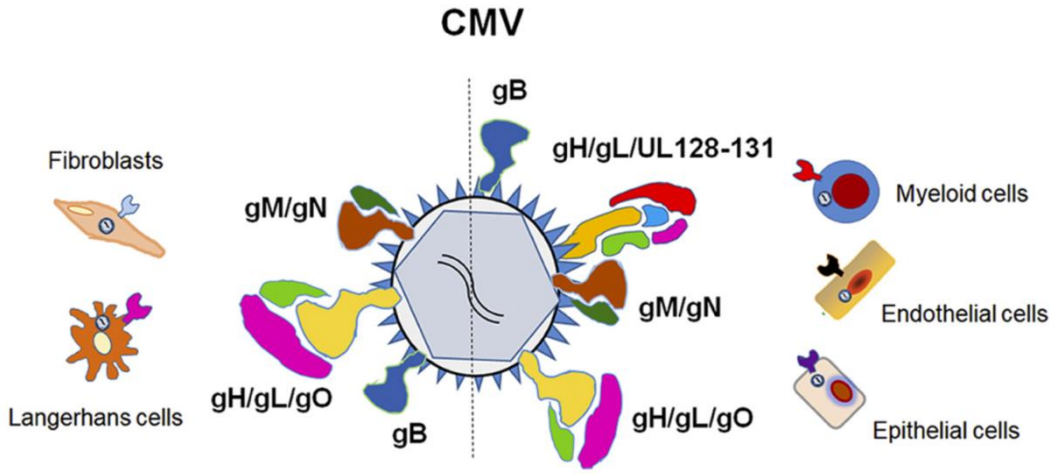

Figure 1: Structure of the cytomegalovirus (CMV). (Adapted from Sandonís, V., García-Ríos, E., et al[2].) CMV's external membrane contains numerous embedded glycoprotein complexes. The gCI complex contains gB, the gCII complex contains gM and gN, and the gCIII complex contains gH, gL, and gO. The pentameric complex is made up of the gH/gL heterodimer attached to three small glycoproteins encoded by UL128, UL130, and UL131.

1.1.1 Pathogenesis of MCMV Infection

CMV exhibits strict host specificity. Mice infected with MCMV are a widely accepted model for investigating HCMV infection since the two infections share similar characteristics. The MCMV infection mouse model has been widely utilized to study the pathogenesis of acute, latent, and recurring viral infections. Similar to HCMV infection, the virus source (tissue culture virus (TCV) and salivary gland-derived virus (SGV)), dose, route of inoculation, immune status of the mouse, age of inoculation, and genetic background of the mouse can all have a significant impact on how the disease manifests during MCMV infection. Adult immunocompetent mice are infected intraperitoneally or through the footpad with TCV (10^5 plaque-forming unit (PFU)), which is usually asymptomatic and does not result in significant organ system damage[12]. In contrast, even in fully immunocompetent hosts, injection of the same amount of SGV

causes damage to various organs and tissues as well as significant mortality[12]. Acute SGV infection is characterized by high levels of virus replication in the spleen and liver[13,14], along with loss of liver function and immunosuppression[15,16]. CMVs are significant opportunistic viruses that attack immunodeficient hosts but generally induce asymptomatic infection in immunocompetent hosts. For instance, HCMV infection causes a variety of clinical symptoms in AIDS patients, such as pneumonitis, hepatitis, retinitis, esophagitis, colitis, and encephalitis[17]. Similarly, TCV infection in immunocompromised mice correlates with increased virus replication and numerous organ damage, leading to severe morbidity and mortality[18]. In congenitally and perinatally newborns, HCMV infections are the primary cause of illness, mortality, and mental retardation[19]. HCMV can be spread from mother to fetus transplacentally during pregnancy. In contrast to HCMV, MCMV cannot be transmitted transplacentally, but it can be transmitted to newborn mice during delivery and later through breastfeeding and saliva. MCMV infection in newborn mice, in contrast to adult mice, is distinguished by virus dispersion throughout the central nervous system (CNS), which is associated with inflammatory alterations and developmental defects[20].

1.1.2 The Innate and Adaptive Response to CMV Infection

The innate immune system is triggered after identifying pathogen-associated molecular patterns (PAMPs), and involves the Toll-like receptors (TLRs) activation. The CMV glycoproteins B and H were discovered to bind to a heterodimer of TLR1 and TLR2, activating professional antigen-presenting cells dendritic cells to release inflammatory cytokines, and attracting NK cells[21]. During primary infection, adaptive immunity was triggered after the identification of CMV proteins, causing the activation and proliferation of functional CMV-specific T cells. APCs process and present CMV antigens in the major histocompatibility complex (MHC) class I, which activates $CD8^+$ T cells and causes them to secrete tumor necrosis factor (TNF) -α or interferon (IFN)-γ to inhibit intracellular virus replication, lyse virally infected cells via the secretion of granzymes and perforins (**Figure 2**) [2,22,23]. APCs present CMV antigens via the MHC

class II pathway and activate $CD4^+$ T cells. The activated $CD4^+$ T cells suppress intracellular virus replication via the release of IFN-γ and IL-2 and promote the proliferation of $CD8^+$ T cells and macrophages[24]. Furthermore, activated $CD4^+$ T cells also induce B cell activation, leading to the generation of CMV-specific antibodies against a variety of viral proteins. Another key function of antibodies is to attract complement to facilitate pathogen lysis after recognizing viral proteins on the surface of the virus or the target antigen expressed on the surface of the infected cell[25]. Alternatively, the infected cell can experience antibody-dependent cell cytotoxicity, promote pathogen phagocytosis, and regulate the downstream response of both the innate and adaptive immune responses[2,26,27].

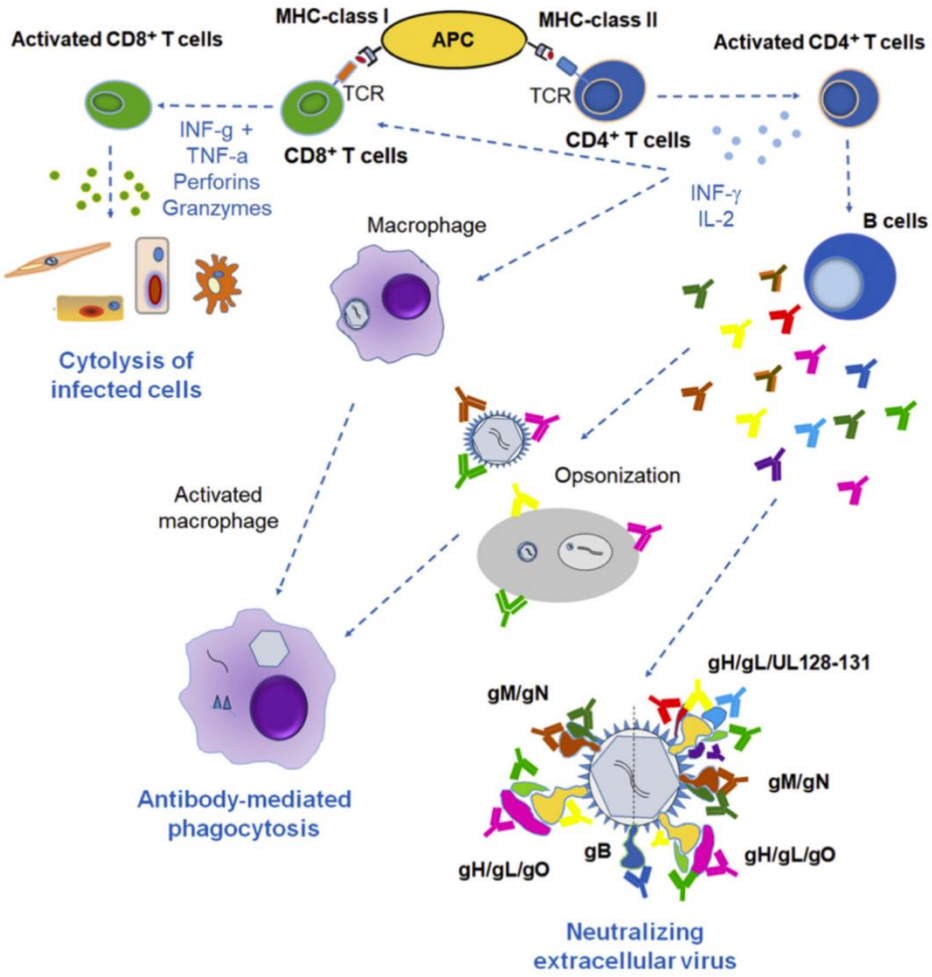

Figure 2: Adaptive Immune Response to CMV Infection. (Adapted from Sandonís, V., García-Ríos, E., et al[2].) APCs process and present CMV antigens in MHC class I, activating $CD8^+$ T cells to suppress intracellular virus replication or induce lysis of virus-infected cells. CMV antigen

presentation via MHC class II activates $CD4^+$ T lymphocytes to suppress intracellular viral replication. Activated $CD4^+$ T cells can also activate B cells, causing them to produce CMV-specific antibodies.

1.2 B cells

B cells are critical components of the adaptive immune system, playing important roles in host defense against infections. In addition to producing antibodies, B cells can also present antigen to T cells, produce proinflammatory cytokines, and secrete anti-inflammatory cytokines that suppress immunological responses. B cell development is accompanied by Immunoglobulin (Ig) gene rearrangements in the bone marrow, resulting in a heterogeneous pool of immature B cells. Those reactive with self-antigens are eliminated or functionally inactivated, and the remaining immature B cells move to the periphery to mature. Antigen stimulation, toll-like receptor signaling, and T cell assistance can activate and mature B cells that are present at the periphery. An essential step in initiating the activation of B cells is antigen binding to the BCR. B cells activated by antigen stimulation and present in the presence of T cells aid in the formation of germinal centers (GCs). In the GCs, B cells then go through Ig V gene somatic hypermutation (SHM) and class switch recombination (CSR) to develop into memory B or antibody-secreting plasma cells (**Figure 3a, 3b**,**3c**). The most well-known function of B cells is antibody production, but beyond that, B cells acting as antigen-presenting cells can uptake and process native antigens and then present degraded peptide fragments to $CD4^+$ T cells in combination with MHC class II molecules (**Figure 3d**). Co-stimulatory molecules, such as CD80, CD86, and CD40, can be expressed by B cells and increase the activation of proinflammatory T cells. In addition, B cells can also secrete a variety of cytokines[28], such as interleukin-6 (IL-6), lymphotoxin-α,and TNF, which help the proliferation and activation of proinflammatory IFN-γ-secreting cells. In contrast, some B cells, identified as B regulatory cells, produce anti-inflammatory cytokines such as IL-10[29,30] and IL-35[31](**Figure 3e**).

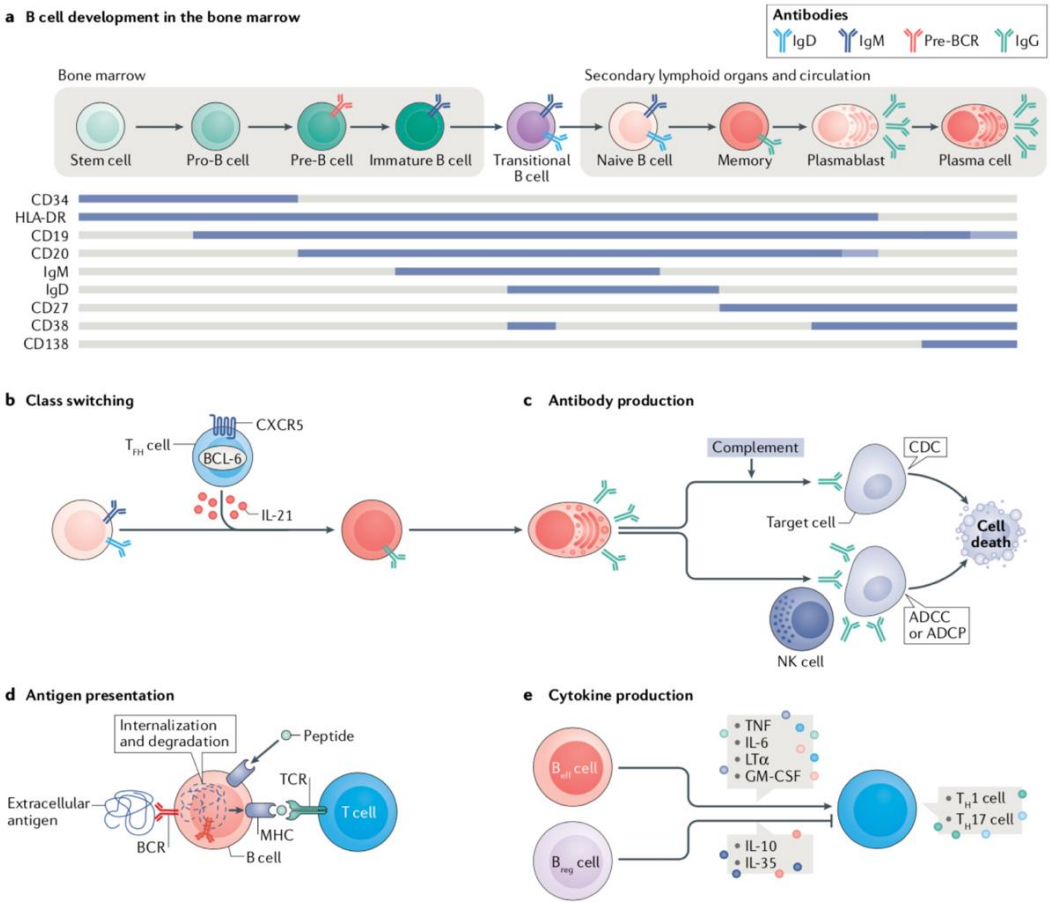

Figure 3: Development and functions of B cells. (Adapted from Sabatino, J. J., Jr., Pröbstel, et al[32].) B cells develop from bone marrow stem cells and undergo B cell receptor (BCR) rearrangement. Pre-B cells express a rearranged heavy chain and a surrogate invariant light chain (pre-BCR). Immature B cells start expressing IgM and leave the bone marrow, transforming into transitional B cells that express IgM with or without IgD. Naive B cells co-express IgM and IgD and can either stay in circulation or move to secondary lymphoid organs. When exposed to antigens, naive B cells differentiate into memory cells, plasmablasts, and ultimately plasma cells (**a**). T follicular helper cells secrete IL-21, which promotes the activation and class switching of naive B cells in lymphoid tissue. Activated B cells can differentiate into memory B cells, some of which develop into long-lived plasma cells. Memory B cells and plasma cells both produce antibodies that bind to antigens on cellular targets, resulting in CDC, ADCP, or ADCC (**b, c**). Through BCR-mediated internalization of extracellular antigens, B cells then processed and presented antigens to T cells through MHC class II molecules (**d**). B cells secrete various kinds of proinflammatory cytokines, leading to the polarization of type 1 T helper cells or IL-17-secreting T helper cells. Breg

cells produce anti-inflammatory cytokines such as IL-10 and IL-35, which may suppress proinflammatory T cells (**d**).

1.2.1 Role of B cells in Viral Infection

The activation of numerous immune cells, such as T cells and natural killer cells, is necessary for effective antiviral immunity. B cells have a unique role by producing antibodies that can both neutralize and eliminate viral particles before they enter the cell[33]. Protective antibodies are produced even before the first pathogen infection via the regulated secretion of so-called natural antibodies, which are produced even in the absence of past microbial exposure[34]. An early round of rapidly produced antibodies from extrafollicular responses relies on the earlier naive or memory repertoire of B cells to generate a powerful protective response that closely follows the clearance of acute infections[33]. Finally, the formation of GCs gives long-term protection by generating long-lived plasma cells and memory B cells, which alter and widen the B cell repertoire for more effective responses in the face of repeated exposures.

1.3 CD169$^+$ macrophages

Macrophages are found in a variety of tissues and organs throughout the body and exhibit a high degree of variability and diversity[35]. Several particular markers expressed on macrophage surfaces, such as F4/80, CD68, SRA1, and CD169, have been utilized to define distinct subsets[36].CD169$^+$ macrophages are a distinct subpopulation of macrophages that is different from M1 and M2 macrophages. CD169$^+$ macrophages have a distinct distribution, particularly in secondary lymphoid organs where blood and lymph enter and exit, such as the metallophilic marginal zone of the spleen, the subcapsular sinus and the medulla of the lymph nodes. CD169$^+$ macrophages have the distinct CD169 molecule on their surface[36]. In contrast to M1 and M2 macrophages, CD169$^+$ macrophages are able to interact directly to T cells, B cells, and dendritic cells

via CD169 molecules for involvement in immunological regulation[37]. $CD169^+$ macrophages are recognized as having essential roles in phagocytosis, antigen presentation[38], viral infection[39,40], inflammatory responses[41], and immune tolerance[42] due to their typical geographic distribution in vivo and the presence of the CD169 molecule on their surfaces. Simultaneously, it has been revealed that $CD169^+$ macrophages may play an important role in antitumor immunity[43,44].

1.3.1 Role of $CD169^+$macrophages in Viral Infection

The marginal zone of the mouse spleen has been proven to play a significant role in the host's resistance against pathogen infections throughout the last decade[45,46]. The primary cell type infected during viral infection is reported to be $CD169^+$ macrophages[47,48], which are able to capture particles of virus in the blood, take in antigens such as viruses and immune complexes, and then present them to follicular B cells, inducing germinal center B cellular responses[49]. In addition, $CD169^+$ macrophages use the CD169 molecule to transport antigens to $CD8a^+$ DCs, which preferentially engage in cell contact, subsequently eliciting an efficient $CD8^+$ T cell response[39]. Furthermore, it has been demonstrated that $CD169^+$ macrophages promote viral replication, resulting in the delivery of many viral antigens and the amplified activity of T and B cell responses[40,50]. Both in vivo and in vitro, type I IFN-induced macrophages express CD169 molecules[51]. During viral infection, $CD169^+$ macrophages can mediate antiviral action by producing type I IFN. Nevertheless, sustained secretion of IFN-I leads to upregulation of programmed death ligand 1 (PD-L1) expression on the surface of these $CD169^+$ cells, potentially leading to $CD8^+$ T cell exhaustion, and the exhaustion of $CD8^+$ T cells has both benefits and drawbacks. In vivo studies of lymphocytic choroidal meningitis virus infection have shown that continuous type I IFN expression led to elevated IL-10 and PD-L1 levels[52]. During chronic infection, type I IFN produced from $CD169^+$ macrophages suppresses the activation of the immune response to secondary infection[53]. However, the lack of

$CD169^+$ macrophages causes insufficient type I IFN production, which lowers antiviral activity and increases virus persistence in the human body.

1.4 Splenic cDC1

The spleen is the body's largest secondary lymphoid organ and is functionally connected to the systemic blood circulation. The spleen is histologically made up of red pulp (RP) and white pulp (WP). Because the spleen is not connected to the afferent lymphatic system, which transports migratory DC from the peripheral tissues to the LN, the splenic DC compartment only contains resident DC. Historically, splenic cDC were divided into at least three distinct subsets according to the expression of the CD8α homodimer or CD4 and CD11b: (1) a CD8-expressing $CD8^+CD11b^-$ cDC1 subset, and a $CD11b^+$ cDC2 subpopulation that can be further classified into (2) $CD4^+CD8^-$ DC and (3) $CD4^-CD8^-$ double-negative DC subsets. A vast majority of splenic $CD8^+$ cDC1 co-express the C-type lectin receptors DEC205 (CD205) and Langerin, according to flow cytometry analysis. At first, staining spleen sections for DEC205 only found $CD8^+$ cDC1 in the T cell-rich periarteriolar lymphoid sheaths[54-59], leading to the assumption that $CD8^+$ cDC1 was located solely in the WP[56,57,60-62]. By contrast, Langerin was found primarily in the MZ and only in small amounts in the RP and the T cell-rich periarteriolar lymphoid sheaths by histology[63-67]. $CD8^+$ cDC1 have a high ability to cross-present cell-associated and soluble Ag[68-73], and they primarily activate Th1-type helper T cell responses[73-75]and also regulatory T cell responses by Transforming Growth Factor (TGF). Furthermore, $CD8^+$ cDC1 is able to activate and polarize classical natural killer T cells through CD1d glycolipid Ag presentation[76].

1.4.1 Splenic $Langerin^+CD8^+$ cDC1

Langerin expression is a characteristic feature of Langerhans cells (LC) in the epidermis and skin-draining lymph nodes[64,77,78]. Nevertheless, Langerin expression is not limited to LC, it is also found in other skin DC subsets[79-82]. $Langerin^+$ DC are also identified as interdigitating cells in the T cell zones of the LN, as well as the gut and the lung[83-86]. In

the spleen, Langerin expression is mostly observed on $CD8^+$ cDC1, but its expression is lower than on LC and is primarily intracellular[87]. $Langerin^+CD8^+$ cDC1 are mostly found in the MZ, where they are interspersed with MZ macrophages and form a ring around the marginal metallophilic $CD169^+$ macrophages. A small amount of $Langerin^+CD8^+$ cDC1 can also be discovered in the RP and T cell-rich periarteriolar lymphoid sheaths[88]. $Langerin^+CD8^+$ cDC1 have a similar morphology to $Langerin^-$ counterparts, as well as a similar expression profile of conventional splenic cDC1 markers such as CD8, CD24, DEC205, CD36, ICAM, Clec9a, and XCR1. Additionally, $Langerin^+CD8^+$ cDC1 co-expresses high levels of the integrin CD103. Splenic cDC exhibit an immature phenotype in steady state and have low levels of MHCII and co-stimulatory molecules[89]. In comparison with $Langerin^-$cDC1, the $Langerin^+CD8^+$ cDC1 subpopulation had a slightly higher baseline expression of the activation markers CD80 and CD86[63,90,91]. Nevertheless, $Langerin^+CD8^+$ cDC1-depleted mice exhibited considerably lower steady-state levels of serum IL-12, showing that these DC are in charge of basal IL-12 production[92]. In conclusion, as a proportion of $CD8^+$ cDC1 in the splenic MZ, $Langerin^+CD8^+$ cDC1 are essential regulators of immune responses to blood-borne Ag in the steady state as well as during inflammation, based on their unique location and phenotypic features.

1.4.2 Role of $Langerin^+CD8^+$ DC During Systemic Infection

Langerin expression of cDC1 in the spleen identifies the cross-presenting $CD8^+$cDC1 subset. $CD8^+$ cDC1 are highly specialized cross-presentation cells that are the most potent providers of IL-12 in a variety of inflammatory situations[73,75,93-96]. NK cell responses and Th1 $CD4^+$T cell differentiation are both affected by the pro-inflammatory cytokine IL-12[97]. Upon systemic stimulation, $Langerin^+$ $CD8^+$ cDC1 produces large quantities of IL-12, whereas $Langerin^-CD8^+$ cDC1 produces less IL-12[92,98]. While $Langerin^-CD8^+$ cDC1 were required for early and transient IL-12 production, $Langerin^+CD8^+$ cDC1 were the primary source of IL-12 later on, at least until three weeks post-infection[93]. Neuenhahn et al. discovered that deleting

Langerin$^+$CD8$^+$ cDC1 reduced protective immunological responses to intravenous mycobacterium bovis infection[93]. Their findings also suggest that Langerin$^+$CD8$^+$ cDC1-depleted mice had higher bacterial burdens as a result of lower IL-12 production along with delayed and impaired CD8$^+$ T cell responses[93]. Invariant natural killer T (iNKT) cells have been linked to infectious illnesses, autoimmune diseases, and cancer. cDC is capable of activating iNKT cells, which in turn induce cDC to generate IL-12. Although Langerin$^+$CD8$^+$ cDC1 are not needed for iNKT cell activation[99], conditioning of Langerin$^+$CD8$^+$ cDC1 by these iNKT cells in conjunction with TLR stimulation synergistically increased cytokine production and maintained T cell priming capacities of Langerin$^+$CD8$^+$ cDC1[100]. To sum up, the main function of Langerin$^+$ CD8$^+$ cDC1 of the spleen during systemic infection is to clear bacteria and viruses and provide protective immunity.

1.5 Aim of this study

My thesis focuses on B cells and MCMV infection, hoping to shed light on how B cells play a role in MCMV infection. CMV is a member of the Herpesviridae family of double-stranded DNA viruses and has coevolved with humans for millions of years. In immune-suppressed hosts, such as transplant recipients, CMV infection can cause significant morbidity and mortality and therefore remains a major health hazard. Being popularly known as one essential immune cell population in the immune system, B cells can mediate humoral immune responses to protect against various viral infections. Surprisingly, increasing evidence also suggests that B cells play an important role in maintaining primary virus-specific CD8$^+$ T cell responses and mouse survival in various virus infection models, such as influenza viruses, lymphocytic choriomeningitis viruses, and vesicular stomatitis viruses[47,101]. However, how B cells influence virus-specific CD8$^+$ T cell responses during virus infection and the relevant mechanisms still remain unclear. In the previous study in our lab that stimulated the splenocytes of the

MCMV-infected mice with virus-specific peptides, in B-cell-deficient J_HT mice, we observed a significant decrease in the percentage of IFN–γ-producing $CD8^+$ T cells seven days-post infection, when compared to $CD8^+$ cells of infected control mice. Therefore, we concluded that B cells are needed for the establishment of the IFN-γ-secreting CTL compartment in the acute response. However, the previous research did not find a rational explanation for this interesting phenomenon. Therefore, in my thesis, I will continue this project in the hope of revealing the role of B cells in MCMV infection and better understanding the mechanisms by which B cells function during MCMV infection.

2 Material and Methods

2.1 Chemicals and buffers

Table 2: List of Chemicals

Chemical Name	Supplier
Agarose	Biozym, Germany
Ammoniumchloride (NH_4Cl)	Sigma-Aldrich, USA
Aqua (water)	B.Braun, Germany
Bovine Serum Albumin (BSA)	Sigma-Aldrich, USA
Collagenase Type IV	Worthington Biochemical, USA
Dimethylsulfoxide (DMSO)	Merck, Germany
DNase I	Roche, Switzerland
DPBS (1X)	Sigma-Aldrich, USA
Ethanol	Applichem, Germany
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich, USA
Fetal calf serum (FCS)	Boehringer GmbH, Germany
Gene ruler 100bp DNA ladder	Thermo Fisher Scientific, USA
HEPES	Gibco® Life technologies™,USA
Isoflurane	Abbott, Germany
Isopropanol	Applichem, Germany
Liberase™-TL	Roche, Switzerland
Lidocaine	Sigma-Aldrich, USA
MEM NEAA	Gibco® Life technologies™,USA
β- Mercaptoethanol (β-ME)	Fluka Chemie GmbH, Switzerland

Chemical Name	Supplier
Midori Green	Nippon Genetics GmbH, Germany
Penicillin/Streptomycin (P/S)	Gibco® Life technologies™,USA
Potassium bicarbonate ($KHCO_3$)	Sigma-Aldrich, USA
Proteinase K	Biozym, Germany
REDTaq® Ready Mix PCR	Sigma-Aldrich, USA
RPMI 1640	Gibco® Life technologies™,USA
Sodium Azide (NaN_3)	Applichem, Germany
Sodium chloride (NaCl)	Carl Roth, Germany
Sodium hydroxide (NaOH)	Merck, Germany
Sodium pyruvate	Gibco® Life technologies™,USA
Sodium dodecyl sulfate (SDS)	Serva Electrophoresis GmbH, Germany
Tissue freezing medium (Tissue Tek)	Leica, Germany
Tris base	Sigma-Aldrich, USA
Tris /HCl	Sigma-Aldrich, USA
Trypanblue	Gibco® Life technologies™,USA
Tween-20	Applichem, Germany

Table 3: List of Buffers

Buffer	Composition
Ammonium-Chloride-Potassium (ACK) lysis buffer -10X (pH 7.4)	150 mM NH_4Cl 100 mM KHCO3 10 mM EDTA

Buffer	Composition
FACS buffer I	PBS 1% BSA 0.1% NaN_3
FACS buffer II	PBS 1% BSA 0.1% NaN_3 20 mM EDTA
MACS buffer	PBS 0.5% BSA 2 mM EDTA
Tail-lysis buffer -10X	50 mM Tris-HCl (pH 8.0) 100 mM EDTA (pH 8.0) 100 mM NaCl 1% SDS
T cell culture medium	RPMI 1640 10% FCS 100 U/mL P/S 1% NEAA 1 mM Sodium pyruvate 1 mM HEPES 50 uM β-ME

Buffer	Composition
Tris-acetate-EDTA (TAE) buffer -50X (pH 8.3)	2 M Tris-HCl 2 M Acetic Acid 50 mM EDTA (pH 8.0)

2.2 Mouse strains

The mice used in this thesis were all on a C57BL/6J background. The following mice have been previously described: J_HT[102], OT-I[103] , C57BL/6J[104] ,CD45.1[105], and bred in our facility (Translational Animal Research Center of the University Medical Center Mainz) under specific pathogen-free (SPF) conditions. For OT-I adoptive transfer experiments, we crossed OT-I mice with CD45.1$^+$ mice to generate CD45.1$^+$OT-I mice. CD169-DTR mice were provided by Lang KS. Lang DTR mice were obtained from Probst HC, and LTβ BM cells were provided by Gommerman JL. For all experiments, mice of both sexes were used at adult age, except when indicated, the appropriate littermate mice were used as WT controls.

2.3 Molecular biology

2.3.1 Genomic DNA isolation

Toe biopsies from pups were digested overnight on the shaker with 500 µl of lysis buffer (50 mM Tris-HCl, pH 8; 100 mM EDTA, pH 8.0; 100 mM NaCl; 1% (w/v) SDS; 0.5 mg/ml proteinase K) at 56°C. Next, the DNA was extracted by adding 500 µl of isopropanol and centrifuging at 13,000 rpm for 15 min at 4°C. Then, discard the supernatant and wash the isolated DNA pellet with 500 µl of 70% (v/v) ethanol, centrifuged at 13,000 rpm for 10 min. Afterwards, discard the supernatant and let it dry at room temperature. After drying, DNA was resuspended in 200 µl of distilled water.

2.3.2 Polymerase chain reaction (PCR)

To determine the genotype of the mice, we prepared the master mix for each gene with 500 µl of REDTaq® Ready Mix PCR Reaction Mix (Sigma-Aldrich, USA), 5 µl of each primer (concentration: 100 µM; primer sequence is shown in Table 4), and 450 µl of water. PCR for each gene was prepared in a 20 µl volume system consisting of 19 µl master mix and 1 µl of DNA. The PCR amplification program is shown in Table 5.

Table 4: List of Primers

Primer Name	Sequence (5′-3′)	T_{ANN} (°C)
J_HT-1	CAG TGA ATG ACA GAT GGA CCT CC	60-63
J_HT-2	GCA GAA GCC ACA ACC ATA CAT TC	60
J_HT-3	CAC AGT AAC TCG TTC TTC TCT GC	63
OT-I-Tcra1	CAGCAGCAGGTGAGACAAAGT	62
OT-I-Tcra2	GGCTTTATAATTAGCTTGGTCC	62

Table 5: List of PCR programs

1				
J_HT WT (primer: J_HT-1+ J_HT-3)				
Step	Action	Temp (°C)	Time (s)	X cycles
1	Denaturation	95	300	
2	Denaturation	94	30	35
3	Annealing	63	45	
4	Amplification	72	120	
5	Amplification	72	600	

2				
J_HT Mut (primer: J_HT-1+ J_HT-2)				
Step	Action	Temp (°C)	Time (s)	X cycles
1	Denaturation	95	300	
2	Denaturation	95	30	35
3	Annealing	60	60	
4	Amplification	72	60	
5	Amplification	72	600	

3				
OT-I				
Step	Action	Temp (°C)	Time (s)	X cycles
1	Denaturation	94	180	
2	Denaturation	94	30	35
3	Annealing	62	60	
4	Amplification	72	60	
5	Amplification	72	120	

2.3.3 Agarose gel electrophoresis

After PCR, the size of the PCR product will be analyzed by running a 2% agarose gel. Agarose gel was prepared using agarose powder (Applichem, Germany) and TAE buffer (40 mM Tris-HCl, 40 mM Acetic Acid, and 1 mM EDTA), as well as adding Midori Green Advance (Nippon Genetics GmbH, Germany). Electrophoresis was carried out at a constant voltage of 120V in TAE buffer for 45 minutes. Meanwhile, Gene ruler 100-bp DNA ladder was added to indicate the size of the PCR product. Upon completion of electrophoresis, the PCR product can be visualized using UV light and the GelDoc imaging system (Gel Doc™, Bio-Rad, USA).

2.4 Cell Biology

2.4.1 Preparation of single cell suspensions

To analyze the DC subpopulation, we first collected spleens from mice, then the spleen was cut into grain-size pieces and shaken for 30 minutes at 37°C in 1 ml of Roswell

Park Memorial Institute (RPMI) 1640 media containing 200 U/ml Collagenase Type IV (Worthington Biochemical) and 0.5 U/ml DNaseI (Roche). After being digested, cell suspension was obtained by passing through a 70 μm cell strainer and washing in PBS with 10 mM EDTA and 2% Fetal Calf Serum (FCS), followed by centrifugation. The cell pellets obtained from centrifugation were incubated with 1ml of ACK lysis buffer (15 mM NH4Cl, 10 mM KHCO3, 1 mM EDTA) for 3 min, and then lysis was stopped by adding 10 ml of PBS containing 2% FCS and subsequent centrifugation. The cell pellets were resuspended in a suitable volume of PBS containing 2% FCS and kept on ice for subsequent analysis. Cell suspensions for analysis of the marginal metallophilic $CD169^+$ macrophages were purified from the spleen. The spleen was cut into small pieces in polypropylene tubes with 0.75 ml of RPMI that included 4 mg/ml Lidocaine (Sigma). In addition, 0.25 ml of RPMI containing 4 WU/ml of Liberase TL (Roche) and 2 U/ml of DNaseI (Roche) was added. Samples were then incubated at 37°C with constant stirring for 15 minutes until digested. After being digested, cells were passed through a 70 μm cell strainer, washed once with ice-cold RPMI plus 10% FCS, 20 mM Hepes, 10 mM EDTA, and 50 μM 2-mercaptoethanol, followed by centrifugation and lysis with ACK lysis buffer. Lymphocytes from OT-I mice were isolated from the spleen and lymph nodes. Spleen and lymph nodes were collected and pushed gently through a sterile 40 μm cell strainer, followed by centrifugation and lysis with ACK lysis buffer.

2.4.2 Sorting cells with the magnetic activated cell sorting (MACS) kit

To purify DC, we used anti-CD11c MACS microbeads (Miltenyi Biotec). For the isolation of B cells from WT mice, we used anti-CD19 MACS microbeads (Miltenyi Biotec). To purify $CD8^+$ T cells, we used the $CD8a^+$ T Cell Isolation Kit (Miltenyi Biotec). Overall, the cell populations we need will be labeled (positive selection) or unlabeled (negative selection) with the appropriate antibodies conjugated by MicroBeads. After incubation, labeled cells are separated from unlabeled cells by the

MACS separator and MACS columns. All operations were performed according to the manufacturer's protocol, and the purity of the cells was checked by FACS.

2.4.3 Sorting cells with FACS

In order to obtain more specific $XCR1^+$ $CD8^+$ cDC1, we first purified DC through MACS sorting, then purified $CD11c^+$ cells were stained for CD8a, CD11c, MHCII, XCR1, SIRPα, and viability dye. Subsequently, the ARIA III cell sorter (BD Biosciences) was used to sort cells based on the strong expression of CD11c and MHCII, as well as the presence of XCR1 and CD8a. After ARIA III sorting, the cells are reconfirmed to have > 95% purity and viability.

2.4.4 Cell counting

The cell number of the single-cell suspension was counted by the LUNA-II automated cell counter (Logos Biosystems, South Korea). First, dilute a small part of the removed cell suspension with Trypan blue solution (Gibco® Life technologies™,USA), which can stain dead cells (live cells cannot be stained), then take 10 μl of diluted cell suspension and add it to the slide, put the slide into the LUNA-II automated cell counter, wait for a few tens of seconds, and the number of live cells per ml will be displayed on the screen.

2.4.5 Flow cytometry

For FACS analysis with 2 million cells per sample. Single cells were suspended in fluorescence-activated cell sorting (FACS) buffer I (PBS, 1% BSA, 0.1% NaN3) and blocked with anti-mouse CD16/32 (BioLegend) for 15 min at 4°C. After blocking, cells were stained with the appropriate surface antibodies at 4°C in the dark for 20 minutes. MCMV-specific $CD8^+$ T cells were stained with M38 or M45 tetramer at 4 °C in the dark for 40 min. Langerin (CD207) requires staining at 4°C for 1 hour or overnight after fixation and permeabilization using the FoxP3 staining kit (Invitrogen) according

to the manufacturer's instructions. Flow cytometry data was required by FACS Canto II (BD Biosciences) or FACS Symphony (BD Biosciences) and analyzed using FlowJo v10 software.

Table 6: List of FACS antibodies

Specificity	**Conjugate**	**Clone**	**Supplier**
CD4	PerCP	GK1.5	BioLegend
CD8a	PE-Cyanine	53-6.7	BioLegend
CD8a	Brilliant Violet 510	53-6.7	BioLegend
CD11b	Brilliant Violet 605	M1/70	BD Biosciences
CD11c	APC	HL3	BD Biosciences
CD11c	APC-R700	N418	BD Biosciences
CD19	PE-Cyanine7	6D5	BioLegend
CD19	PE-Cyanine5	1D3	BD Biosciences
CD44	PerCP-Cyanine5.5	IM7	BioLegend
CD45	Brilliant Ultra Violet 805	30-F11	BD Biosciences
CD45.1	FITC	A20	BioLegend
CD62L	APC	MEL-14	BioLegend
CD64	Brilliant Ultra Violet 737	X54-5/7.1	BD Biosciences

Specificity	Conjugate	Clone	Supplier
CD103	PerCP-Cyanine5.5	2 E7	BioLegend
CD135	Brilliant Violet 421	A2F10.1	BD Biosciences
CD169	PE	SER-4	ThermoFisherScientific
CD172a	PerCP-eFluor 710	P84	eBioscience
CD172a	PE/Cyanine7	P84	BioLegend
CD207	ALEXA 647	122D5.03	Eurobio Scientific
ESAM	PE	1G8/ESAM	BioLegend
F4/80	Brilliant Blue 790	T45-2342	BD Biosciences
FceRIa	PE/Dazzle 594	MAR-1	BioLegend
Ly-6C	FITC	AL-21	BD Biosciences
Ly-6G	Brilliant Violet 750	1A8	BD Biosciences
MHCII (I-A/I-E)	eFluor 450	M5/114.15.2	eBioscience
MHCII (I-A/I-E)	Brilliant Violet 786	M5/114.15.2	BD Biosciences
TCR V alpha 2	FITC	B20.1	eBioscience
TCR Vbeta5.1 5.2	PE	MR9-4	BioLegend
XCR1	Brilliant Violet 510	ZET	BioLegend
XCR1	Brilliant Violet 650	ZET	BioLegend

2.4.6 DC phagocytosis assay

$CD11c^+$ DC were isolated by MACS and diluted in medium to a concentration of 1.5 $x10^6$ cells/ml, and 100 µl of cells were plated in a 96-well round bottom plate. OVA-488 of the gradient concentration diluted with PBS (0 µg/ml, 0.3 µg/ml, 1 µg/ml, 3 µg/ml, 5 µg/ml, 10 µg/ml, 30 µg/ml, and 60 µg/ml) was added to the $CD11c^+$ DC and incubated for 30 minutes at 37 °C in the cell culture incubator. The plate of an identical assay was kept on ice as a negative control. Excess fluorochrome bound to the cell surface was quenched by adding 0.1% Trypan blue stain and incubating for three minutes at room temperature. Afterwards, the cells were washed three times with PBS and then stained for flow cytometry.

2.4.7 DC processing assay

$CD11c^+$ DC were isolated by MACS and diluted in medium to a concentration of 1.5 $x10^6$ cells/ml, and 100 µl of cells were plated in a 96-well round bottom plate. OVA-DQ was diluted in medium to a concentration of 2 µg/ml or 10 µg/ml. Diluted OVA-DQ was incubated with $CD11c^+$ DC in a 37 °C cell culture incubator according to the following time points: 0 (OVA-DQ was added right before centrifugation), 30, 60, 90, and 120 minutes. The plate of an identical assay was kept on ice as a negative control. After incubation, the cells were centrifuged and stained for flow cytometry.

2.4.8 Co-culture experiments

OVA-specific T cell transgenic (TCR) $CD8^+$ T cell isolation was performed on the spleen and peripheral LNs from OT-I mice using the Mouse $CD8a^+$ T Cell Isolation Kit (Miltenyi Biotec) according to the manufacturer's instructions. For the cDC1/OVA or SIINFEKL/OT-I co-culture experiment, purified $4x10^4$ $XCR1^+$ cDC1 was incubated with OVA or SIINFEKL peptide for 60 min at 37°C in the cell culture incubator; subsequently, $XCR1^+$cDC1 were vastly washed and cultured with $1x10^5$ cell trace Cell

Trace Violet (ThermoFisher) labeled OT-I $CD8^+$ T cells. After 3 days, the proliferation of OT-I $CD8^+$ T cells was analyzed by flow cytometry.

In the MCMV-SIINFEKL-infected cDC1 and OT-I $CD8^+$ T cell co-culture experiment, purified $XCR1^+$cDC1 from the spleen 18 hours after MCMV-SIINFEKL infection was cultured with cell trace violet (ThermoFisher) labeled OT-I $CD8^+$ T cells with different ratios. The dilution of the cell dye was analyzed by flow cytometry on day 3 of culture.

For the MCMV-SIINFEKL-infected B cells and OT-I $CD8^+$ T cell co-culture experiment, B cells were purified 18 hours after MCMV-SIINFEKL infection in WT mice using the MACS CD19 microbeads (Miltenyi Biotec) according to the manufacturer's protocol. Purified B cells were cultured with cell trace violet (ThermoFisher) labeled OT-I $CD8^+$ T cells with different ratios. After 3 days, the proliferation of OT-I $CD8^+$ T cells was analyzed by flow cytometry.

2.5 Immunofluorescence

Whole spleens were cut into 7-mm sections with a cryostat. Sections were dried overnight at room temperature, then stored at -80 degrees until staining began. Non-specific staining was blocked using PBS plus 5% normal goat serum and 0.5% Triton-100 for 1 hour at room temperature, then sections were stained at 4 °C overnight with the primary antibodies. The next day, sections were washed and further labeled with the appropriate secondary antibodies for 1 hour at RT, followed by mounting with Fluoroshield mounting media with DAPI (Sigma-Aldrich). Images were captured by whole-slide scanning with a Zeiss SP8 and analyzed using Fiji software.

Table 7: List of Immunofluorescence primary antibodies

Specificity	Clone	Supplier
B220	RA3-6B2	BD Biosciences
CD169	MOMA-1	BMA BIOMEDICALS
GFP	N/A	Novus

Table 8: List of Immunofluorescence secondary antibodies

Specificity	Conjugate	Supplier
Donkey Anti-Chicken IgY (H+L)	CF488A	Sigma-Aldrich
Goat Anti-Rat IgG (H+L)	CF555	Sigma-Aldrich

2.6 Mouse experiments

2.6.1 MCMV infection

Mice were infected via left hind footpad with $1x10^5$ plaque-forming units (PFU) of cell-culture-derived MCMV (strain Smith) or intraperitoneal injection (I.P.) with the $2x10^5$ PFU of MCMV-SIINFEKL or I.P. with the $1x10^6$ PFU of MCMV-EGFP.

2.6.2 OT-I $CD8^+$ T cells adoptive transfer

OVA-specific T cell transgenic $CD8^+$ T cell isolation was performed on the spleen and peripheral LNs from $CD45.1^+$ OT-I mice using the Mouse $CD8a^+$ T Cell Isolation Kit (Miltenyi Biotec) according to the manufacturer's instructions. After FACS testing for

purity, CD45.1$^+$ OT-I CD8$^+$ T cells were washed in PBS, counted, and adjusted to the appropriate concentration. 5x10^6 CD45.1$^+$ OT-I CD8$^+$ T cells in PBS were injected into gender-matched host mice via the tail vein. Recipient mice were infected by intraperitoneal injection with 2x10^5 PFU of MCMV-SIINFEKL after 24 hours. Spleens were removed 3 days after infection, and the proliferation of transferred CD45.1$^+$ OT-I CD8$^+$ T cells were analyzed using FACS.

2.6.3 B-cell transfer experiment

B cell isolation was performed from WT mice using the MACS CD19 microbeads (Miltenyi Biotec) according to the manufacturer's instructions. After FACS testing for purity, B cells were washed in PBS, counted, and adjusted to the appropriate concentration. 50x10^6 B cells in PBS were intravenously (i.v.) injected into the tail vein of gender-matched host J_HT mice. After 7 days, spleens from reconstructed J_HT mice were taken to be analyzed by FACS.

2.6.4 Bone marrow chimeras

To generate B cells lacking Ltβ expression mice, lethally irradiated C57BL/6J recipients were reconstituted with 80% J_HT bone marrow plus 20% Ltβ$^{-/-}$ bone marrow. Controls were created by reconstituting lethally irradiated C57BL/6J recipients with 80% J_HT bone marrow plus 20% WT bone marrow. Bone marrow cells were from the tibia and femur of mice, and bone marrow cells were flushed out of the bone with 5 ml of cold PBS plus 2% FCS using a 5 ml syringe with a 24 G needle. The obtained single-cell suspension was passed through a 40 µm cell strainer, and the erythrocytes were removed with ACK lysis buffer. Bone marrow cells diluted to the appropriate concentration were injected via the tail vein into lethally irradiated C57BL/6J recipients. The recipient mice received antibiotic drinking water starting 10 days before being lethally irradiated and continuing for 6 weeks following bone marrow reconstitution. 8 weeks after reconstitution, mice were infected with MCMV and analyzed.

2.7 Bulk RNA-Sequencing and analysis

Complementary DNA (cDNA) was generated from MCMV-M45 tetramer^{+} CD8^{+} T cells using the Smart-Seq V4 Ultra Low Input RNA Kit (Takara). The amplified cDNA was fragmented, and libraries were made using the Nextera-XT DNA Sample Preparation Kit (Illumina) according to the manufacturer's instructions. The quantity of the cDNA libraries was assessed with the Qubit flex fluorometer (Invitrogen) and the average library size was determined using Agilent's 2100 Bioanalyzer HS DNA assay. Quantified libraries were sent to Novogene (Cambridge, UK) and sequenced on NovaSeq 6000 (Illumina) to generate around 30 million PE150 reads for each sample. The sequence reads were trimmed for adaptor sequences before being analyzed with Qiagen's CLC Genomics Workbench (v23.0.2 with CLC's default settings for RNA-Seq analysis). Raw RNA sequencing reads were mapped to the reference genome GRCm38.

2.8 Statistical Analysis

Data in all experiments are presented as the mean ± SEM. Statistical analyses were assessed with two-tailed, unpaired Student's t test to compare two groups or one-way ANOVA to compare multiple groups. $P < 0.05$ was considered statistically significant. Significance is indicated as: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, and ns (non-significant). Statistical analyses were performed using GraphPad Prism (version 9.4.0).

3 Results

3.1 B cells enhance primary CD8$^+$ T cell responses to MCMV infection

B cells are at the core of the adaptive humoral immune system, mediating the generation of antigen-specific Ig directed against invading pathogens. Interestingly, previous studies in many models have extensively shown that the presence of B cells has an effect on the initial CD8$^+$ T cell response, including influenza, modified vaccinia virus Ankara (MVA) infection[106], and subunit vaccination[107].However, there are still a lot of questions waiting to be answered, such as the mechanism.

In order to better understand the involvement of B cells in the virus-specific primary CD8$^+$ T cell response, we first infected control and B cell-deficient J_HT mice with MCMV. After 7 days, we found a reduction in MCMV tetramer-positive CD8$^+$ T cells in the spleen of J_HT mice compared to control mice (**Figure 4A and 4B**). Similar to the phenotype in the spleen, we also observed attenuated MCMV tetramer-positive CD8$^+$ T cells in the blood of J_HT mice (**Figure 4C**). In addition, we stimulated the splenocytes of the infected mice with the previously reported MCMV immunodominant peptides[108]. To test for the function of CD8$^+$ T cells, we measured their capacity to produce IFN-γ as the best proxy for their activity. In J_HT mice, we observed a significant decrease in the percentage of IFN-γ-producing CD8$^+$ T cells when compared to the cells of the infected control mice (**Figure 4D**). To test if the decreased primary CD8$^+$ T cell response was due to the lack of antibodies in the J_HT mice, we also infected IgMi mice with MCMV. These mice, as we reported previously, have a polyclonal population of B cells, but they are not able to class switch or produce secreted antibodies[109]. Interestingly, we found that the percentage of IFN-γ-producing CD8$^+$ T cells in the IgMi mice is not different as compared to control mice (**Figure 4D**), suggesting that secreted antibodies do not play a role in the primary CD8$^+$ T cell response. Collectively,

we concluded that B cells are important for maintaining MCMV-specific primary CD8$^+$ T cell responses, and this regulation is independent of the antibodies secreted by B cells.

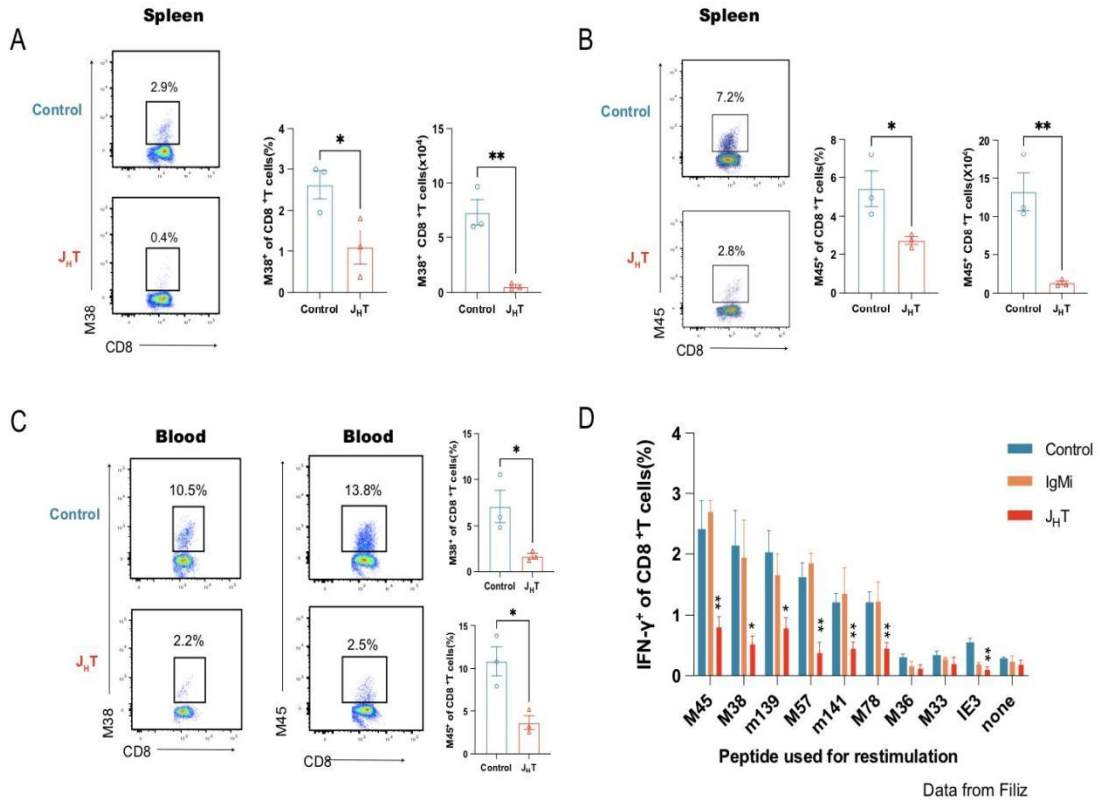

Figure 4: B cell deficiency attenuates primary CD8$^+$ T cell responses to MCMV infection. (**A**) Representative flow cytometry dot plots of MCMV-M38 tetramer$^+$ CD8$^+$ T cells in the spleens of control and J_HT mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of MCMV-M38 tetramer$^+$ CD8$^+$ T cells (n = 3). (**B**) Flow cytometry dot plot showing MCMV-M45 tetramer$^+$ CD8$^+$ T cells in the spleens of control and J_HT mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of MCMV-M45 tetramer$^+$ CD8$^+$ T cells (n = 3). (**C**) Flow cytometry dot plot showing MCMV-M38 tetramer$^+$ CD8$^+$ T cells (left) and MCMV-M45 tetramer$^+$ CD8$^+$ T cells (right) in the blood of control and J_HT mice. Bar graphs indicate the frequency of MCMV-M38 tetramer$^+$ CD8$^+$ T cells (top) and the frequency of MCMV-M45 tetramer$^+$ CD8$^+$ T cells (bottom) (n = 3). (**D**) Frequency of IFN-γ-producing CD8$^+$ T cells after stimulation with different dominant MCMV peptides at 7 days post infection of control, J_HT, or IgMi mice (n = 3-12). Differences in Figure 4D come from comparing each group to the control.

Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure 4A- 4C. *P < 0.05 and **P < 0.01. one-way ANOVA, Figure 4D. *P < 0.05, **P < 0.01.

3.2 B-cell deficiency has no effect on CD8$^+$ T cell function

Given that the percentage of IFN-γ-producing CD8$^+$ T cells in J_HT mice showed a significant decrease, we wondered whether the absence of B cells leads to alterations in the CD8$^+$ T cells themselves, for example, for their effector function. To test this possibility, we first analyzed CD8$^+$ T cells in the steady state, and we found that the frequencies and numbers of CD8$^+$ T cells were not changed in the spleen of J_HT mice when compared to control mice (**Figure 5A**). Next, we sorted equal numbers of MCMV tetramer-positive CD8$^+$ T cells and subjected them to deep RNA sequencing to determine the molecular pathways resulting in the defective CD8$^+$ T cell responses. The RNA-sequencing data showed that 1018 genes were up- or down-regulated at least twofold in the splenic MCMV tetramer-positive CD8$^+$ T cells isolated from J_HT mice as compared to the control splenic MCMV tetramer-positive CD8$^+$ T cells (**Figure 5B**). Nevertheless, when we analyzed the MCMV-specific CD8$^+$ cells of the J_HT mice for molecules related to anti-viral activity of T cells, such as CD28, KLRG1, perforin, granzyme B, and IFN-γ[110], we observed no differences or even increased expression of these molecules in comparison with CD8$^+$ T cells of control mice (**Figure 5C**), suggesting that CD8$^+$ T cells in J_HT mice are able to develop into effector CD8$^+$ T cells after recognizing MCMV antigen and that the decrease in primary CD8$^+$ T cell response in J_HT mice is not attributable to alterations in CD8$^+$ T cells themselves. The increased expression of effector molecules such as perforin and granzyme B in virus-specific CD8$^+$ T cells of the J_HT mice may be due to the greater infectious load as well as the reduced number of virus-specific CD8$^+$ T cells, which require that the CD8$^+$ T cells that have already recognized the antigen compensate for the generation of more fully armed effector cells.

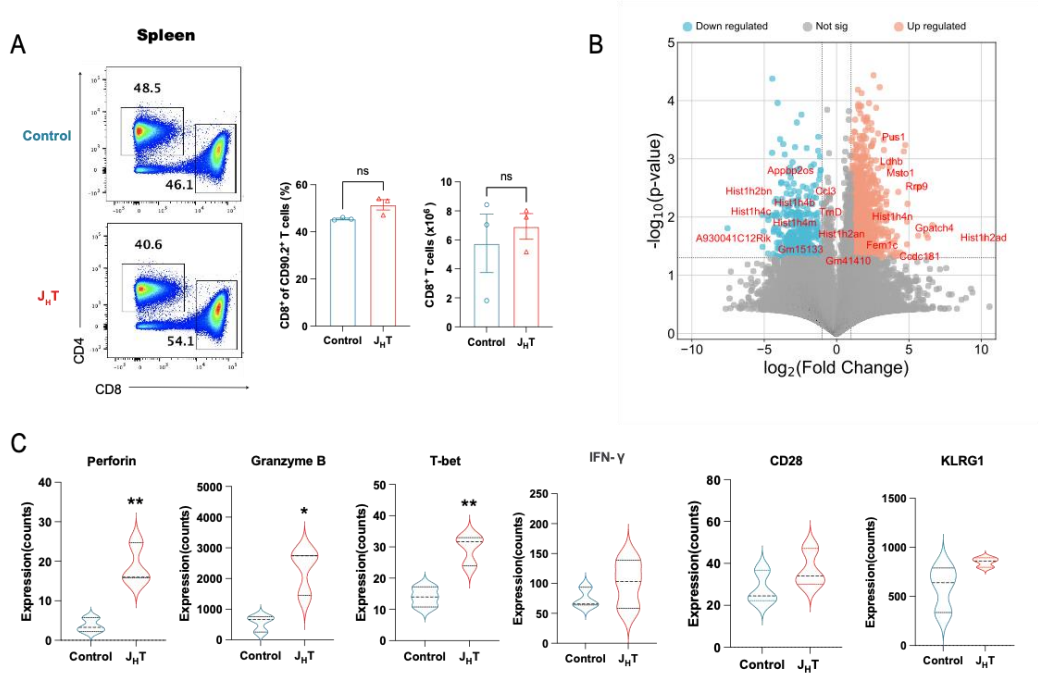

Figure 5: Loss of B cells does not affect CD8⁺ T cell function. (A) Flow cytometry dot plot showing splenic CD8⁺ T cells in control and J_HT mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of CD8⁺ T cells (n = 3). (**B**) Volcano plots depicting differentially expressed genes (DEGs) in splenic MCMV-M45 tetramer⁺ CD8⁺ T cells of J_HT versus control mice (P value versus log2 fold change). The top 10 upregulated and downregulated DEGs were shown. Genes with corrected P-value <0.05 and log2 [fold change] > 1 or < -1 were considered differentially expressed. **(C)** Violin plots showing the expression of selected genes associated with anti-viral activity of T cells in splenic MCMV-M45 tetramer⁺ CD8⁺ T cells. Data are presented as the mean ± SEM. Bulk RNA sequencing data are from three control and three J_HT mice. Statistical analysis: Student's t test, Figure 5A. ns (non-significant).

3.3 The absence of B cells leads to a decrease in virus-specific cytotoxic T lymphocyte prime

Changes in the function of antigen-presenting cells (APCs) have been linked to the virus-specific primary CD8⁺ T cell response. To test if alternations in the function of these cells is responsible for the reduction in MCMV-specific CD8⁺ T cells in the J_HT

mice, we adoptively transferred Cell Trace Violet-labeled CD8$^+$ T lymphocytes from OVA-specific TCR OT-I mice into J_HT or control mice. A day later, we infected the mice with MCMV-SIINFEKL. At 3 days post-infection, we assessed the proliferation of donor OT-I CD8$^+$ T cells (expressing Vα2 Vβ5 TCR) from the recipient mice's spleen by flow cytometry (**Figure 6A**). When compared to uninfected mice, infected control mice showed strong OT-I CD8$^+$ T cell expansion, whereas transferred OT-I CD8$^+$ cells in J_HT mice expanded significantly less (**Figure 6B**). Furthermore, we also observed a reduction in the percentage and the number of CD62L$^-$CD44$^+$ effector CD8$^+$ T cells in the OT-I CD8$^+$ T cells transferred to B cell-deficient mice as compared to control mice (**Figure 6C**). These results suggest that T cells cannot be properly activated in the J_HT mice, possibly due to the lack of functional APC in these mice.

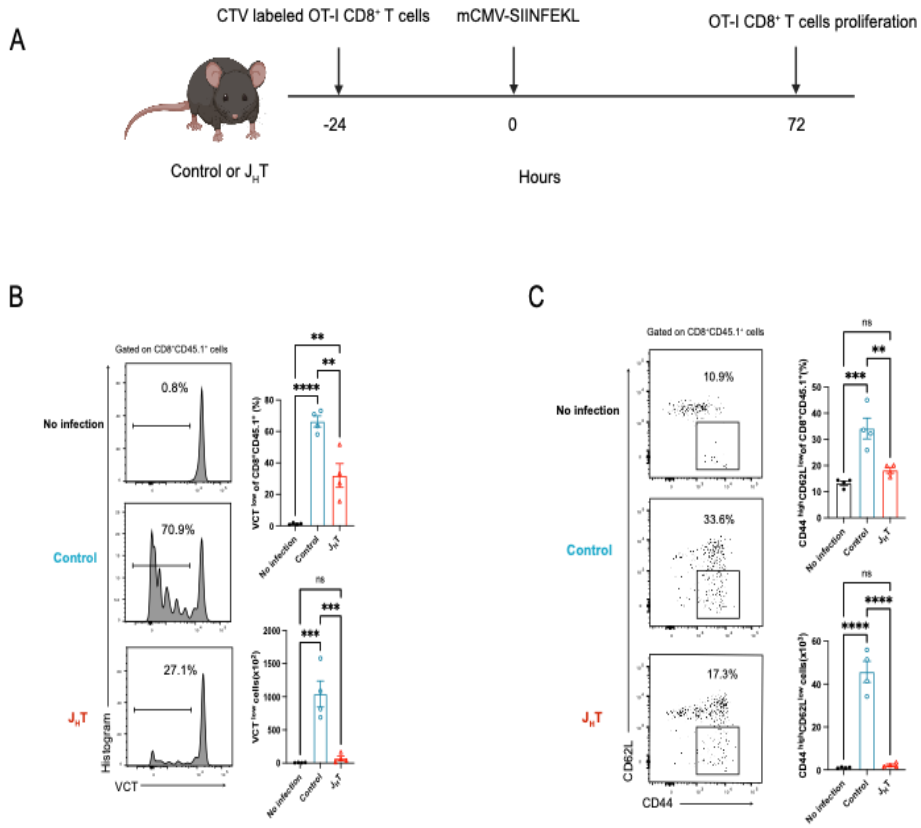

Figure 6: Diminished Virus-Specific Cytotoxic T Lymphocyte Priming in J_HT Mice. (A) Schematic of infecting control or J_HT mice with MCMV- SIINFEKL 24 h after intravenous transfer of 5 × 10^6 OT-I CD45.1$^+$ CD8$^+$ T cells. **(B)** Proliferation of adoptively intravenous transferred OVA-

specific transgenic OT-I CD45.1$^+$ CD8$^+$ T cells at 3 days after MCMV-SIINFEKL infection. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of proliferating transferred CD45.1$^+$ OT-I CD8$^+$ T cells (n = 4). **(C)** Flow cytometry dot plot showing CD62L$^-$CD44$^+$ effector OT-I CD45.1$^+$ CD8$^+$ T cells at 3 days after MCMV-SIINFEKL infection. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of CD62L$^-$CD44$^+$ effector OT-I CD45.1$^+$ CD8$^+$ T cells (n = 4). Data are presented as the mean ± SEM. Statistical analysis: one-way ANOVA, Figure 6B and 6C. **P < 0.01, ***P < 0.001, ****P < 0.0001, and ns (non-significant).

3.4 B cells fail to directly cross-present viral particles to CD8$^+$ T cells

Based on the phenotypes we observed, we next had to focus on the antigen-presenting cells of the J_HT mice. The B cells themselves are one potential APC candidate. B cells that express both MHC class I and class II can interact directly with T cells, a process that is critical in the germinal centers[111,112]. To test whether the decreased CD8$^+$ T cell priming observed in J_HT mice was due to the lack of B cells to do antigen presentation, we purified B cells from MCMV-SIINFEKL-infected WT mice and then cultured them with Cell Trace Violet-labeled OT-I CD8$^+$ T cells for 3 days (**Figure 7A**). We found that the B cells isolated from MCMV-SIINFEK-infected mice were not able to induce OT-I CD8$^+$ T cell expansion, indicating that B cells aren't the primary antigen-presenting cells that directly cross-present antigens to CD8$^+$ T cells after MCMV infection (**Figure 7B**). These results support the conclusion that defective CD8$^+$ T cell responses after viral infection in the J_HT mice are not attributable to B cells acting as APCs, which directly induce CD8$^+$ T cell responses.

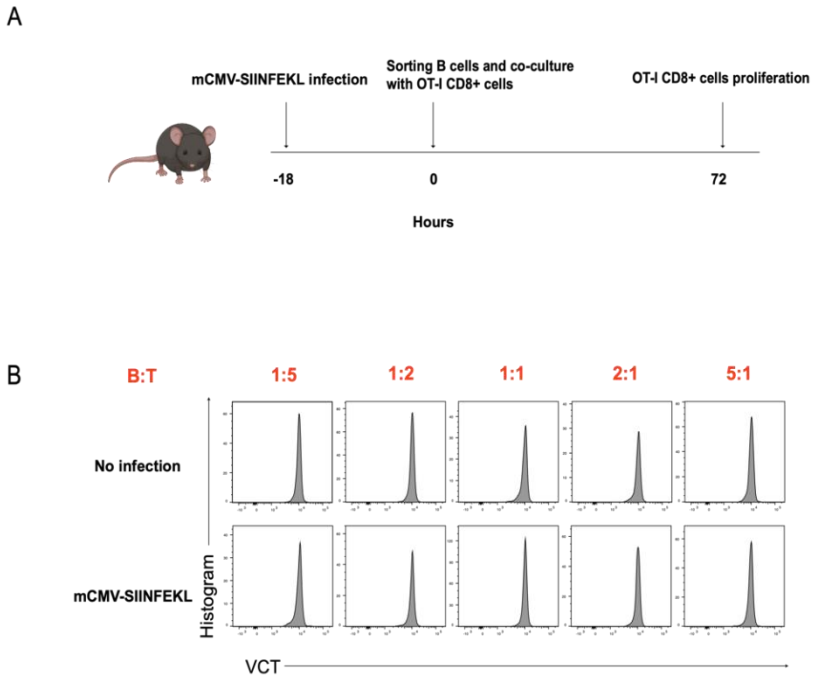

Figure 7: B cells are unable to cross-present antigen to CD8$^+$ T cells directly after MCMV infection. (A) Schematic diagram outlining the program for co-culturing OVA-specific transgenic OTI CD8$^+$ T cells with B cells isolated from MCMV-SIINFEKL-infected WT mice. **(B)** Flow cytometry histograms showing the proliferation of OVA-specific transgenic OTI CD8$^+$ T cells co-cultured with B cells isolated from uninfected or MCMV-SIINFEKL-infected-WT mice (n = 5).

3.5 B cells are critical to the persistence and function of splenic marginal metallophilic CD169$^+$ macrophages

As we could not find a direct role for B cells as APCs in priming of MCMV-specific CD8$^+$ T cells, we reckoned that the absence of B cells might affect other APCs, critical for priming of anti-MCMV T cell responses. The major antigen-presenting cells other than B cells are macrophages and dendritic cells. To test how the absence of B cells can affect other APCs in immune organs, we used flow cytometry and histology. First, we observed that the frequencies and numbers of F4-80$^+$CD11b$^+$ macrophages in the spleen

of J_HT mice were comparable to those of control mice (**Figure 8A**). Following that, we characterized marginal metallophilic CD169$^+$ macrophages that have been strategically located in the splenic marginal zone at the entering site of blood, and thus might serve as a port of entry for MCMV into the spleen, and therefore are critical to mounting an appropriate T cell response to it. Histological and flow cytometry investigations of the spleen showed that the frequencies and number of marginal metallophilic CD169$^+$ macrophages were significantly decreased in the J_HT mice when compared with control mice at steady state. (**Figure 8B and 8C**). These data provide evidence that the presence of B cells is important for the maintenance of marginal metallophilic CD169$^+$ macrophages homeostasis.

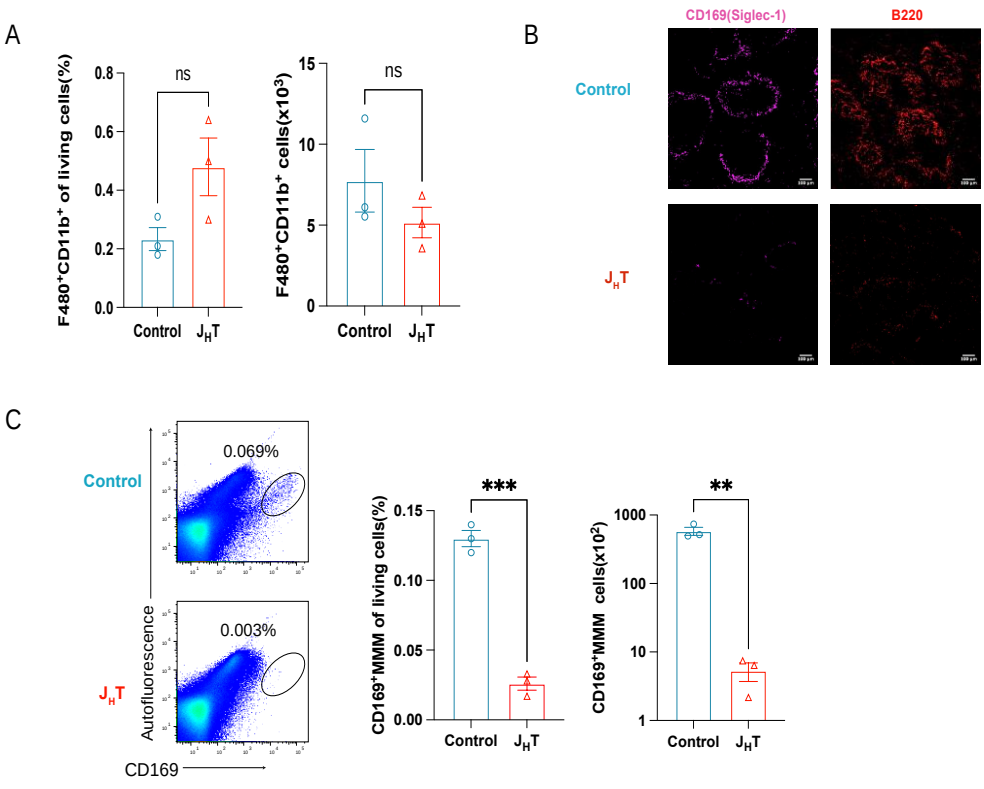

Figure 8: B-cell deficiency leads to a decrease in splenic metallophilic CD169$^+$ macrophages. **(A)** Bar graphs indicate the frequency (left) and absolute numbers (right) of F4-80$^+$CD11b$^+$ macrophages in the spleens of control and J_HT mice (n = 3). **(B)** Immunofluorescence images of CD169 (Siglec-1) and B220 in the spleens of control and J_HT mice (scale bar, 100 µm; n = 4). **(C)** Flow cytometry dot plot showing marginal metallophilic CD169$^+$ macrophages in the spleen of

control and J_HT mice. Marginal metallophilic $CD169^+$ macrophages were identified as living autofluorescent $CD169^+$ cells. Bar graphs indicate the frequency (left) and absolute numbers (right) of marginal metallophilic $CD169^+$ macrophages (n = 3). Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure 8A and 8C. **P < 0.01, ***P < 0.001, and ns (non-significant).

Previous studies show that marginal metallophilic $CD169^+$ macrophages play a role in antigen presentation and the induction of adaptive immune responses[113]. To determine whether marginal metallophilic $CD169^+$ macrophages were the target cells of CMV infection, we infected WT mice with recombinant murine CMV-expressing EGFP (MCMV-EGFP). After 48 hours, histological analysis of the spleen indicated that marginal metallophilic $CD169^+$ macrophages and MCMV-EGFP were co-located (**Figure 9A**). Next, to define whether the marginal metallophilic $CD169^+$ macrophages can directly contribute to virus-specific $CD8^+$ T cell responses in primary MCMV infection, we used the CD169-DTR mice, whose treatment with diphtheria toxin (DT) causes the deletion of marginal metallophilic $CD169^+$ macrophages. We first treated CD169-DTR and control mice with DT prior to their infection with MCMV. Histological examination of the spleen has shown that CD169-DTR mice exhibit almost no presence of marginal metallophilic $CD169^+$ macrophages after DT treatment (**Figure 9B**).Interestingly, 7 days after MCMV infection, we found a significantly reduced virus-specific $CD8^+$ T cell response in both the spleen and blood of CD169-DTR mice, as detected by MCMV tetramer staining (**Figure 9C, 9D, 9E**), which indicates that marginal metallophilic $CD169^+$macrophages are very important to mediate priming of MCMV-specific $CD8^+$ T cells.

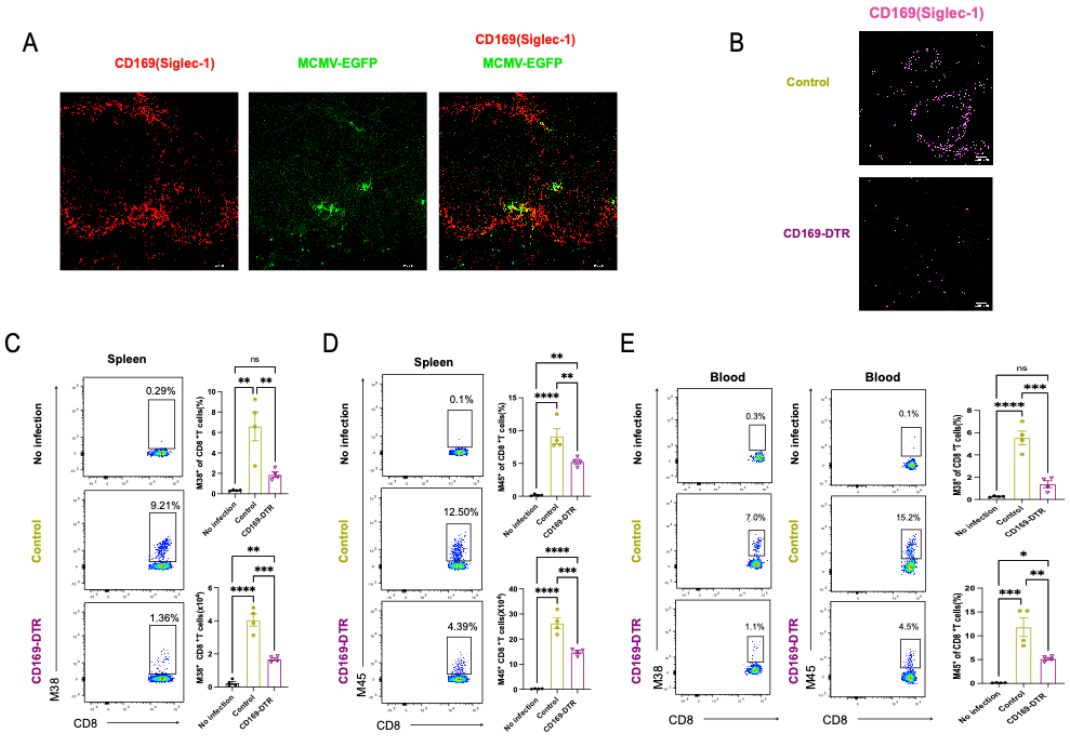

Figure 9: Marginal metallophilic CD169$^+$ macrophages are essential to initiate priming of virus-specific CD8$^+$ T cells. (A) Immunofluorescence images for CD169 and fluorescent MCMV (MCMV-EGFP) in the spleens of WT mice 48 hours after MCMV-EGFP infection (scale bar, 100µm; n = 3). **(B)** Immunofluorescence images showing marginal metallophilic CD169$^+$ macrophages in the spleen of control and CD169-DTR mice after diphtheria toxin treatment (scale bar, 100µm; n = 4). **(C)** Representative Flow cytometry dot plots of splenic MCMV-M38 tetramer$^+$ CD8$^+$ T cells in uninfected, control, and CD169-DTR mice. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of MCMV-M38 tetramer$^+$ CD8$^+$ T cells (n = 4). **(D)** Flow cytometry dot plot showing splenic MCMV-M45 tetramer$^+$ CD8$^+$ T cells in uninfected, control, and CD169-DTR mice. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of MCMV-M45 tetramer$^+$ CD8$^+$ T cells (n = 4). **(E)** Flow cytometry dot plot showing MCMV-M38 tetramer$^+$ CD8$^+$ T cells (left) and MCMV-M45 tetramer$^+$ CD8$^+$ T cells (right) in blood of uninfected, control, and CD169-DTR mice. Bar graphs indicate the frequency of MCMV-M38 tetramer$^+$ CD8$^+$ T cells (top) and the frequency of MCMV-M45 tetramer$^+$ CD8$^+$ T cells (bottom) (n = 4). Data are presented as mean ± SEM. Statistical analysis: one-way ANOVA, Figure 9C-9E. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, and ns (non-significant).

3.6 B cells expressing LTβ control splenic marginal metallophilic CD169$^+$ macrophages homeostasis

It was shown previously that the homeostasis of splenic marginal metallophilic CD169$^+$ macrophages relies on the non-canonical NFB signaling pathway through the NFB-inducing kinase (NIK)[114]. A handful of molecules, such as lymphotoxin (LTβ), which is produced by B cells[115-118], have the ability to activate NIK[119]. In order to find whether B cell-expressed LTβ controls the development of marginal metallophilic CD169$^+$ macrophages in the spleen, we generated mixed bone marrow (BM) chimera using BM from WT mice mixed with that of J_HT mice (control) or using BM from LTβ-deficient mice mixed with that of J_HT mice, generating mice where only the B cells are not capable of producing LTβ (**Figure 10A**). In comparison to control mice, the absence of LTβ expressed by B cells resulted in a significant reduction of marginal metallophilic CD169$^+$ macrophages in the spleen (**Figure 10B**). Next, we infected these mice with MCMV. After 7 days, we found that, similar to the mice without B cells, the frequency of MCMV-specific-tetramer-positive CD8$^+$ T cells reduced significantly in the absence of LTβ expressed by B cells as compared to control mice (**Figure 10C, 10D, and 10E**). Collectively, these data highlight that the absence of LTβ expressed by B cells results in a reduction in CD8$^+$ T cell priming following MCMV infection, probably due to the lack of marginal metallophilic CD169$^+$ macrophages.

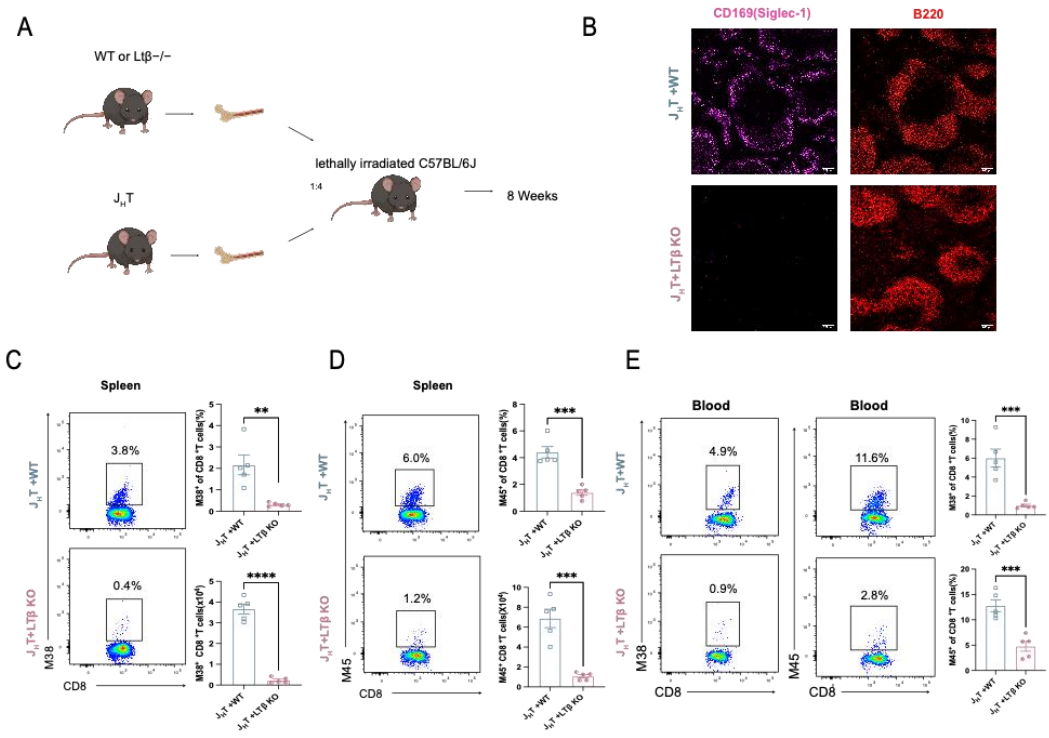

Figure 10: B cells that express LTβ regulate the homeostasis of splenic marginal metallophilic CD169$^+$ macrophages. (A) Schematic of the bone marrow chimera experiment to get B cells failed to express LTβ mice. **(B)** Immunofluorescence images of CD169 (Siglec-1) and B220 in the spleens of J_HT mixed WT BM chimera mice and J_HT mixed LTβ-deficient BM chimera mice (scale bar, 100 µm; n = 3). **(C)** Representative flow cytometry dot plots of splenic MCMV-M38 tetramer$^+$ CD8$^+$ T cells in the J_HT mixed WT BM chimera mice and J_HT mixed LTβ-deficient BM chimera mice. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of MCMV-M38 tetramer$^+$ CD8$^+$ T cells (n = 5). **(D)** Flow cytometry dot plot showing splenic MCMV-M45 tetramer$^+$ CD8$^+$ T cells in J_HT mixed WT BM chimera mice and J_HT mixed LTβ-deficient BM chimera mice. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of MCMV-M45 tetramer$^+$ CD8$^+$ T cells (n = 5). **(E)** Flow cytometry dot plot showing MCMV-M38 tetramer$^+$ CD8$^+$ T cells (left) and MCMV-M45 tetramer$^+$ CD8$^+$ T cells (right) in the blood of J_HT mixed WT BM chimera mice and J_HT mixed LTβ-deficient BM chimera mice. Bar graphs indicate the frequency of MCMV-M38 tetramer$^+$ CD8$^+$ T cells (top) and the frequency of MCMV-M45 tetramer$^+$ CD8$^+$ T cells (bottom) (n = 5). Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure 10C-10E. **P < 0.01, ***P < 0.001, ****P < 0.0001.

3.7 B cell deficiency affects the function of XCR1$^+$ CD8$^+$cDC1 to prime CD8$^+$ T cells

Previous studies have demonstrated that the primary functions of marginal metallophilic CD169$^+$ macrophages are pathogen uptake, followed by the transfer of antigens to CD8$^+$ DCs for the initiation of effective CD8$^+$ T cell responses[39,120]. Since we observed a reduced number of marginal metallophilic CD169$^+$ macrophages in mice lacking B cells, we hypothesized that CD8$^+$ cDC1 in J_HT mice do not acquire sufficient antigen to initiate an effective CD8$^+$ T cell response after MCMV infection. To verify this hypothesis, we infected control and J_HT mice with MCMV-SIINFEKL. After 18 h, we isolated XCR1$^+$ CD8$^+$ cDC1 from the spleens of J_HT and control mice and co-cultured them with Cell Trace Violet-labeled CD8$^+$ T cells from OT-I mice in different ratios for 3 days (**Figure 11A**). In line with our hypothesis, we found that the OT-I CD8$^+$ T cells co-cultured with XCR1$^+$ CD8$^+$ cDC1 from infected J_HT mice showed a weak expansion compared to those incubated with XCR1$^+$ CD8$^+$ cDC1 from infected control mice (**Figure 11B**). We next asked whether the reduced expansion of the OT-I CD8$^+$ T cells was simply due to not having sufficient marginal metallophilic CD169$^+$ macrophages to transfer antigen to the CD8$^+$ cDC1 or whether the absence of B cells also concurrently led to altered CD8$^+$ cDC1 antigen-presenting function. Due to this, we purified XCR1$^+$ cDC1 from the spleens of naive J_HT and control mice and incubated them with the OVA protein. Following one hour of incubation at 37°C, XCR1$^+$cDC1 was thoroughly washed and cultured with CD8$^+$ T cells labeled with Cell Trace Violet, which were obtained from OT-I mice. After 3 days, we observed that OT-I CD8$^+$ T cells co-cultured with XCR1$^+$ cDC1 from J_HT mice showed weak expansion at different concentrations of OVA protein compared to OT-I cells co-cultured with XCR1$^+$ cDC1 from control mice (**Figure 11C**).

To further define how the antigen-presenting function of XCR1$^+$cDC1 is changed in J_HT mice, we first assessed *in vitro* antigen uptake utilizing OVA conjugated with Alexa Fluor 488 dye^R. We observed that XCR1$^+$cDC1 of J_HT mice are similar in antigen

phagocytosis, detected as green fluorescence cells compared to the same cellular population taken from control mice (**Figure 11D**). In order to present antigen on MHC molecules to $CD8^+$ T cells, after uptaking the antigen, DC need to process the uptaken antigen into peptides. To assess if the $XCR1^+$cDC1 cells of the B cell-deficient mice can properly process antigen to further present it by MHC class I, we cultured $XCR1^+$ cDC1 with OVA conjugated to the pH-insensitive BODIPY FL dye (OVA-DQ). Upon proteolytic degradation, the processed elements of OVA-DQ exhibit bright fluorescence that could be assessed by flow cytometry. As Figure 11E shows, we couldn't find any differences in fluorescence, suggesting that J_HT splenic $XCR1^+$ cDC1 did not significantly change in their capacity to process phagocytosed OVA-DQ when compared with control splenic $XCR1^+$ cDC1 (**Figure 11E**). Together, these data suggest that cDC1 of B-cell-deficient mice are able to uptake and process protein antigen similarly to control mice. In order to investigate the potential impacts of B cell deletion on cDC1 function after phagocytosis and processing, we co-cultured $XCR1^+$ cDC1, OT-I $CD8^+$ T cells, and SIINFEKL peptide to analyze $XCR1^+$ cDC1 performance in T cell priming. Indeed, we found that OT-I $CD8^+$ T cells co-cultured with $XCR1^+$ cDC1 from J_HT mice exhibited reduced expansion at low concentrations of SIINFEKL peptide as compared to OT-I cells incubated with $XCR1^+$ cDC1 from control mice. (**Figure 11F**). Thus, our results confirm that B cell deficiency impaired the capacity of splenic $XCR1^+$ cDC1 to present antigen to $CD8^+$ T cells.

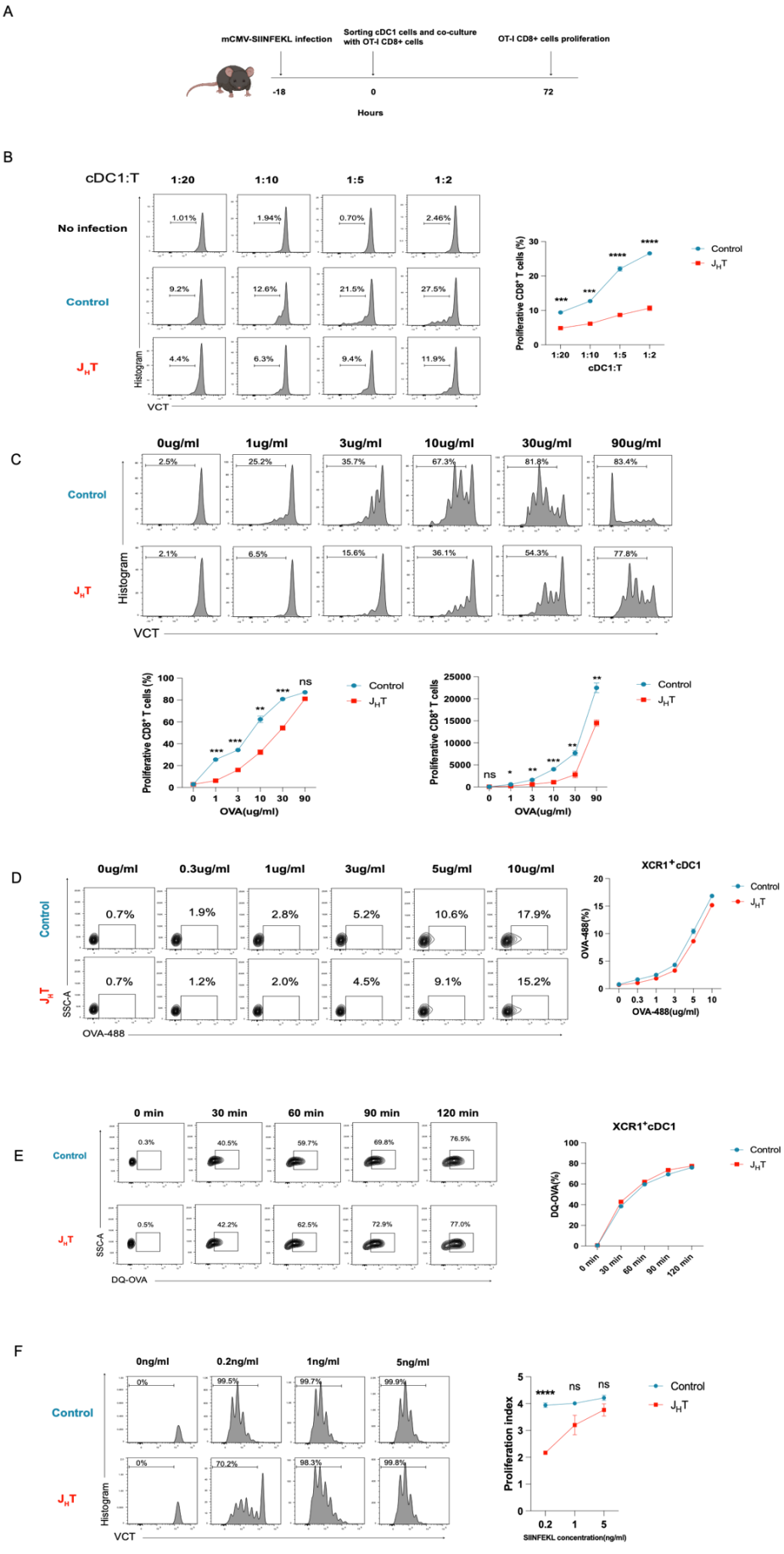
A
mCMV-SIINFEKL infection
Sorting cDC1 cells and co-culture with OT-I CD8+ cells
OT-I CD8+ cells proliferation
-18
0
72
Hours
B
cDC1:T
1:20
1:10
1:5
1:2
No infection
Control
J_HT
1.01%
1.94%
0.70%
2.46%
9.2%
12.6%
21.5%
27.5%
4.4%
6.3%
9.4%
11.9%
Histogram
VCT
Proliferative CD8+ T cells (%)
cDC1:T
Control
J_HT
C
0ug/ml
1ug/ml
3ug/ml
10ug/ml
30ug/ml
90ug/ml
2.5%
25.2%
35.7%
67.3%
81.8%
83.4%
2.1%
6.5%
15.6%
36.1%
54.3%
77.8%
Histogram
VCT
Proliferative CD8+ T cells (%)
OVA(ug/ml)
Proliferative CD8+ T cells
D
0ug/ml
0.3ug/ml
1ug/ml
3ug/ml
5ug/ml
10ug/ml
0.7%
1.9%
2.8%
5.2%
10.6%
17.9%
0.7%
1.2%
2.0%
4.5%
9.1%
15.2%
SSC-A
OVA-488
XCR1+cDC1
OVA-488(%)
OVA-488(ug/ml)
E
0 min
30 min
60 min
90 min
120 min
0.3%
40.5%
59.7%
69.8%
76.5%
0.5%
42.2%
62.5%
72.9%
77.0%
SSC-A
DQ-OVA
XCR1+cDC1
DQ-OVA(%)
F
0ng/ml
0.2ng/ml
1ng/ml
5ng/ml
0%
99.5%
99.7%
99.9%
0%
70.2%
98.3%
99.8%
Histogram
VCT
Proliferation index
SIINFEKL concentration(ng/ml)

Figure 11: B cells are required for XCR1$^+$CD8$^+$cDC1 to prime CD8$^+$ T cells. (A) Schematic diagram outlining the program used to co-culture OVA-specific transgenic OTI CD8$^+$ T cells with cDC1 isolated from MCMV-SIINFEKL-infected control and J_HT mice. **(B)** Proliferation of OVA-specific transgenic OTI CD8$^+$ T cells co-cultured at various ratios with cDC1 isolated from uninfected or MCMV-SIINFEKL-infected control and J_HT mice. The line graph indicates the frequency of proliferating OTI CD8$^+$ T cells (n = 5). **(C)** Proliferation of OVA-specific transgenic OTI CD8$^+$ T cells co-cultured with cDC1 isolated from control or J_HT mice and pulsed with OVA protein. Line graphs indicate the frequency (left) and absolute numbers (right) of proliferating OTI CD8$^+$ T cells (n =5). **(D)** Flow cytometry plot showing the frequencies of OVA-488$^+$ XCR1$^+$ cDC1s at each incubated concentration of OVA-488. Briefly, cDC1 was isolated from control or J_HT mice and incubated with increasing concentrations of OVA-488 for 30 minutes at 37 °C and 5% CO2. The line graph indicates the frequencies of OVA-488$^+$ XCR1$^+$ cDC1 in each group (n = 4). **(E)** Representative flow cytometry plots of OVA-DQ$^+$ XCR1$^+$ cDC1 exposed to OVA-DQ for different lengths of time. Briefly, cDC1 was isolated from control or J_HT mice and incubated with 10 µg/ml OVA-DQ for 0, 30, 60, 90, and 120 minutes at 37 °C and 5% CO2. The line graph indicates the frequency of OVA-DQ$^+$ XCR1$^+$ cDC1 in each group (n = 4). **(F)** Proliferation of OVA-specific transgenic OTI CD8$^+$ T cells co-cultured with cDC1 isolated from control or J_HT mice and pulsed with SIINFEKL peptide. The line graph indicates the proliferation index of OTI CD8$^+$ T cells (n = 6). Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure11B-11F. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, and ns (non-significant).

3.8 Loss of B cells reduces the number of Langerin^{+}XCR1^{+} cDC1

Given that the above data point to the fact that B-cell deficiency results in cDC1 not priming T cells well, we then tried to explore what contributed to this phenomenon. First, we observed that a lack of B cells had no effect on the frequency or absolute numbers of DCs in the spleen (**Figure 12A**). Furthermore, the division of DC into XCR1^{+} cDC1 and SIRP-α^{+} cDC2 subsets was also not affected in the spleen of J_HT mice as compared to control mice (**Figure 12B**). To have a deeper comprehension of the complexity of DC subsets in the spleen and how they might be influenced by B cell deficiency, we utilized complex multi-dimensional flow cytometry. Using t-distributed stochastic neighbor embedding (t-SNE) for showing these data, we detected notable alterations in the J_HT mice's splenic cDC1 and cDC2 populations (**Figure 12C**). Further analysis reveals that the altered splenic cDC1 subpopulation in the J_HT mice was Langerin^{+}XCR1^{+} cDC1, while the altered splenic cDC2 subpopulation was endothelial cell specific adhesion marker (ESAM)^{hi} SIRP-α^{+} cDC2 (**Figure 12D**). In agreement with t-SNE data, the specific flow cytometry analysis of cDC1 or cDC2 revealed that the frequencies and numbers of Langerin^{+}XCR1^{+} cDC1 and ESAM^{hi} SIRP-α^{+} cDC2 in the J_HT mice were significantly decreased (**Figure 12E and 12F**).

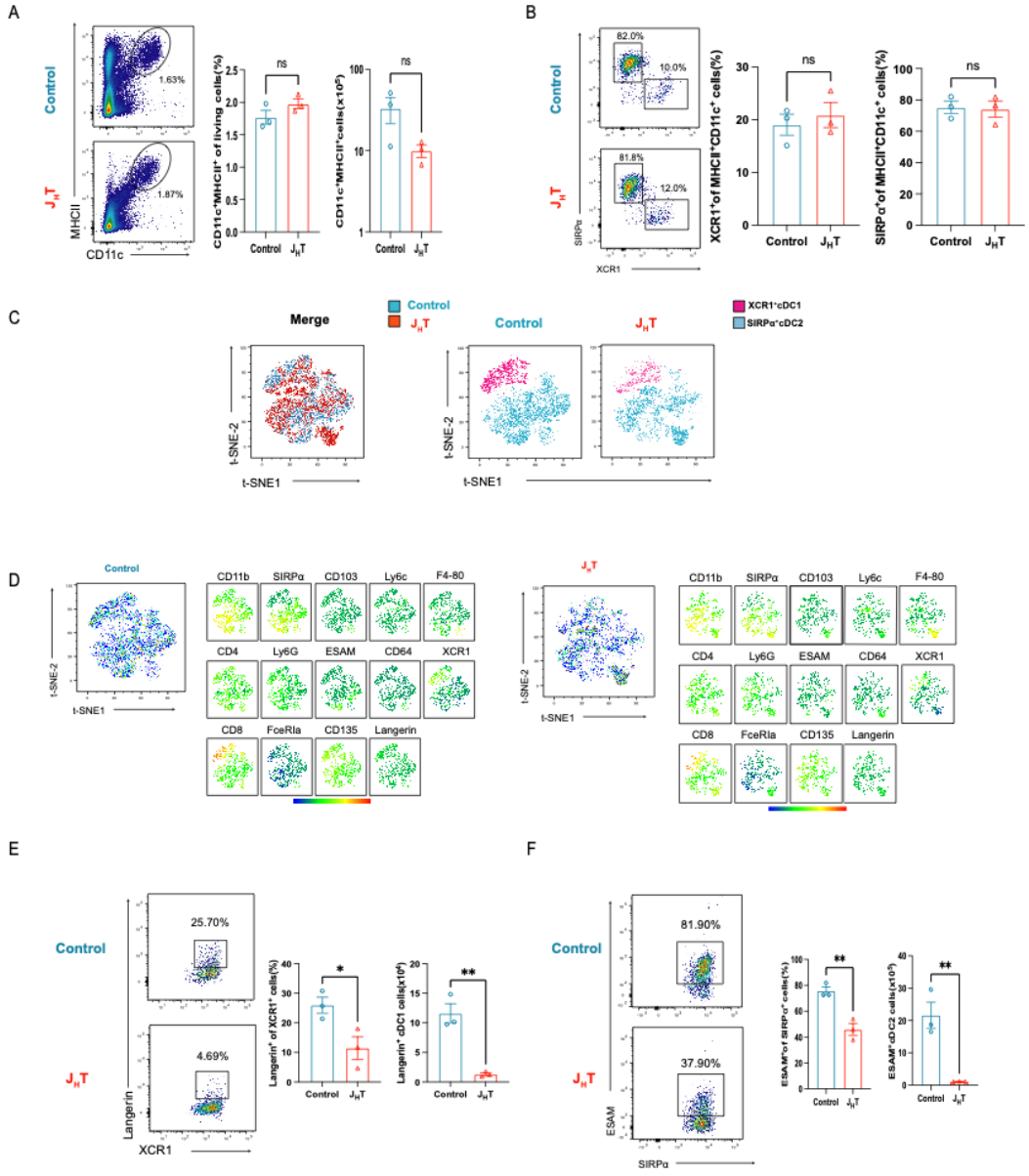

Figure 12: B cells control the homeostasis of splenic Langerin$^+$ cDC1. (A) Flow cytometry dot plot showing CD11c$^+$MHCII$^+$ cDC in the spleen of control or J_HT mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of CD11c$^+$MHCII$^+$ cDC (n = 3). **(B)** Flow cytometry plot showing XCR1$^+$ cDC1 and SIRPα$^+$ cDC2 subsets in the spleens of control or J_HT mice. Bar graphs indicate the frequency of XCR1$^+$ cDC1 (left) and the frequency of SIRPα$^+$ cDC2 (right) (n = 3). **(C)** Three control and three J_HT concatenated samples with 5.000 cDC (CD11c$^+$ MHCII$^+$ CD19$^-$) each were embedded using t-distributed stochastic neighbor embedding (t-SNE). cDC1 (pink) and cDC2 (blue) were classified according to XCR1 and SIRPα expression (n = 3). **(D)** t-

SNE of the expression of selected markers associated with the DC subgroup in the control (left) and J_HT (right) DC populations (n = 3). **(E)** Flow cytometry dot plot showing splenic Langerin$^+$ cDC1 cells in control or J_HT mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of Langerin$^+$ cDC1 cells (n = 3). **(F)** Flow cytometry dot plot showing splenic ESAM$^+$ cDC2 cells in control or J_HT mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of ESAM$^+$ cDC2 cells (n = 3). Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure 12A, 12B, 12E, and 12F. *P < 0.05, **P < 0.01, ns (non-significant).

To further determine if splenic Langerin$^+$XCR1$^+$ cDC1 were required for control of MCMV infection, we treated Lang-DTR[121] and control mice with DT and then infected them with MCMV (**Figure 13A**). Multiple DT doses were tolerated well and successfully depleted Langerin$^+$XCR1$^+$ cDC1 in the spleen (**Figure 13B**). 7 days after infection, we assessed the virus-specific CD8$^+$ T cell response by MCMV tetramer staining. We found that the M38 and M45 tetramer-positive CD8$^+$ T cells were significantly decreased in both the spleen and blood of Lang-DTR mice as compared to control mice (**Figure 13C, 13D, and 13E**), demonstrating that the decreased virus-specific CD8$^+$ T cell response following MCMV infection can be attributed to a decrease in Langerin$^+$XCR1$^+$ cDC1.

In summary, we discovered that the lack of B cells resulted in a reduction in splenic Langerin$^+$XCR1$^+$ cDC1, which results in changes in the antigen-presenting function of remaining cDC1 cells in J_HT mice and ultimately leads to a significant decrease in the virus-specific CD8$^+$ T cell response.

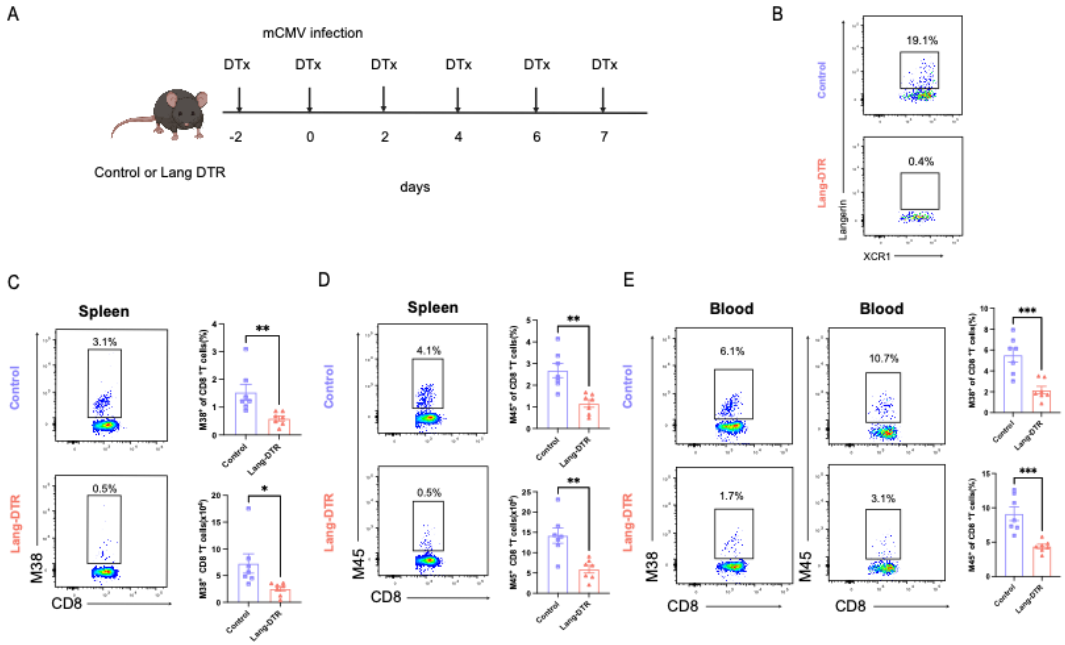

Figure 13: Langerin$^+$ cDC1 is essential for the virus-specific CD8$^+$ T cells. (A) Schematic of DT treatment of Lang-DTR mice to delete Langerin$^+$cDC1 and validation of the role of Langerin$^+$cDC1 in MCMV infection. **(B)** Flow cytometry dot plot showing splenic Langerin$^+$ cDC1 cells in control and Lang-DTR mice after DT treatment. **(C)** Representative flow cytometry dot plots of splenic MCMV-M38 tetramer$^+$ CD8$^+$ T cells in control and Lang-DTR mice. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of splenic MCMV-M38 tetramer$^+$ CD8$^+$ T cells (n = 7). **(D)** Flow cytometry dot plot showing splenic MCMV-M45 tetramer$^+$ CD8$^+$ T cells in control and Lang-DTR mice. Bar graphs indicate the frequency (top) and absolute numbers (bottom) of splenic MCMV-M45 tetramer$^+$ CD8$^+$ T cells (n = 7). **(E)** Flow cytometry dot plot showing MCMV-M38 tetramer$^+$ CD8$^+$ T cells (left) and MCMV-M45 tetramer$^+$ CD8$^+$ T cells (right) in the blood of control and Lang-DTR mice. Bar graphs indicate the frequency of MCMV-M38 tetramer$^+$ CD8$^+$ T cells (top) and the frequency of MCMV-M45 tetramer$^+$ CD8$^+$ T cells (bottom) (n = 7). Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure 13C, 13D, and 13E. *P < 0.05, **P < 0.01, and ***P < 0.001.

3.9 B-cell reconstitution partially rescues the reduced primary CD8$^+$ T-cell response in J_HT mice

In order to investigate whether the transfer of B cells could rescue the primary CD8$^+$ T response in the B cell-deficient mice, we reconstituted J_HT mice with magnetically sorted splenic WT B cells, as previously shown[122]. One week after the transfer, we analyzed the marginal metallophilic CD169$^+$ macrophages and splenic Langerin$^+$XCR1$^+$ cDC1. As expected, we observed that marginal metallophilic CD169$^+$ macrophages and splenic Langerin$^+$XCR1$^+$ cDC1 can be partially rescued (**Figure 14A and 14B**). Meanwhile, we infected control, B-cell-reconstituted J_HT mice, and J_HT mice with MCMV a week after the transfer, and the virus-specific CD8$^+$ T cell response was analyzed 7 days after infection (**Figure 14C**). As we showed with marginal metallophilic CD169$^+$ macrophages and splenic Langerin$^+$XCR1$^+$ cDC1 being able to partially restore, we found that transfer of B cells could partially reconstituted the primary CD8$^+$ response of the J_HT mice (**Figure 14D**).

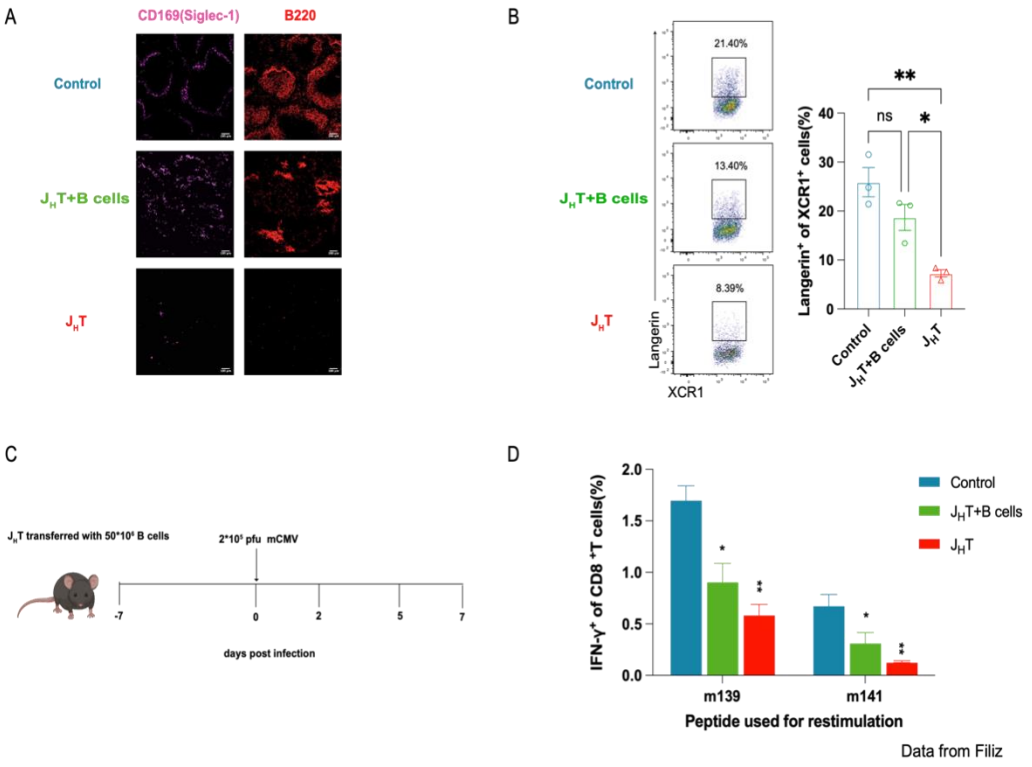

Figure 14: Impaired primary CD8$^+$ T-cell response is partly restored by B-cell reconstitution in J_HT mice. (A) Immunofluorescence images of CD169 (Siglec-1) and B220 in the spleens of control, J_HT, and B-cell-reconstituted J_HT mice (scale bar, 100 µm; n = 3). **(B)** Representative flow cytometry dot plots of splenic Langerin$^+$ cDC1 cells in control, J_HT, and B-cell-reconstituted J_HT mice. Bar graphs indicate the frequency of Langerin$^+$ cDC1 cells (n = 3). **(C)** Schematic of reconstituted J_HT mice with splenic WT B cells, and one week after the transfer, the mice were infected with MCMV. **(D)** Frequency of IFN-γ-producing CD8$^+$ T cells after stimulation with different MCMV-dominant peptides of control, J_HT, and B-cell-reconstituted J_HT mice 7 days-post infection (n = 3- 4). Differences in Figure 14D come from comparing each group to the control. Data are presented as the mean ± SEM. Statistical analysis: one-way ANOVA, Figure 14B and 14D. *P < 0.05, **P < 0.01, and ns (non-significant).

3.10 B cells expressing LTβ control marginal metallophilic CD169$^+$ macrophages to maintain splenic Langerin$^+$ cDC1 homeostasis

The majority of Langerin$^+$XCR1$^+$ cDC1 are located in the splenic marginal zone, where they form a ring surrounding the marginal metallophilic CD169$^+$ macrophages[123]. Considering that B cells are required for the development of marginal metallophilic CD169$^+$ macrophages and Langerin$^+$XCR1$^+$ cDC1 and marginal metallophilic CD169$^+$ macrophages can interact with cross-presenting CD8$^+$ DCs, we next investigated whether B cells regulate the homeostasis of Langerin$^+$XCR1$^+$ cDC1 directly or via marginal metallophilic CD169$^+$ macrophages. Interestingly, through assessment of the CD169-DTR mice after DT treatment, we observed that following marginal metallophilic CD169$^+$ macrophages depletion, there was a significant decrease in both the frequencies and numbers of Langerin$^+$XCR1$^+$cDC1 (**Figure 15A**), demonstrating that marginal metallophilic CD169$^+$ macrophages play an essential role in maintaining homeostasis of splenic Langerin$^+$XCR1$^+$ cDC1. As expected, the frequencies and

numbers of Langerin$^+$XCR1$^+$ cDC1 were also significantly decreased in the mixed BM chimera mice where B cells lack expressed LTβ when compared to control mice (**Figure 15B**). Collectively, these data highlight that in the absence of LTβ expressed by B cells, the spleen structure and specifically the placement of marginal metallophilic CD169$^+$ macrophages are disrupted, which leads to the reduction of Langerin$^+$XCR1$^+$ cDC1.

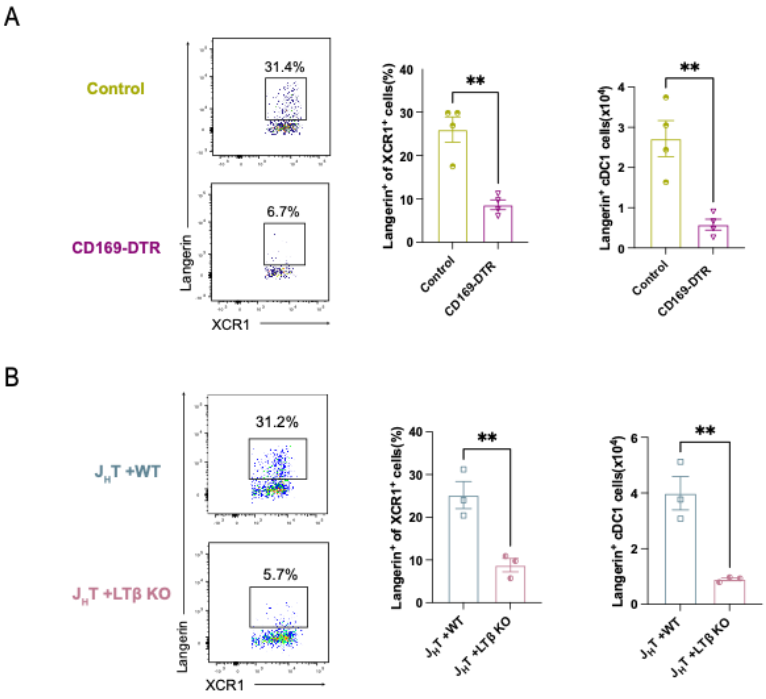

Figure 15: B cells that express LTβ regulate marginal metallophilic CD169$^+$ macrophages in the spleen to maintain the homeostasis of splenic Langerin$^+$ cDC1. (A) Representative flow cytometry dot plots of splenic Langerin$^+$ cDC1 cells in control and CD169-DTR mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of Langerin$^+$ cDC1 cells (n = 4). **(B)** Flow cytometry dot plot showing splenic Langerin$^+$ cDC1 cells in J_HT mixed WT BM chimera mice and J_HT mixed LTβ-deficient BM chimera mice. Bar graphs indicate the frequency (left) and absolute numbers (right) of Langerin$^+$ cDC1 cells (n = 3). Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure 15A and 15B. **P < 0.01.

So far, the data show that the absence of marginal metallophilic CD169$^+$ macrophages leads to a decrease in Langerin$^+$XCR1$^+$ cDC1. Given those results, we wanted to determine if the XCR1$^+$ cDC1 function was impacted after deleting the marginal metallophilic CD169$^+$ macrophages. We first used the OVA conjugated with Alexa Fluor 488 dye^R to test antigen uptake. As with the B cell-deficient mice, we found that antigen uptake of cDC1 was not affected by the depletion of marginal metallophilic CD169$^+$ macrophages (**Figure 16A**). Likewise, no significant differences in processing of OVA-DQ were observed in the XCR1$^+$ cDC1 between CD169-DTR and control mice (**Figure 16B**) at different incubation time points. Notably, similar to the J_HT mice, we found that after deletion of marginal metallophilic CD169$^+$ macrophages, cDC1 does not prime OVA-specific T cell transgenic OT-I CD8$^+$ T cells well by incubating XCR1$^+$ cDC1 from the spleens of naive CD169-DTR or control mice with OVA protein (**Figure 16C**).

Taken together, these findings indicate that deleting marginal metallophilic CD169$^+$ macrophages would disrupt the homeostasis of splenic Langerin$^+$XCR1$^+$ cDC1 and have an impact on the function of XCR1$^+$ cDC1 to prime CD8$^+$ T cells. These findings, combined with earlier in vitro and in vivo model results, clearly and strongly demonstrate that B cells expressing LTβ would control splenic metallophilic CD169$^+$ macrophages to further maintain splenic Langerin$^+$ cDC1 homeostasis.

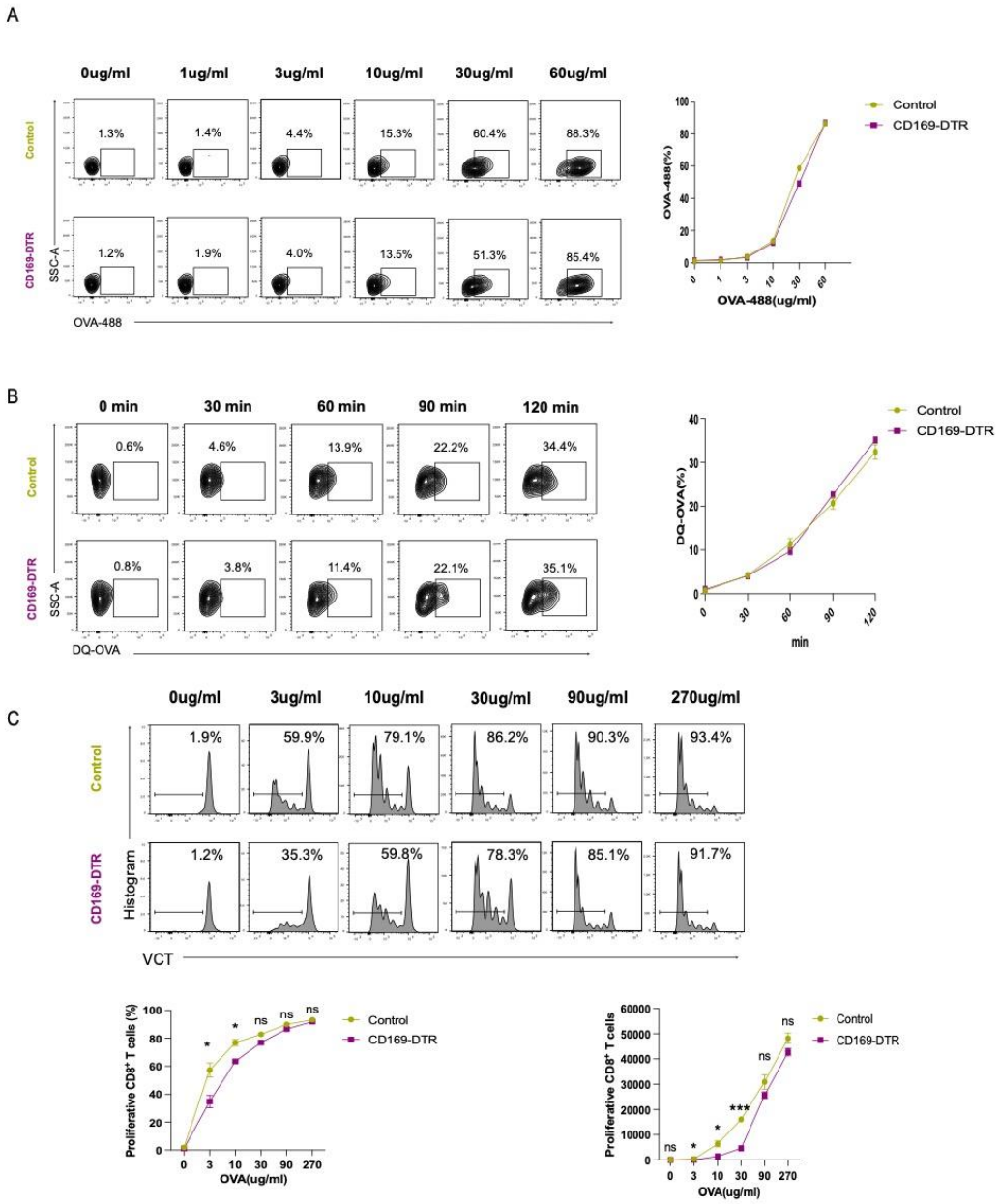

Figure 16: The absence of marginal metallophilic CD169$^+$ macrophages impair the function of XCR1$^+$ cDC1. (A) Representative flow cytometry plots of OVA-488$^+$ XCR1$^+$ cDC1 at each incubated concentration of OVA-488. Briefly, cDC1 was isolated from control and CD169-DTR and incubated with increasing concentrations of OVA-488 for 30 minutes at 37 °C and 5% CO2. The line graph indicates the frequency of OVA-488$^+$ XCR1$^+$ cDC1 (n = 5). **(B)** Flow cytometry plots showing OVA-DQ$^+$ XCR1$^+$ cDC1 exposed to OVA-DQ for different lengths of time. Briefly, cDC1 was isolated from control and CD169-DTR mice and incubated with 2 µg/ml OVA-DQ for 0, 30, 60, 90, and 120 minutes at 37 °C and 5% CO2. The line graph indicates the frequency of

OVA-DQ^{+} XCR1^{+} cDC1 (n = 5). **(C)** Proliferation of OVA-specific transgenic OTI CD8^{+} T cells co-cultured with cDC1 isolated from control or CD169-DTR mice and pulsed with OVA protein. Graphs indicate the frequency (left) and absolute numbers (right) of proliferating OTI CD8^{+} T cells (n = 5). Data are presented as the mean ± SEM. Statistical analysis: Student's t test, Figure 16A, 16B, and 16C. *P < 0.05, ***P < 0.001, and ns (non-significant).

4 Discussion

CMV infection remains an important but under-addressed medical problem because of the high mortality risk in immunologically deficient persons, such as hematopoietic cell transplantation recipients and solid organ transplantation recipients[124,125].Virus-specific $CD8^+$ T cells are critical in the control of both acute and persistent CMV infections[126]. Virus-specific $CD8^+$ T cells, which suppress intracellular viral replication by secreting TNF-α or IFN–γ, or lyse cells infected with viruses by secreting granzymes and perforins[23]. Activated B cells produce CMV-specific antibodies against a variety of viral proteins. A fraction of antibodies generated during infection identify glycoproteins on the CMV envelope and show significant virus-neutralizing activity[127,128]. Neutralizing antibodies work by inhibiting CMV envelope glycoproteins from interacting with their cellular receptors, blocking both CMV access and cell-to-cell dissemination. Recruiting complement to enhance the lysis of the pathogen is another crucial role of antibodies that detect proteins presented on the surface of the virus or the target antigen when presented on the surface of an infected cell[25]. Interestingly, increasing research reveals that B cells also play a significant role in maintaining primary virus-specific $CD8^+$ T cell responses in diverse virus infection models, including influenza, lymphocytic choriomeningitis, and vesicular stomatitis viruses[47,101]. However, it is still unknown how B cells regulate $CD8^+$ T cell responses after viral infection. Therefore, my thesis focuses on exploring whether B cells play a role in the virus-specific $CD8^+$ T cell response after MCMV infection and the related mechanisms.

4.1 The presence of B cells is important for MCMV-specific $CD8^+$ T cell responses

We first infected control and B cell-deficient J_HT mice with MCMV to investigate the contribution of B cells in the primary virus-specific $CD8^+$ T cell response. Indeed, we noticed a decrease in MCMV tetramer-positive $CD8^+$ T cells in J_HT mice in comparison

to control mice. Further, we stimulated the infected mice's spleenocytes with virus-specific peptides, and we demonstrated a significant reduction in the percentage of IFN-γ-producing $CD8^+$ T cells in J_HT mice when compared to infected control mice. The above experimental results fully illustrate that B cells are important for maintaining MCMV-specific primary $CD8^+$ T cell responses.

As previously mentioned, antibodies play an important role in defense against MCMV infection. The study by Klenovsek et al. showed that the adoptive transfer of MCMV-specific memory B cells into immunodeficient hosts can protect against MCMV-induced morbidity and mortality[129]. To investigate whether antibodies play a role in the primary MCMV-specific $CD8^+$ T cell responses, we infected IgMi mice with MCMV, and these mice had polyclonal populations of B cells, but they could not class switch or produce secreted antibodies. Our findings show that the percentage of IFN-γ-producing $CD8^+$ T cells in IgMi mice is not different as compared to control mice after stimulation with MCMV-specific peptides. Furthermore, we reconstituted J_HT mice with either normal mouse serum (NMS) or mouse serum from MCMV-infected WT mice (IMS), and we found that immunoglobulin transferred from infected WT mice exhibited no impact on the primary virus-specific $CD8^+$ T cell response after MCMV infection. Therefore, we exclude a role for B cells in the virus-specific $CD8^+$ T cell response via antibodies.

In addition, we are interested in whether the absence of B cells has an effect on $CD8^+$ T cell function. Interestingly, our bulk RNA-seq analysis for MCMV tetramer-positive $CD8^+$ T cells suggested that $CD8^+$ T cells in J_HT mice can develop into effector $CD8^+$ T cells after recognizing MCMV antigen, and that means the decrease in acute $CD8^+$ T cell response in J_HT mice is not attributable to alterations in $CD8^+$ T cell effector function.

A key point of this thesis is that we demonstrated that reduced primary $CD8^+$ T cell responses in J_HT mice are possibly due to weakened APC function in these mice. Here, our data suggest that OVA-specific T cell transgenic OT-I $CD8^+$ T cells were adaptively

transferred into J_HT and control mice and infected them with MCMV-SIINFEKL, and the OT-I $CD8^+$ T cells transferred into the J_HT mice did not proliferate well. Moreover, this interesting finding provides us with new clues for subsequent studies on how B cells regulate primary $CD8^+$ T cell responses.

4.2 Viral particles cannot be directly cross-presented by B cells to $CD8^+$ T cells

B cells are able to serve as professional APCs by expressing cell surface MHC II molecules. We identified that the decreased primary virus-specific $CD8^+$ T cell response in B cell deficiency mice was associated with attenuated antigen-presenting cell function. This has led us to investigate whether B cells act as antigen-presenting cells in virus-specific $CD8^+$ T cell responses.

Unlike typical APCs (DCs and macrophages), which are largely endocytic in nature, B cells can only take up and process the specific antigen identified by their BCR. As a consequence, B cells can effectively take up and deliver specific antigens to activate cognate $CD4^+$ T cells, thereby initiating immune responses or inducing their differentiation into TFH cells[130]. Furthermore, an ex vivo study of B cells from mice infected with Friend retrovirus (FV) revealed that infected B cells increased the expression of co-stimulatory markers CD40, CD80, and CD86, as well as MHC II molecules[131]. The study also showed that infected B cells had significantly improved APC function, which was evaluated by their ability to stimulate T cell activation and proliferation in vitro in comparison with uninfected B cells [131]. In addition, there is also a research study that shows activation of B3Z hybridoma cells (expressing OVA-derived $CD8^+$ T cell epitope SIINFEKL) indicates that in vitro loading of B cells with OVA in combination with the presence of CpG oligonucleotides induces OVA cross-presentation[132]. However, little is known about the ability of B cells to directly present antigen to $CD8^+$ T cells after viral infection. With co-culture of B cells derived from MCMV-SIINFEKL WT mice and OT-I $CD8^+$ T cells, we found that B cells are not the

main antigen-presenting cells that directly cross-present antigens to $CD8^+$ T cells after MCMV infection. Consistent with this finding, it's been reported before that FO and MZ B cells have the capacity to capture virus-like particles similarly to DCs and efficiently present the MHC class II-restricted virus-like particle peptide to Th cells but failed to cross-present the MHC class I-restricted virus-like particle peptide to cytotoxic T cells in vitro and in vivo[133].

Interestingly, Castiglioni et al. demonstrate that B cells have the ability to directly prime $CD4^+$ T cells, while $CD8^+$ T cell activation necessitates the assistance of $CD4^+$ T cells[134].Their study demonstrated that activated $CD4^+$ T cells can help B cells either through cell contact or soluble substances from the supernatants of activated $CD4^+$ T cells to prime naive $CD8^+$ T cells [134]. So whether B cells can present antigens to $CD8^+$ T cells with the help of activated $CD4^+$ T cells after MCMV infection remains a question to be considered in the future.

4.3 Splenic marginal metallophilic $CD169^+$ macrophages rely on B lymphocytes for development

$CD169^+$ macrophages, also known as metallophilic marginal zone macrophages and SCS macrophages, are located on top of B cell follicles surrounding the marginal sinus in the spleen and the SCS in the lymph nodes. The successful generation of mice with CD169 gene deletion and their application for different disease models is increasingly elucidating the roles of $CD169^+$ macrophages in immune regulation. During virus infection, by capturing viruses and enhancing viral replication, $CD169^+$ macrophages play a key role in activating the innate and adaptive immune systems[37,49,135,136]. Due to decreased interferon production and restricted activation of adaptive immune cells, the absence of $CD169^+$ macrophages results in a catastrophic illness outcome[136-138]. In some autoimmune diseases, several research studies have found that activated $CD169^+$ macrophages play a role in inflammatory responses. In the dextran sulfate sodium (DSS)- induced IBD model, $CD169^+$ macrophages have been shown to contribute to colitis development[139,140]. Human multiple sclerosis study has demonstrated that

$CD169^+$ macrophages are abundant in MS patients and can be used as targeted markers for microglia and macrophages, which get activated early in MS lesions[141]. Moreover, $CD169^+$ macrophages in the lymph nodes and spleen have been reported to play an important role in antitumor immunity. In animals without $CD169^+$ macrophages, the activation of $CD8^+$ T cells in response to tumor antigens and subsequent anti-tumor responses was significantly compromised[142,143]. Collectively, all of the above publications confirm the need for research on $CD169^+$ macrophages. Complete splenic architecture is necessary for $CD169^+$ macrophage development and function. A number of chemokines and cytokines like LTβ and RANK preserve the splenic architecture and hence impact $CD169^+$ macrophages' development[116,144,145]. For the past years, antibody-independent B-cell functions, such as cytokine production, have received increasing attention from researchers. Interestingly, LTβ produced by B cells can lead to the reconstitution of secondary lymphoid organs and contribute to the establishment of an effective immune response[117,118]. Using a bone marrow chimera model, we performed the mice where only the B cells were not capable of expressing LTβ. We then confirmed that B cells can express LTβ, which helped in maintaining marginal metallophilic $CD169^+$ macrophages in the spleen. Importantly, our data demonstrated attenuation of virus-specific $CD8^+$ T cell responses in bone marrow-reconstituted mice with B-cell-specific deletion of LTβ, further confirming the essential role played by marginal metallophilic $CD169^+$ macrophages in resistance to viral infection. In the previously mentioned publications, $CD169^+$ macrophages play an imperative role in different diseases, and given that $CD169^+$ macrophages are reduced in B-cell-deficient mice, this may also provide a completely new perspective for exploring how B plays a role in the previously mentioned diseases.

4.4 The splenic cDCs' homeostasis depends on B cells

DC can be categorized as conventional DC (cDC), monocyte-derived DC, and plasmacytoid DC. The cDC is further subdivided into cDC1 and cDC2, which are mainly involved in initiating cellular and humoral immune responses[146-148]. The paper

published by MacPherson and colleagues brought researchers' attention for the first time to the fact that DCs can provide natural antigens to B cells to promote humoral responses[149-151]. Subsequent studies have identified direct interactions between antigen-bearing DCs and transgenic B cells when DCs carrying egg lycopene (HEL) were co-cultured with HEL-specific B cells in vitro[152]. Thanks to advances in technology, the interaction between DCs and B cells was visualized in vivo using two-photon microscopy and over-transfer in a very intriguing study[153]. Overall, the above studies collectively confirm that dendritic cells are capable of interacting with B cells and delivering antigens to activate B cells. However, little is known about whether B cells can influence the development as well as the function of DC. Our observation of an attenuated proliferation of OT-I cells when co-cultured with cDC1 derived from B-cell deficiency mice as well as OVA proteins suggests that deletion of B cells leads to impaired antigen-presenting function of cDC1. Beyond changes in cDC1 function, our complex multi-dimensional flow cytometry work further delineates that B-cell absence leads to disruption of DC homeostasis in the spleen. We found that B-cell deficiency results in a reduction of Langerin$^+$cDC1 and ESAM$^+$cDC2. Some research has demonstrated that this Langerin$^+$ subset of cDC1 is particularly important for cDC1 functions such as cross-presentation and CD4$^+$ Th1 cell stimulation[63,90,92,123]. Prendergast et al. found that Langerin$^+$ cDC1 is essential for the initiation of CD8$^+$ T cell responses in response to bacteremia[92]. In line with earlier studies, we utilized Lang DTR mice to confirm the importance of Langerin$^+$cDC1 to maintain MCMV-specific CD8$^+$ T cell responses. These findings also enrich our understanding of B cells and Langerin$^+$cDC1. cDC2 has been shown to be a more effective CD4$^+$ T cell activator and trigger of Th2, Th17, and T-follicular helper cell differentiation[68,154-156]. ESAM$^+$cDC2 is the major cDC2 subpopulation in the splenic marginal zone, and they play an important role in the activation of CD4$^+$T cells as well as the formation of germinal centers[157,158]. Therefore, using diverse disease models to investigate whether B cells might modulate CD4$^+$T cell responses via ESAM$^+$cDC2 is also an intriguing future study.

4.5 Marginal metallophilic CD169$^+$ macrophages are crucial for maintaining homeostasis of splenic Langerin$^+$XCR1$^+$ cDC1

Many studies have shown that CD169$^+$ macrophages are essential for T cell initiation[49,136,137,159-161], and the interplay between CD169$^+$ macrophages and cDC1s has been shown to enhance antiviral T cell responses[39,161]. More recently, the synergistic interaction between CD169$^+$ and cDC1s has been studied in more detail[39]. This study showed that the CD169 receptor brings cells into contact with salivating ligands on cDCs, thereby facilitating antigen transfer to cDCs. Overall, all of these studies are elucidating how CD169$^+$ macrophages cooperate with cDC1 to promote CD8$^+$ T cell responses, but little is known about whether CD169$^+$ macrophages have a regulatory relationship with cDC1 or subpopulations of cDC1. Langerin$^+$CD8$^+$ cDC1 is found mainly in the marginal zone, where they are interspersed with marginal zone macrophages to form a ring around marginal metallophilic CD169$^+$ macrophages[123]. These fascinating phenomena led us to ponder whether there is a direct regulatory relationship between marginal metallophilic CD169$^+$ macrophages and Langerin$^+$ cDC1. Indeed, after we deleted marginal metallophilic CD169$^+$ macrophages by treating CD169-DTR mice with DT, we found a concomitant reduction in Langerin$^+$cDC1, implying that marginal metallophilic CD169$^+$ macrophages are exceptionally important for the homeostatic maintenance of Langerin$^+$ cDC1. Furthermore, follow-up experiments with cDC1, OT-I$^+$CD8$^+$ T cells, and OVA protein co-cultures confirmed that the absence of metallophilic CD169$^+$ macrophages results in diminished cDC1 antigen presentation due to a decrease in the Langerin$^+$cDC1 subpopulation. However, after we successfully deleted Langerin$^+$ cDC1 by treating Lang-DTR mice with DT, we found that marginal metallophilic CD169$^+$ macrophages were not significantly altered, demonstrating that the regulation of Langerin$^+$ cDC1 by marginal metallophilic CD169$^+$ macrophages exists unidirectionally. In the future, we will continue to explore how metallophilic CD169$^+$ macrophages regulate the homeostasis of Langerin$^+$ cDC1. By performing single-cell RNA sequencing of

Langerin$^+$ cDC1 from metallophilic CD169$^+$ macrophages-deficient mice and control mice and comparing differentially expressed genes and pathways, it may help us draw better conclusions.

In summary, we first used a model of MCMV infection to confirm that B cells play an important role in MCMV-specific CD8$^+$ T-cell responses. In terms of mechanisms, our in vitro and in vivo studies clearly and strongly indicate that B cells expressing LTβ could maintain marginal metallophilic CD169$^+$ macrophages to further regulate splenic Langerin$^+$ cDC1 homeostasis. In other words, we demonstrate that B cells maintain marginal metallophilic CD169$^+$ macrophages by expressing LTβ, and the presence of marginal metallophilic CD169$^+$ macrophages is essential for the regulation of Langerin$^+$XCR1$^+$ cDC1 homeostasis, which in turn is critical for the development of an efficient CD8$^+$ T cell response to the virus.

5 Summary

CMV belongs to the β-herpesviridae family, which causes a chronic, asymptomatic infection[1]. Effective reconstitution of $CD8^+$ T lymphocytes is correlated with the recovery from HCMV infection[162]. Likewise, transplantation of virus-specific $CD8^+$ T cells provides protection in immunocompromised mice[163,164]. In contrast to T cells, the role of B cells in CMV infection is less clear.

B cells are essential for antibody secretion and mediating the humoral immune response to protect against viral infection. Moreover, B cells are crucial for the splenic architecture's development, especially for the maintenance of the marginal zone[165]. The splenic MZ is where blood-borne antigens first interact with immune cells. Here, innate and adaptive immune cells come together to guarantee an effective response to pathogenic antigens[166,167]. APCs, such as $CD169^+$ marginal metallophilic macrophages, SIGN-$R1^+$ MZ macrophages, and cDC, are dispersed throughout the splenic MZ and play an important role in the immune response to blood-borne viral infections. These cells capture antigens from the circulation for clearance and degradation, as well as for inducing the adaptive immune response[123,168,169]. According to publications, marginal metallophilic $CD169^+$ macrophages are the main cell type that is infected by viruses[170,171]. Furthermore, marginal metallophilic $CD169^+$ macrophages transfer antigens to $CD8^+$ cDCs via the CD169 molecule, which then induces an effective $CD8^+$ T cell response[39]. cDC are mainly divided into cDC1 and cDC2 lineages[172], and they can also be further classified into several distinct cDC subsets that each have different immune-modulatory functions[146,173-176]. Among the $CD8^+$ cDC1 subpopulations, $Langerin^+$cDC1s, which are predominantly located in the MZ, are the predominantly specialized cross-presenting subset capable of initiating $CD8^+$ T cell responses[88]. However, it remains unknown whether B cells can be involved in antiviral $CD8^+$ T cell responses through the APC present in the MZ.

In this study, we found that B cells are a critical factor in supporting virus-specific $CD8^+$ T cell responses. Interestingly, the diminished antigen-presenting capacity of

APC in mice lacking B cells provides a reasonable mechanistic explanation for this phenomenon. We reveal that B cells, via the expression of LTβ, are able to maintain the presence of marginal metallophilic $CD169^+$ macrophages within the splenic MZ. Moreover, using unsupervised visualization of high-dimensional flow cytometry, we showed that the absence of B cells and consequently a reduction in the marginal metallophilic $CD169^+$ macrophage population leads to a decrease in the number of $Langerin^+XCR1^+$ cDC1s, which are important in triggering MCMV-specific $CD8^+$ T cell responses.

In conclusion, the studies presented here provide new insights into the function of B cells in antiviral immunity. Besides contributing to viral clearance through the production of antibodies and cytokines, our findings confirm that B cells are able to maintain virus-specific $CD8^+$ T cell responses by controlling other APC like macrophages and dendritic cells. We hope that our findings could contribute to the understanding of the function of B cells in the immune response and offer novel ideas for disease therapy.

6 Zusammenfassung

CMV gehört zur Spezies Familie der β-Herpesviridae, die eine chronische, asymptomatische Infektionen verursachen können[1]. Eine Rekonstitution von $CD8^+$ T-Lymphozyten korreliert dabei mit der Genesung von HCMV-Infektionen[162]. Ebenso bietet die Transplantation von virusspezifischen $CD8^+$ T-Zellen Schutz vor CMV-Infektionen bei immungeschwächten Mäusen[163,164]. Im Gegensatz zu den T-Zellen ist die Rolle der B-Zellen bei der CMV-Infektionen jedoch deutlich weniger erforscht.

B-Zellen spielen eine entscheidende Rolle bei der Sekretion von Antikörpern, tragen maßgeblich zur humoralen Immunantwort bei und dienen somit dem Schutz gegen virale Infektionen. Darüber hinaus sind B-Zellen entscheidend für die Entwicklung des funktionalen Aufbaus der Milz, insbesondere für die Aufrechterhaltung der sogenannten Marginalzone[165]. In der MZ interagieren angeborene und adaptive Immunzellen mit durch Blut übertragenen Antigenen, um eine Immunreaktion unter anderem gegen virale Pathogene einzuleiten[166,167]. Auch milz-residente APCs wie $CD169^+$ marginale metallophile Makrophagen, SIGN-$R1^+$ MZ-Makrophagen und cDC spielen eine wichtige Rolle in der Abwehr von Virusinfektionen. Diese Zellen internalisieren Antigene aus dem Blutkreislauf, degradieren sie und initialisieren somit eine adaptive Immunantwort [123,168,169]. Veröffentlichungen zufolge sind marginale metallophile $CD169^+$ Makrophagen der Zelltyp, der am meisten von Viren infiziert wird[170,171]. Marginale metallophile $CD169^+$ Makrophagen übertragen Antigene über das CD169-Molekül auf $CD8^+$ cDCs, die darauf folgend eine wirksame $CD8^+$ T-Zell-Antwort auslösen können[39]. cDC werden in die Untergruppen cDC1- und cDC2[172] unterteilt, können aber auch in weitere cDC-Untergruppen unterteilt werden, mit jeweils unterschiedlichen immunmodulatorischen Funktionen haben[146,173-176]. Unter den $CD8^+$ cDC1-Subpopulationen sind Langerin$^+$cDC1, die sich vorwiegend in der milzspezifischen MZ befinden, die am stärksten im Auslösen einer $CD8^+$-T-Zell-Immunantwort spezialisierte kreuzpräsentierende [88]. Jedoch ist noch nicht bekannt, ob

B-Zellen, über die in der MZ vorhandenen APC eine Rolle bei antiviralen $CD8^+$ T-Zellantworten modulieren.

Durch diese Studie stellte sich heraus, dass B-Zellen die virusspezifischen $CD8^+$ T-Zell-Antworten begünstigen. Interessanterweise bietet die verringerte Antigenpräsentationskapazität der APC in Mäusen ohne B-Zellen eine vernünftige mechanistische Erklärung für dieses Phänomen. Wir zeigenen, dass B-Zellen durch die Expression von LTβ in der Lage sind, die Präsenz von marginalen metallophilen $CD169^+$ Makrophagen in der Milz aufrechtzuerhalten. Darüber hinaus haben wir mit Hilfe der hochdimensionalen Durchflusszytometrie gezeigt, dass die Abwesenheit von B-Zellen und folglich eine Verringerung der marginalen metallophilen $CD169^+$ Makrophagenpopulation zu einer Reduktion von $Langerin^+XCR1^+$ cDC1s führt, welche für das Auslösen einer MCMV-spezifischen $CD8^+$ T-Zell-Antworten erantwortlich sind.

Zusammenfassend lässt sich sagen, dass die hier vorgestellten Studien neue Erkenntnisse über die Funktion von B-Zellen im Kontext der antiviralen Immunität liefern. Neben dem Beitrag zur Virusbeseitigung durch die Produktion von Antikörpern und Zytokinen zeigen unsere Ergebnisse, dass B-Zellen in der Lage sind, virusspezifische $CD8^+$ T-Zellantworte aufrechtzuerhalten, indem sie APC wie Makrophagen und dendritische Zellen modulieren kontrollieren. Wir hoffen, dass unsere Erkenntnisse tragen somit zum weiteren Verständnis der Funktion von B-Zellen in der virusspezifischen Immunantwort bei und bilden demnach Grundlagen für neue Therapieansätze zur Behandlung von Erkrankungen.

7 References

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9 Eidesstattliche Versicherung

Hiemit versichere ich, dass ich die von mir vorgelegte Dissertation angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit, einschließlich Tabellen und Abbildungen, die andere Werke im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keener anderen Fakultät oder Universität zur Prüfung vorgelegen hat, dass sie noch nicht veröffentlicht worden ist. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Herrn Prof. Dr. Ari Waisman betreut worden.

Mainz, January 2024 Xinyuan Liu

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11 Research and Publications

1. Gao, P*., Ji, M*., **Liu, X***., Chen, X*., Liu, H., Li, S., Jia, B., Li, C., Ren, L., Zhao, X., et al. (2022). Apolipoprotein E mediates cell resistance to influenza virus infection. Sci Adv 8, eabm6668. 10.1126/sciadv.abm6668.
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*Contributed equally