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Research paper

Wiskott-Aldrich syndrome protein may be critical for CD8$^+$ T cell function following MCMV infection

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ABSTRACT

Wiskott-Aldrich syndrome (WAS) patients are characterized by immunodeficiency and viral infections. T cells derived from WAS patients and WAS protein (WASP)-deficient mice have various defects. However, whether WASP plays a role in immune control of cytomegalovirus (CMV) infection remains unclear. We analyzed the distribution of CD8$^+$ T subsets and the pathological damage to various organs and tissues in MCMV infected *Was* knockout (KO) mice. A relatively high number of MCMV-specific cytotoxic T cells (CTLs) were observed in the spleen of *Was* KO mice. In MCMV infected *Was* KO mice, the late differentiated CD8$^+$ T subset (CD27$^-$CD28$^-$) decreased in lungs, compared with those in the spleen and peripheral blood. Additionally, we found that the most severe pathological lesions occurred in the lungs, the main target organ of MCMV infection. By stimulating the spleen-derived CD8$^+$ T lymphocytes of *Was* KO mice, we found that IL-2 and granzyme B production declined compared with that in wild- type mice. Moreover, the number of apoptotic CD8$^+$ T cells increased in *Was* KO mice compared with the number in wild-type mice. Therefore, our results demonstrate that WASP may be involved in regulating cytotoxic function and apoptosis in CD8$^+$ T cells following MCMV infection, which is supported by the distribution and memory compartment of MCMV-specific T cells in MCMV infected *WAS* mice.

Wiskott-Aldrich syndrome (WAS) is a severe X-linked recessive immune deficiency disorder caused by mutations in the gene encoding the Wiskott-Aldrich syndrome protein (WASP), a key regulator of actin polymerization and cytoskeletal reorganization in hematopoietic cells [1–3]. Mutations in WASP result in a wide spectrum of clinical manifestations, ranging from the relatively mild X-linked thrombocytopenia to the classic WAS phenotype, characterized by microthrombocytopenia, infections caused by immunodeficiency, eczema, and high susceptibility to lymphoproliferative tumors and autoimmune diseases [2,4,5]. WASP can promote motility, adhesion, migration and activation via cytoskeletal reconstitution and actin polymerization in a variety of hematopoietic cells [6]. For T cells activated by T cell receptor (TCR) and costimulatory molecules, WASP is involved in the recruitment of actin and other synaptic proteins, cytoskeletal rearrangement and immunological synapse formation to promote the effective transmission of TCR signals and T cell proliferation [7,8].

Human cytomegalovirus (HCMV) is a low-pathogenic, opportunistic virus that is normally asymptomatic during latent infection. However, in fetuses, newborns and immunosuppressed individuals, HCMV can avoid host immune attacks to inflict damage [9], such as birth defects, intellectual impairment and systemic disseminated infection. Moreover, cytomegalovirus (CMV) infection is a crucial contributor to the death of patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) despite the existence of advanced detection and treatment methods [10–12]. CMV cytotoxic T lymphocytes (CTLs) are essential for inhibiting CMV reactivation, and effective control of CMV infection ultimately depends on CMV-specific CTL reconstitution [13]. HSCT is currently the only therapeutic option for curing WAS patients [14]. Furthermore, previous studies have shown that WAS patients have an increased risk of recurrent infections of herpes virus family members including CMV [15,16], which is not controlled by antiviral drugs [17].These data suggest that WASP might be involved in host immune control of CMV infection.

In the present study, we observed the function and distribution of CMV-specific CTLs and the percentage of CD8$^+$ T cell subsets in the main organs via a CMV infection mouse model. In addition, we

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compared the functionalities of the $CD8^+$ T cells of *Was* KO and wild type mice, aiming to explore the role of WASP in CMV-specific immune responses.

1. Materials and methods

1.1. Animals

Was knockout ($Was^{-/-}$) mice (*129S6/SvEvTac*$^{-}$*Was*$^{tm1Sbs/}$*J*) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and treated either with or without murine CMV (MCMV). Wild-type $Was^{+/+}$ mice (*129S1/SvImNJ*) were purchased from the National Resource Center of Model Mice (NRCMM) (Nanjing University, China) and treated with MCMV. The genotype of *Was* knockout mice was confirmed by PCR (Genotyping primers: $Was^{-/-}$ gene and $Was^{+/+}$ gene common forward primer: 5′-CCCATCAGGTGGTCCACTA-3′; reverse primer for $Was^{-/-}$: 5′-GCTATCAGGACATAGCGTTGG-3′; reverse primer for $Was^{+/+}$:5′-TTTGAATCCACTCGGTGCTC-3′.) (Fig. S1a, b). Both the $Was^{-/-}$ and $Was^{+/+}$ groups contained equal number of male and female mice that were between 6 and 8 weeks of age and weighing 19–22 g. The mice were fed and housed in the Chongqing Medical University Laboratory SPF Animal Center. Brood mice were placed in a cage to breed at a female to male ratio of 2:1. All animal studies were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

1.2. Cell lines and virus strains

NIH 3T3 cells were purchased from China Center for Type Culture Collection (CTCC) (Wuhan University, China). The MCMV Smith strain was purchased from American Center for Type Culture Collection (ATCC) (Manassas, VA, USA). NIH 3T3 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% bovine fetal serum (Gibco, BRL, Life Technologies) and passaged at a cell density of 1×10^6/ml. Once cells reached 80%～90% confluence, MCMV was added to propagate and passage the virus. The cells were maintained in a controlled incubator (37 °C, 5% CO_2) until 80–90% of the cells exhibited cytopathic effects (CPE), at which point the virus was collected and preserved in a −80 °C ultra-low-temperature freezer. MCMV virulence was titrated by the 50% tissue culture infectious dose (TCID50) method and calculated by the Reed-Muench method [18]. The proportionate distance (PD) was calculated as PD = [(%next above 50%) − 50%]/[(%next above 50%) − (%next below 50%)]; X = PD + (dilution next above 50%) and TCID50/ml = 10ˆX/0.1.

1.3. Establishment of the MCMV systemic dissemination model

Both $Was^{-/-}$ and $Was^{+/+}$ mice were injected intraperitoneally (ip.) with a 1×10^5 PFU MCMV suspension (0.2 ml), and additional $Was^{-/-}$ mice were injected ip. with sterile phosphate-buffered saline (PBS) as a control. The body weights, appetites, activities and reactions of the mice were observed and recorded daily. At 9 days post-MCMV infection, the mice were sacrificed, and their liver, spleen, lung, kidney, and heart tissues and salivary glands were dissected under aseptic conditions. Tissues were fixed overnight in 10% formaldehyde solution, paraffin imbedded, sectioned, hematoxylin and eosin (H&E) stained and examined for pathological damage under a light microscope. Lung lesions were scored semi-quantitatively as absent (0), minimal (1), slight (2), moderate (3), marked (4), or severe (5) [19]. MCMV gB from *Was* knockout mice was qualified by RT-PCR (forward primer: 5′-GTCGGCCATCTACGAGAGAC-3′, reverse primer: 5′-GACCAGCGGTCTCGAATAAC-3′.).

The salivary glands of the mice were fully homogenized in 1 ml of DMEM and centrifuged at 1000 × g/min for 20 min at 4 °C to separate MCMV, and the virus was then titrated for virulence. For titration, the virus was serially diluted in D-Hank's buffer solution in 10-fold increments, and 100 µl of each test homogenate was aspirated and added to an NIH 3T3 cell monolayer in a 96-well plate. In total, each dilution was tested 8 times, and the cells were observed daily. Homogenate virulence was also calculated by the Reed-Muench method.

1.4. Memory compartment of the $CD8^+$ T cell subsets

Mice from each group were sacrificed at 9 days post infection. Their spleens, lungs, and peripheral blood were immediately used to prepare single cell suspensions; 2×10^6 cells from each sample were dispensed into properly numbered tubes. The cells were washed with PBS and centrifuged, and the supernatants were aspirated. The pellets were resuspended in approximately 50 µl of the remaining PBS, and the cells were surface-stained with fluorochrome-labeled anti-CD3-PE (145-2C11, BD PharMingen, San Diego, CA, USA), anti-CD8-PerCP (53–6.7, BD PharMingen, San Diego, CA, USA), anti-CD27-FITC (LG.7F9, eBioscience, San Diego, CA, USA), and anti-CD28-APC (37.51, eBioscience, San Diego, CA, USA) at 4 °C in the dark for 30 min. Red blood cell lysis buffer (Beyotime Biotechnology, China) was added to peripheral blood, which was then washed and evaluated for the percentage of cytotoxic T cell subsets among the effector cells by flow cytometry. Data were analyzed by FlowJo Version 7.0 software (Tree Star, Inc.).

1.5. MCMV-specific CTL detection by pentamer staining

$Was^{+/+}$and $Was^{-/-}$mice were sacrificed at 9 days post infection, and their spleens, lungs, and peripheral blood were immediately harvested to prepare single cell suspensions; 2×10^6 cells from each sample were dispensed into several tubes. After being washed with PBS, the cell supernatants were aspirated, and MHC-H-2Db/HGIRNASFI (murine CMV m45 985–993) R-PE-labeled Pro MHC pentamers (ProImmune, Oxford, United Kingdom) were added to the cell pellet. The mixture was well mixed with a vortexer and incubated for 10 min in the dark at room temperature. After incubation, anti-CD8-PerCP (53–6.7, BD PharMingen, San Diego, CA, USA) was added, and the mixture was incubated for 30 min on ice. Next, 3 ml of red blood cell lysis buffer was added to each sample for 5 min, and the percentage of CTLs in the $CD8^+$ T cell subset was measured by flow cytometry. MCMV CTLs were defined as being double-positive for CD8 cells and a pentamer. The percentage of MCMV CTLs among $CD8^+$ T cells was calculated as ($CD8^+$/$pentamer^+$)/$CD8^+$ cells.

1.6. Intracellular cytokine staining and CD107a mobilization assay

$CD8^+$T cells were obtained from the spleens of $Was^{-/-}$ and $Was^{+/+}$ mice using a $CD8a^+$ T cell isolation kit (Miltenyi Biotech, Bergisch, Germany). Briefly, a biotin-antibody cocktail was added to the spleen suspension, the mixture was incubated in the dark for 10 min at 4 °C, and anti-biotin microbeads were added for 15 min. The cells were washed with MACS buffer, centrifuged, and resuspended before $CD8^+$T cells were negatively isolated using an LS column and MACS separator. Flow-through cells containing enriched murine $CD8^+$ T cells were collected, stained with trypan blue and evaluated for purity. The isolated $CD8^+$ T cells were cultured overnight in a 24-well plate, which was coated with 1 µg/ml anti-CD3 and 5µg/ml anti-CD28 (BD Bioscience). Before fluorescence staining, the protein transfer inhibitor GolgiStop™ (BD Bioscience, Palo Alto, CA, USA) and anti-CD107a-FITC (1D4B, BD PharMingen) were added to each well for 4 h. The cells were then collected, and anti-CD8-PerCP (53–6.7, BD PharMingen, San Diego, CA, USA) was added for 30 min in the dark on ice. After 30 min, the cells were fixed and permeabilized with a fixation/permeabilization solution kit (BD Bioscience). The intracellular cytokine antibodies anti-

IFN-γ-FITC (XMG1.2, BD PharMingen), anti-IL-2-PE (JES6-5H4, BD PharMingen) or anti-granzymeB-FITC (NGZB, eBioscience) antibodies were then added, and the mixture was incubated in the dark for 30 min at 4 °C. The cells were washed with FACS buffer, and the percentages of $CD8^+$/CD107a$^+$,$CD8^+$/IFN-γ$^+$,$CD8^+$/IL-2$^+$ and $CD8^+$/granzymeB$^+$ cells among $CD8^+$ T lymphocytes were detected by flow cytometry.

1.7. Apoptosis assay

Freshly isolated $CD8^+$ T cells were incubated for 48 h in a 24-well culture plate coated with 1 μg/ml anti-CD3 and 5 μg/ml anti-CD28. Cells were collected, resuspended in annexin V binding buffer, and stained with FITC-labeled annexin V and propidium iodide (PI) (KeyGEN BioTECH company, China) in the dark for 30 min at 4 °C. The apoptosis rates of $CD8^+$ T cell subsets were then detected by flow cytometry. Annexin V/PI co-staining was performed to discriminate among early apoptotic, late apoptotic/secondary necrotic and necrotic cells, or between apoptotic and non-apoptotic cells, respectively. Annexin V-/PI− were regarded as live cells, Annexin V+/PI− as apoptic, and Annexin V+/PI+ as late apoptic cells. The frequency of each apoptotic cell category described above was determined by FlowJo Version 7.0.

1.8. Statistical analysis

Student's *t*-test and ANOVA were applied using SPSS 12.0 software (SPSS Inc., Chicago, IL). Data are shown as mean ± SD. P-values < 0.05 were considered statistically significant.

2. Results

2.1. MCMV infection severely damaged the lungs of $Was^{-/-}$ mice

Mice that were i.p. injected with 1×10^5 PFU MCMV exhibited obviously poor appetites, less activity, somnolence, fur sparsity and lags in response to stimulation that gradually appeared on the 2nd day post injection. Lower body mass and pilomotor fur were particularly evident in the treated $Was^{-/-}$ mice, while the $Was^{+/+}$ mice showed all the above symptoms to a lesser extent. Beginning on the 5th day after infection, the body masses of the MCMV-infected $Was^{-/-}$ mice were much more reduced than those of $Was^{-/-}$ mice without MCMV infection ($p < 0.05$). After the 7th day, the body masses of the treated $Was^{-/-}$ mice were reduced more than those of MCMV-infected $Was^{+/+}$ mice ($p < 0.05$) (Fig. 1a).

Compared with $Was^{+/+}$ mice, *Was* KO mice had more evident pathological lesions on their hearts, livers, lungs and kidneys after infection with MCMV (Fig. 1b). Histopathological examination revealed interstitial edema, partial cytolysis and disordered arrangement in myocardial cells, and hepatocytes with vacuole-like denaturation, structural derangement and lytic necrosis. Both groups of mice showed enlarged glomerular sizes and smaller glomerular capsules, but *Was* KO mice exhibited greater vacuolar degeneration, necrosis and renal tubule epithelial cell edema. The lung tissues of *Was* KO mice were pathologically characterized by interstitial pneumonia (with an even thicker alveolar septum), sharply increased cellular components, interstitial congestion, edema (with inflammatory cell infiltration comprising mostly mononuclear cells), and less alveolar exudate in the alveolar space (Fig. 1c, d). Furthermore, MCMV virulence was titrated by TCID50 in salivary gland ($WAS^{-/-}$: 4.59 ± 0.15, $WAS^{+/+}$: 4.47 ± 0.12, p > 0.05). However, the level of MCMV gB was higher in lung of MCMV-infected $Was^{-/-}$ mice than in $Was^{+/+}$ mice (p < 0.05) (Fig. S2a, b).

2.2. Fewer MCMV-specific CTLs resident in the lung

The percentage of MCMV CTLs was higher in the spleen than in the lung or peripheral blood of MCMV-treated *Was* KO *129S6* mice [(2.71 ± 0.97) % vs (0.31 ± 0.15) %, respectively, $p < 0.001$; and (2.71 ± 0.97) % vs (0.6 ± 0.69) %, respectively, $p < 0.001$], while the percentage of MCMV CTLs in the spleen, lung and peripheral blood was not significantly different in $Was^{+/+}$ mice. In the lung, the percentage of MCMV CTLs was (1.48 ± 0.71)% and (0.31 ± 0.15)% in $Was^{+/+}$ and $Was^{-/-}$ mice, respectively ($p = 0.007$). However, the percentage of MCMV CTLs in the spleen or peripheral blood of $Was^{-/-}$ mice was not significantly different from that of $Was^{+/+}$ mice [(2.71 ± 0.97)% vs (4.86 ± 2.48)%, respectively, $p = 0.055$ and (0.60 ± 0.69)% vs (2.92 ± 3.32)%, respectively, $p = 0.117$] (Fig. 2).

2.3. Fewer $CD27^-CD28^-CD8^+$ T cell subsets in the lung

The percentage of $CD27^+CD28^-CD8^+$ T cells was higher in the lungs of treated $Was^{-/-}$ mice than in the spleen [(64.14 ± 18.50) % vs (32.17 ± 20.71) %, respectively, $p = 0.006$] and peripheral blood [(64.14 ± 18.50) % vs (29.75 ± 11.85) %, respectively, $p = 0.004$]. However, no distinct differences among the spleen, lung and peripheral blood were found in $Was^{+/+}$ mice. Moreover, in the lung, spleen and peripheral blood, the ratio of $CD27^+CD28^-CD8^+$ T cells to $CD8^+$ T lymphocytes was not markedly different between treated $Was^{-/-}$ and $Was^{+/+}$ mice (Fig. 3a–c).

The ratio of $CD27^-CD28^-CD8^+$ T cells to $CD8^+$ T cells in the lung was (24.28 ± 13.45) % in treated $Was^{-/-}$ mice and (43.16 ± 7.87) % in treated $Was^{+/+}$ mice ($p = 0.033$). The percentages of $CD27^-CD28^-CD8^+$ T cells in the spleen and peripheral blood of treated $Was^{-/-}$ mice were not significantly different than those in $Was^{+/+}$ mice. In $Was^{-/-}$ mice, the percentage of $CD27^-CD28^-CD8^+$ T cells was lower in the lung than in the spleen and peripheral blood [(24.28 ± 13.45) % vs (53.11 ± 11.44) %, respectively, $p = 0.001$] and [(24.28 ± 13.45) % vs (56.54 ± 10.03) %, respectively, $p < 0.001$]. In $Was^{+/+}$mice, the percentage of $CD27^-CD28^-CD8^+$ T cells was not significantly different among the lung, spleen and peripheral blood (Fig. 3a, d).

2.4. Impaired $CD8^+$ T lymphocyte functionality in Was KO mice

Anti-CD3 and anti-CD28 antibodies were combined to stimulate sorted $CD8^+$ T lymphocytes isolated from spleen in vitro for 48 h. Histograms were generated to compare the two groups of $Was^{-/-}$ mice and $Was^{+/+}$ mice. The percentage of $CD8^+$ T cells expressing IL-2 was lower in treated $Was^{-/-}$ mice [(3.35 ± 1.47)%] than in $Was^{+/+}$ mice [(9.14 ± 4.15)%, $p = 0.009$] (Fig. 4a). In *Was* KO mice, fewer $CD8^+$ T lymphocytes produced granzyme B compared with $Was^{+/+}$ mice [(1.74 ± 1.33) % vs (3.40 ± 1.47) %, $p = 0.048$] (Fig. 4b). However, IFN-γ and CD107a expression in $CD8^+$ T lymphocytes was not significantly different between $Was^{-/-}$ and $Was^{+/+}$ mice (Fig. 4c, d).

2.5. Apoptosis of $CD8^+$ T lymphocytes in Was KO mice

Two days after co-culturing mouse $CD8^+$ T lymphocytes with anti-CD3 and anti-CD28 monoclonal antibodies in vitro, we analyzed the cells by flow cytometry, and the apoptosis rate was calculated. We defined apoptotic cells as those in the early and late fractions. The apoptosis rate of $CD8^+$ T lymphocytes from $Was^{+/+}$ mice was (30.50 ± 4.42) %, which was lower than that of $CD8^+$ T lymphocytes from treated $Was^{-/-}$ mice [(40.44 ± 5.79) %, $p = 0.007$]. Furthermore, the apoptosis rate of $CD8^+$ T lymphocytes from $Was^{+/+}$ [(16.40 ± 2.19)%] and treated $Was^{-/-}$ mice [(25.09 ± 5.44) %] without anti-CD3 and anti-CD28 antibody stimulation was significantly different ($p = 0.009$), potentially due to spontaneous apoptosis (Fig. 5).

3. Discussion

The clinical manifestations of CMV infection in WAS patients are

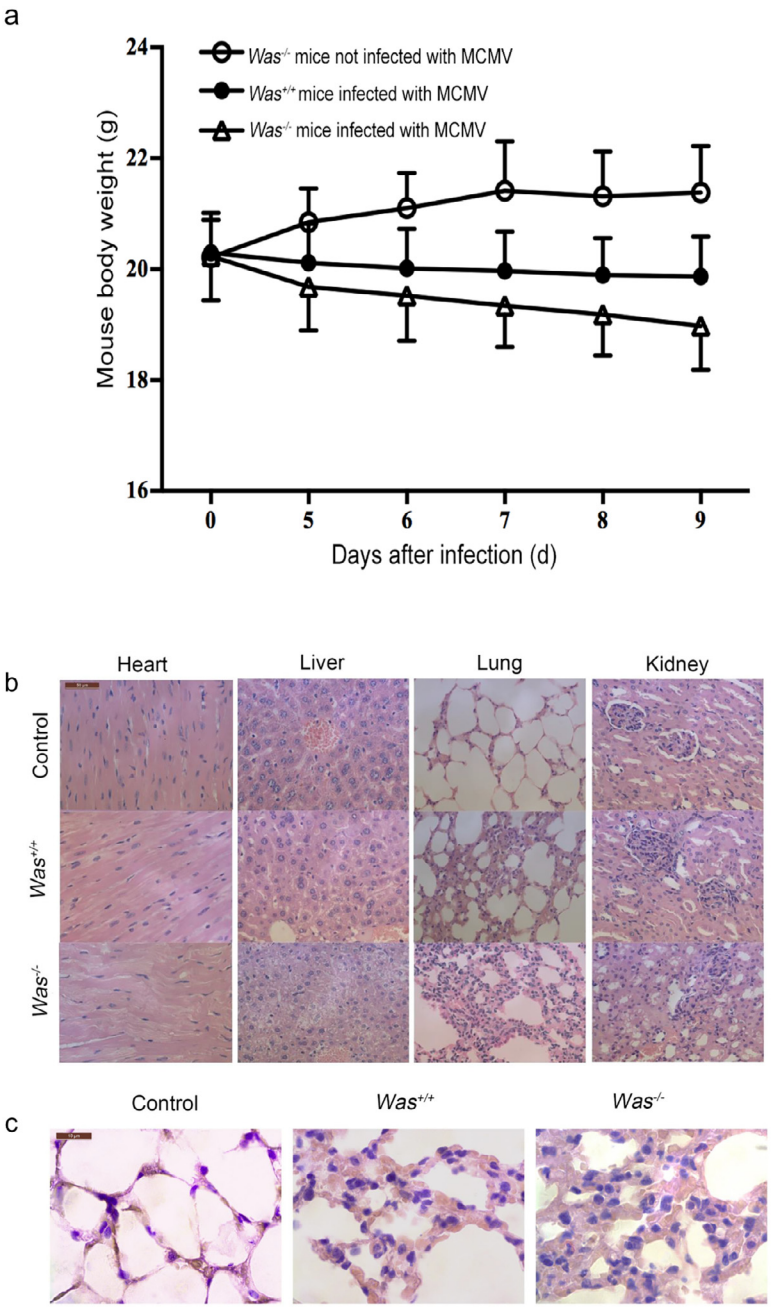

Fig. 1. MCMV infection severely damaged the lungs of *Was*$^{-/-}$ mice. Beginning on the 5th day post-infection, the body weight of treated *Was*$^{-/-}$ mice was clearly reduced compared with that of uninfected *Was*$^{-/-}$ mice and MCMV-infected *Was*$^{+/+}$ mice (Fig. 1a). Histological examination of the organs from WT 129S1 mice and *Was* KO mice infected with MCMV, as determined by hematoxylin & eosin staining; 400 × magnification (Fig. 1b); 1000 × magnification (Fig. 1c). Fig. 1b shows pathological changes in heart, liver, lung and kidney tissue. Fig. 1c shows that *Was* KO mice have more obvious inflammatory changes in their lungs, including a thicker alveolar septum, more cellular components, interstitial hyperemia and edema with marked infiltration of mononuclear cells. Fig. 1d indicated severity scores of pulmonary lesions of mice after infection with MCMV. Indicated pulmonary lesions are scored from absent (0) to severe (5) (n = 4/group).

more severe than those of other patients with CMV [20–22]. Alternatively, the effective reconstitution of CMV-specific CTLs is crucial for clearing CMV infection in patients with CMV infection; which indicates that WASP may be involved in regulating the distribution and function of CMV-specific CTLs. Since MCMV has the same tissue tropism as HCMV which is closely related to host age and immune status [23,24], we analyzed the main organs and functionality of CD8$^+$ T cell subsets in MCMV-infected *Was* KO mice to elucidate the role of WASP in CMV-specific CTL function.

The lungs are frequently challenged by viruses including CMV [25,26], and lethal pneumonia is among the most common complications in WAS patients [15]. Several case reports also indicated that WAS patients suffered from recurrent CMV pneumonia [22,27–29]. Andreansky et al. [30] reported that *Was* KO mice infected with three different pathogenic microorganisms, influenza A virus, *Streptococcus pneumoniae* and Bacillus Calmette-Guerin vaccine showed increasing lung susceptibility and dysfunctional pathogen elimination. Consistent with these findings, our study showed that the lungs of MCMV-infected *Was* KO mice exhibited the most serious pathological damage and slower clearance of virus from lung. It is supported by the fact that

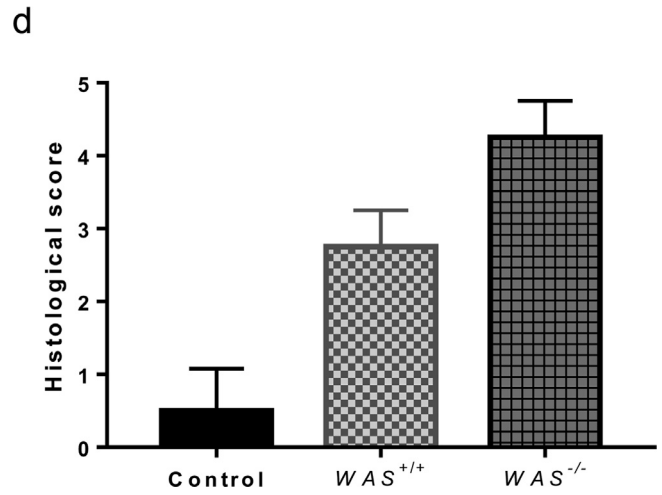

Fig. 1. (*continued*)

T cells ($CD27^+CD28^+$), intermediately differentiated T cells ($CD27^+CD28^-$) and late-differentiated T cells ($CD27^-CD28^-$) [37]. $CD27^-CD28^-CD8^+$ T cells are mostly cytotoxic effector cells [38,39] that express cytotoxic factors and produce cytokines, including granzyme B and IL-2 [40,41]. Compared with late-differentiated T cells, intermediately differentiated T cells display decreased cytotoxic capacity, and their functionality lies between that of naïve and late-differentiated T cells [37]. Hiroko et al. [41] identified an inverse correlation between the quantitative expression of CD27 and cytotoxic effector function in peripheral blood after infection with human CMV. In our study, $CD8^+$ T lymphocytes were found to mainly reside in the late-differentiated T cell subsets in $Was^{+/+}$ mice, but not $Was^{-/-}$ mice, after MCMV infection. These findings illustrate that WASP may impact the differentiation of $CD8^+$ T cells from intermediately differentiated T cells to late-differentiated T cells and further affect the antiviral efficacy of late $CD8^+$ T cells. Previous studies had shown that viral load determines the memory $CD8^+$ T cell phenotype [42]. High frequency of virus antigen encountered at the site of infection changes the CMV-

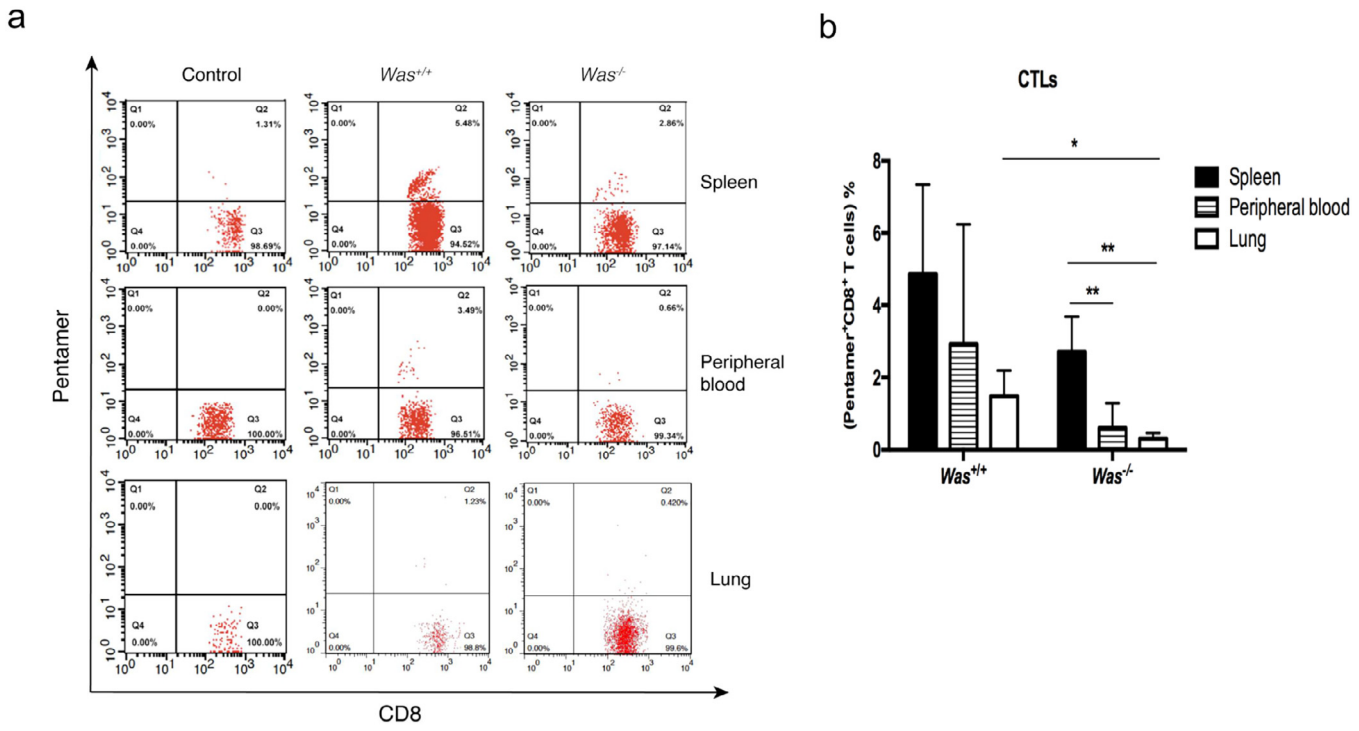

Fig. 2. Fewer MCMV-specific CTLs resident in the lung after MCMV infection. MCMV CTLs identified by pentamer and CD8 staining in spleen, peripheral blood and lung cells of three groups of mice (Fig. 2a). Comparison of the percentages of MCMV CTLs in the spleens, peripheral blood and lungs of $Was^{+/+}$ and $Was^{-/-}$ mice (Fig. 2b) (*$p < 0.05$; **$p < 0.01$) (n = 7). Fewer MCMV CTLs were present in the lungs of treated $Was^{-/-}$ mice. In addition, more MCMV CTLs were observed in the spleens of $Was^{-/-}$ mice than in the peripheral blood and lungs.

MCMV-specific CTLs and late-differentiated effector T cells were reduced when the *Was* gene was knocked out, which can lead to impaired surveillance of this virus. Interestingly, we found that the percentage of MCMV CTLs in the spleen was a bit higher than those in the lung and peripheral blood. Because MCMV establishes latency in a variety of tissues similar to HCMV, including the spleen, lungs, and bone marrow [26,31], tissue-specific immune response as well as those in peripheral blood behavior differently and probably contribute the control CMV infection [32,33]. As an MCMV latency site, the spleen was determined to be the first tissue to eradicate MCMV infection because of the presence of CTLs [34–36], which could also play a vital role during the infection stage.

Based on expression of the costimulatory receptors CD27 and CD28, $CD8^+$ T lymphocytesare divided into three types of T cells, early naïve

specific $CD8^+$ T cell phenotype and results in the late-differentiation phenotype playing a dominant role in non-immunodeficient individuals [32]. In contrast to $Was^{+/+}$ mice, MCMV treated $Was^{-/-}$ mice had lower levels of late-differentiated $CD8^+$ T cells in the lung, which indicated that the WAS protein was positively correlated with the effector ability of $CD8^+$ T cells.

WASP has been implicated in a number of intrinsic T cell functions, including cell proliferation, differentiation and survival, through both actin-dependent and independent mechanisms [43]. WASP is crucial for the maintenance of immunological synapse (IS) symmetry, while the breakdown of SMACs (supramolecular activation clusters) plays a vital role in IS migration [44,45]. The dynamic IS balance between WASP and SMACs contributes to T cell activation and effector function. Dysfunctional lymphocytes in *Was*-deficient mice and patients have been

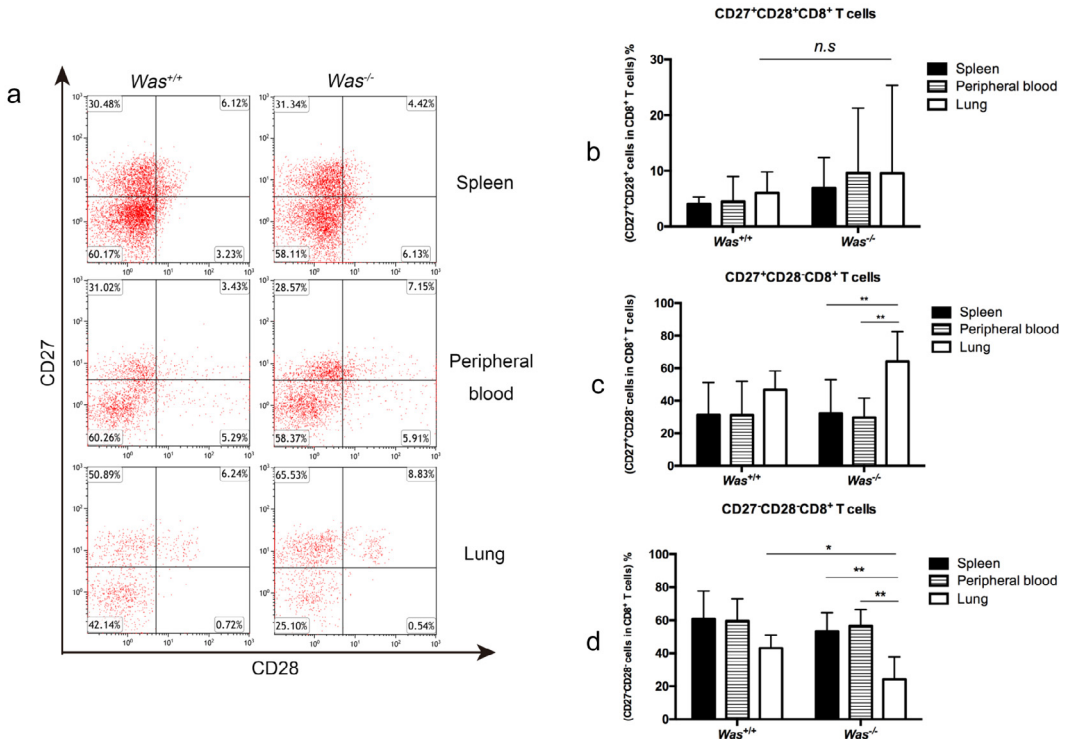

Fig. 3. Distribution of CD27/CD28 in $CD8^+$ T cells in various organs and tissues. CD27/CD28 gated on $CD8^+$ *T*-cell populations of distinct organs in $Was^{+/+}$and $Was^{-/-}$ mice (Fig. 3a). $CD27^+CD28^+$ T cell populations were not significantly different between the $Was^{+/+}$ and $Was^{-/-}$ mice in terms of various organs. The lungs of $Was^{-/-}$ mice had a higher percentages of $CD27^+CD28^-$ T cells and a lower percentages of $CD27^-CD28^-$ T cells than the spleens and peripheral blood. The $CD27^-CD28^-$ T cell population in the lungs also decreased when the *Was* gene was knocked out (Fig. 3b–d) ($*p < 0.05$; $**p < 0.01$) (n = 7).

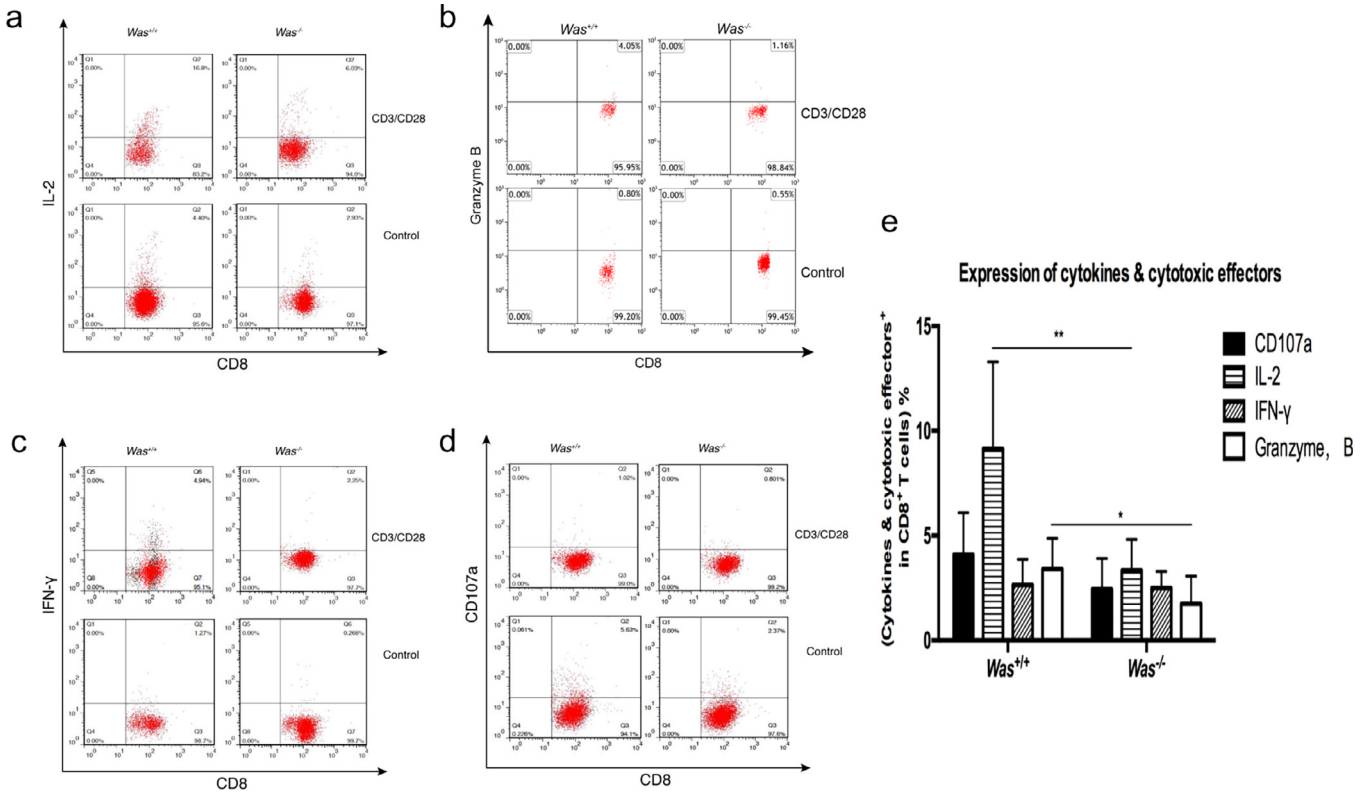

Fig. 4. Functional changes in the spleen lymphocyte $CD8^+$ T cells of $Was^{+/+}$ and $Was^{-/-}$ mice cultured in vitro. Multicolor (IL-2 (Fig. 4a), granzyme B (Fig. 4b), IFN-γ (Fig. 4c), and CD107a (Fig. 4d)) staining of spleen $CD8^+$ T cells stimulated in vitro and statistical analysis of the percentages of, IL-2^+, granzyme B^+, IFN-γ^+ and CD107a^+ T cells (Fig. 4e) among $CD8^+$ T cells from $Was^{+/+}$ and $Was^{-/-}$ mice ($*p < 0.05$) (n = 7). Background values were subtracted. The histograms show decreased IL-2 and granzyme B levels in *Was* $^{-/-}$ mice.

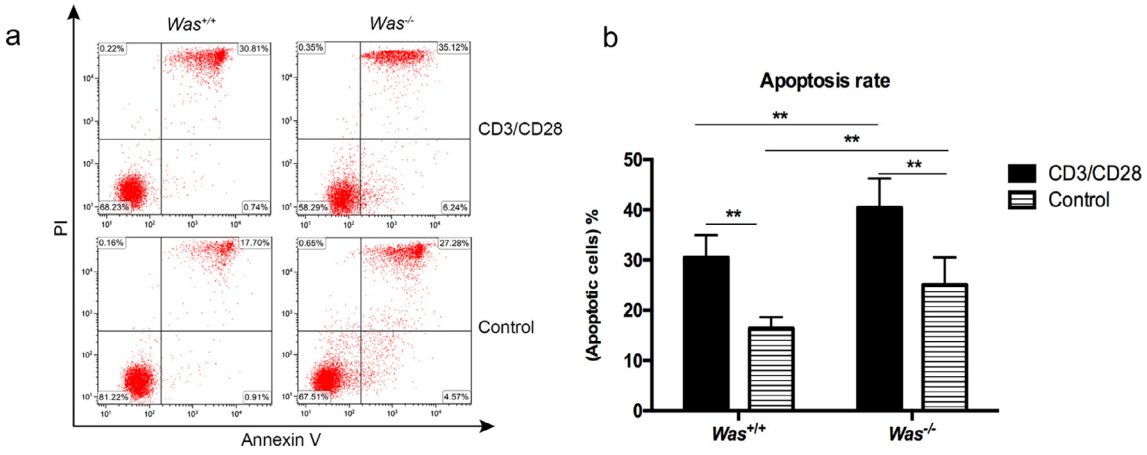

Fig. 5. Apoptosis rates of spleen CD8$^+$T lymphocytes cultured in vitro. Apoptosis rates of spleen CD8$^+$ T lymphocytes from *Was*$^{+/+}$ and *Was*$^{-/-}$ 129S6 mice (either coated or not coated with anti-CD3 and anti-CD28) as detected with PI and Annexin V staining (Fig. 5a). The apoptosis rate of CD8+ T lymphocytes from *Was*$^{+/+}$ mice was lower than that of CD8+ T lymphocytes from treated *Was*$^{-/-}$ mice. Furthermore, the apoptosis rate of CD8+ T lymphocytes from *Was*$^{+/+}$ and treated *Was*$^{-/-}$ mice without anti-CD3 and anti-CD28 antibody stimulation was significantly different, potentially due to spontaneous apoptosis (Fig. 5b) (n = 6) (*$p < 0.05$, **$p < 0.01$).

studied multiple times, including by our group [46–51]. WAS KO mice showed reduced viral clearance and enhanced immunopathology during LCMV infection [50]. This was attributed to both an intrinsic CD8$^+$ T cell defect and defective priming of CD8$^+$ T cells by dendritic cells. Consistent with Lang's result, we confirmed these findings in WAS KO mice infected with MCMV. However, Lang et al. found virus-induced hepatic immunopathology in WAS KO mice with LCMV, while our data indicated severe lung damage induced directly by MCMV in WAS KO mice. T cells from WAS patients harbor defects in antigen-driven proliferation and cytokine production [52], while these cells proliferate poorly, secrete low levels of IL-2 in response to anti-CD3 mAb and display abnormal cell morphology [53,54]. In Wiskott-Aldrich syndrome patients or mice, low cytokine expression might be induced by broken IS symmetry, low *T*-bet mRNAexpression, aberrant AP-1 (activating protein-1) expression or dysfunctional NFAT-1/2 (nuclear factor of activated T cells) nuclear translocation [45,49,55]. Furthermore, WASP deficiency affects the cytotoxic activity of CD8$^+$ T cells [56]. Our results further show that CD8$^+$ T lymphocytes from *Was*$^{-/-}$ mice were defective in secreting cytokines and cytotoxic effectors after MCMV infection. Consequently, IL-2 and granzyme B secretion and CD8$^+$ T cell differentiation were damaged, and late-differentiated cells were reduced, indicating that WASP deficiency indirectly influences CD8+ T cell subsets and leads to viral clearance failure.

Lymphocytes isolated from WASP-deficient patients reportedly exhibit strong actin polymerization in response to phorbol-12-myristate-13-acetate (PMA) and bryostatinstimulation, whereas WASP-deficient lymphocytes are incapable of polymerizing actin when stimulated with anti-CD3 [57,58]. We found that the apoptosis rate of lymphocytes from *Was* KO mice was increased upon anti-CD3 and anti-CD28 stimulation, indicating that WASP plays a vital role in regulating lymphocyte apoptosis. WASP-deficient lymphocytes showed upregulated FAS expression and increased caspase-3 activation, which mediate accelerated apoptosis [58]. In addition, cytoskeleton-associated proteins are closely associated with cell apoptosis regulation [59,60]. Because WASP might delay apoptosis using cytoskeleton accessories, apoptosis is increased when *Was* is deficient [61]. Thus, WASP might affect the activation and function of CD8$^+$ T lymphocytes, perhaps by altering the number and structure of microvillion WASP-deficient T lymphocytes, which structurally damages the actin cytoskeleton [62,63]. Therefore, when stimulated with an anti-CD3 monoclonal antibody, T cells from *Was*$^{-/-}$ mice can not polymerize actin or recombine the actin cytoskeleton, ultimately affecting CTL activation [48,64].

In conclusion, our results suggest that MCMV infection most prominently affects the lungs of *Was* KO mice. We also confirm previous finding that the production of cytokines and cytotoxic effectors by CD8$^+$ T cells from *Was* KO deficient mice is defective. Further studies are needed to more deeply explore the roles of WASP in lung viral clearance and CD8$^+$ T cell subsets in WAS patients infected with CMV.

Declaration of interests

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellimm.2019.03.004.

References

[1] Y. Jin, C. Mazza, J.R. Christie, S. Giliani, M. Fiorini, P. Mella, et al., Mutations of the Wiskott-Aldrich Syndrome Protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation, Blood 104 (13) (2004) 4010–4019.
[2] H.D. Ochs, Mutations of the wiskott-aldrich syndrome Protein affect protein expression and dictate the clinical phenotypes, Immunol. Res. 44 (1–3) (2009) 84–88.
[3] M.J. Massaad, N. Ramesh, R.S. Geha, Wiskott-Aldrich syndrome: a comprehensive review, Ann. N. Y. Acad. Sci. 1285 (2013 May) 26–43.
[4] M. Bosticardo, F. Marangoni, A. Aiuti, A. Villa, M. Roncarolo Grazia, Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome, Blood 113 (25) (2009) 6288–6295.
[5] K. Imai, S. Nonoyama, H.D. Ochs, WASP (Wiskott-Aldrich syndrome protein) gene mutations and phenotype, Curr. Opin. Allergy Clin. Immunol. 3 (6) (2003) 427–436.
[6] A.J. Thrasher, S.O. Burns, WASP: a key immunological multitasker, Nat. Rev. Immunol. 10 (3) (2010 Mar) 182–192.
[7] L. Dupre, A. Aiuti, S. Trifari, S. Martino, P. Saracco, C. Bordignon, et al., Wiskott-Aldrich syndrome protein regulates lipid raft dynamics during immunological synapse formation, Immunity 17 (2) (2002) 157–166.
[8] O. Matalon, B. Reicher, M. Barda-Saad, Wiskott-Aldrich syndrome protein-dynamic regulation of actin homeostasis: from activation through function and signal termination in T lymphocytes, Immunol. Rev. 256 (1) (2013 Nov) 10–29.
[9] P.D. Griffiths, S. Walter, Cytomegalovirus, Curr. Opin. Infect. Dis. 18 (3) (2005) 241–245.
[10] E.J. Ariza-Heredia, L. Nesher, R.F. Chemaly, Cytomegalovirus diseases after hematopoietic stem cell transplantation: a mini-review, Cancer Lett. 342 (1) (2014

Jan 01) 1–8.
[11] V. Bhat, A. Joshi, R. Sarode, P. Chavan, Cytomegalovirus infection in the bone marrow transplant patient, World J. Transplant. 5 (4) (2015) 287–291.
[12] M. Boeckh, W.G. Nichols, G. Papanicolaou, R. Rubin, J.R. Wingard, J. Zaia, Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges, and future strategies, Biol Blood Marrow Transplant. 9 (9) (2003) 543–558.
[13] X.H. Luo, X.J. Huang, K.Y. Liu, L.P. Xu, D.H. Liu, Protective immunity transferred by infusion of cytomegalovirus-specific CD8(+) T cells within donor grafts: its associations with cytomegalovirus reactivation following unmanipulated allogeneic hematopoietic stem cell transplantation, Biol Blood Marrow Transplant. 16 (7) (2010 Jul) 994–1004.
[14] H. Ozsahin, M. Cavazzana-Calvo, L.D. Notarangelo, A. Schulz, A.J. Thrasher, E. Mazzolari, et al., Long-term outcome following hematopoietic stem-cell transplantation in Wiskott-Aldrich syndrome: collaborative study of the European Society for Immunodeficiencies and European Group for Blood and Marrow Transplantation, Blood. 111 (1) (2008) 439–445.
[15] K.E. Sullivan, C.A. Mullen, R.M. Blaese, J.A. Winkelstein, A multiinstitutional survey of the Wiskott-Aldrich syndrome, J Pediatr. 125 (6 Pt 1) (1994 Dec) 876–885.
[16] K. Imai, T. Morio, Y. Zhu, Y. Jin, S. Itoh, M. Kajiwara, et al., Clinical course of patients with WASP gene mutations, Blood 103 (2) (2004) 456–464.
[17] W.I. Lee, J.L. Huang, T.H. Jaing, K.H. Wu, Y.H. Chien, K.W. Chang, Clinical aspects and genetic analysis of taiwanese patients with wiskott-Aldrich syndrome protein mutation: the first identification of x-linked thrombocytopenia in the chinese with novel mutations, J. Clin. Immunol. 30 (4) (2010 Jul) 593–601.
[18] S. Welkos, A. O'Brien, Determination of median lethal and infectious doses in animal model systems, Methods Enzymol. 235 (1994) 29–39.
[19] M. Barends, M. van Oosten, C.G.H. de Rond, J.A.M.A. Dormans, A.D.M.E. Osterhaus, H.J. Neijens, et al., Timing of infection and prior immunization with respiratory syncytial virus (RSV) in RSV-enhanced allergic inflammation, J. Infect. Dis. 189 (10) (2004) 1866–1872.
[20] W.G. Nichols, L. Corey, T. Gooley, C. Davis, M. Boeckh, High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection, J. Infect. Dis. 185 (3) (2002 Feb 01) 273–282.
[21] E. Haastrup, K. Muller, H. Baekgaard, C. Heilmann, Cytomegalovirus infection after allogeneic stem cell transplant in children, Pediatr. Transplant. 9 (6) (2005 Dec) 734–740.
[22] K.Y. Huang, M.W. Lai, W.I. Lee, Y.C. Huang, Fatal cytomegalovirus gastrointestinal disease in an infant with Wiskott-Aldrich syndrome, J. Formos Med. Assoc. 107 (1) (2008 Jan) 64–67.
[23] C. Sinzger, G. Jahn, Human cytomegalovirus cell tropism and pathogenesis, Intervirology 39 (5–6) (1996) 302–319.
[24] E.S. Mocarski, R.F. Pass, Human cytomegalovirus: general features, Encycl. Virol. (2008) 474–485.
[25] M.J. Reddehase, F. Weiland, K. Munch, S. Jonjic, A. Luske, U.H. Koszinowski, Interstitial murine cytomegalovirus pneumonia after irradiation: characterization of cells that limit viral replication during established infection of the lungs, J. Virol. 55 (2) (1985 Aug) 264–273.
[26] M. Balthesen, M. Messerle, M. Reddehase, Lungs are a major organ site of cytomegalovirus latency and recurrence, J. Virol. 67 (9) (1993) 5360–5366.
[27] M. Kaneko, T. Watanabe, H. Watanabe, M. Kimura, H. Suzuya, Y. Okamoto, et al., Successful unrelated cord blood transplantation in an infant with Wiskott-Aldrich syndrome following recurrent cytomegalovirus disease, Int. J. Hematol. 78 (5) (2003 Dec) 457–460.
[28] D. Richter, W. Hampl, F. Pohlandt, Vertical transmission of cytomegalovirus, most probably by breast milk, to an infant with Wiskott-Aldrich syndrome with fatal outcome, Eur. J. Pediatr. 156 (11) (1997 Nov) 854–855.
[29] M. Balthesen, M. Messerle, M.J. Reddehase, Lungs are a major organ site of cytomegalovirus latency and recurrence, J. Virol. 67 (9) (1993 Sep) 5360–5366.
[30] S. Andreansky, H. Liu, S. Turner, J.A. McCullers, R. Lang, R. Rutschman, et al., WASP- mice exhibit defective immune responses to influenza A virus, Streptococcus pneumoniae, and Mycobacterium bovis BCG, Exp. Hematol. 33 (4) (2005 Apr) 443–451.
[31] T. Collins, C. Pomeroy, M.C. Jordan, Detection of latent cytomegalovirus DNA in diverse organs of mice, J. Infect. Dis. 168 (3) (1993) 725–729.
[32] G.J. de Bree, E.M. van Leeuwen, T.A. Out, H.M. Jansen, R.E. Jonkers, R.A. van Lier, Selective accumulation of differentiated CD8+ T cells specific for respiratory viruses in the human lung, J. Exp. Med. 202 (10) (2005 Nov 21) 1433–1442.
[33] M. Almanan, J. Raynor, A. Sholl, M. Wang, C. Chougnet, R.D. Cardin, et al., Tissue-specific control of latent CMV reactivation by regulatory T cells, PLoS Pathog. 13 (8) (2017) e1006507.
[34] S. Jonjic, W. Mutter, F. Weiland, M.J. Reddehase, U.H. Koszinowski, Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes, J. Exp. Med. 169 (4) (1989 Apr 01) 1199–1212.
[35] J. Mercer, C. Wiley, D. Spector, Pathogenesis of murine cytomegalovirus infection: identification of infected cells in the spleen during acute and latent infections, J. Virol. 62 (3) (1988) 987–997.
[36] J.A. Mercer, C.A. Wiley, D.H. Spector, Pathogenesis of murine cytomegalovirus infection: identification of infected cells in the spleen during acute and latent infections, J. Virol. 62 (3) (1988) 987–997.
[37] V. Appay, P.R. Dunbar, M. Callan, P. Klenerman, G.M. Gillespie, L. Papagno, et al., Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections, Nat. Med. 8 (4) (2002) 379–385.
[38] D.N. Posnett, J.W. Edinger, J.S. Manavalan, C. Irwin, G. Marodon, Differentiation of human CD8 T cells: implications for in vivo persistence of CD8+ CD28- cytotoxic effector clones, Int. Immunol. 11 (2) (1999) 229–241.
[39] D. Hamann, P.A. Baars, M.H. Rep, B. Hooibrink, S.R. Kerkhof-Garde, M.R. Klein, et al., Phenotypic and functional separation of memory and effector human CD8+ T cells, J. Exp. Med. 186 (9) (1997) 1407–1418.
[40] P. Romero, A. Zippelius, I. Kurth, M.J. Pittet, C. Touvrey, E.M. Iancu, et al., Four functionally distinct populations of human effector-memory CD8+ T lymphocytes, J. Immunol. 178 (7) (2007) 4112–4119.
[41] H. Tomiyama, H. Takata, T. Matsuda, M. Takiguchi, Phenotypic classification of human CD8+ T cells reflecting their function: inverse correlation between quantitative expression of CD27 and cytotoxic effector function, Eur. J. Immunol. 34 (4) (2004) 999–1010.
[42] L.E. Gamadia, E.M. van Leeuwen, E.B. Remmerswaal, S.L. Yong, S. Surachno, P.M. Wertheim-van Dillen, et al., The size and phenotype of virus-specific T cell populations is determined by repetitive antigenic stimulation and environmental cytokines, J Immunol. 172 (10) (2004) 6107–6114.
[43] E. Rivers, A.J. Thrasher, Wiskott-Aldrich syndrome protein: emerging mechanisms in immunity, Eur. J. Immunol. (2017).
[44] T.N. Sims, T.J. Soos, H.S. Xenias, B. Dubin-Thaler, J.M. Hofman, J.C. Waite, et al., Opposing effects of PKCtheta and WASp on symmetry breaking and relocation of the immunological synapse, Cell. 129 (4) (2007 May 18) 773–785.
[45] J.L. Cannon, J.K. Burkhardt, Differential roles for Wiskott-Aldrich syndrome protein in immune synapse formation and IL-2 production, J Immunol. 173 (3) (2004 Aug 01) 1658–1662.
[46] X. Zhang, R. Dai, W. Li, H. Zhao, Y. Zhang, L. Zhou, et al., Abnormalities of follicular helper T-cell number and function in Wiskott-Aldrich syndrome, Blood. 127 (25) (2016) 3180–3191.
[47] Wu J, Liu D, Tu W, Song W, Zhao X. T-cell receptor diversity is selectively skewed in T-cell populations of patients with Wiskott-Aldrich syndrome. Journal of Allergy and Clinical Immunology. 2015;135(1):209-16. e8.
[48] J. De Meester, R. Calvez, S. Valitutti, L. Dupre, The Wiskott-Aldrich syndrome protein regulates CTL cytotoxicity and is required for efficient killing of B cell lymphoma targets, J Leukoc Biol. 88 (5) (2010 Nov) 1031–1040.
[49] S. Trifari, G. Sitia, A. Aiuti, S. Scaramuzza, F. Marangoni, L.G. Guidotti, et al., Defective Th1 cytokine gene transcription in CD4+ and CD8+ T cells from Wiskott-Aldrich syndrome patients, J Immunol. 177 (10) (2006 Nov 15) 7451–7461.
[50] Lang PA, Shaabani N, Borkens S, Honke N, Scheu S, Booth S, et al. Reduced type I interferon production by dendritic cells and weakened antiviral immunity in patients with Wiskott-Aldrich syndrome protein deficiency. Journal of Allergy and Clinical Immunology. 2013;131(3):815-24. e2.
[51] T.S. Strom, S.J. Turner, S. Andreansky, H. Liu, P.C. Doherty, D.K. Srivastava, et al., Defects in T-cell-mediated immunity to influenza virus in murine Wiskott-Aldrich syndrome are corrected by oncoretroviral vector-mediated gene transfer into repopulating hematopoietic cells, Blood. 102 (9) (2003) 3108–3116.
[52] S. Trifari, G. Sitia, A. Aiuti, S. Scaramuzza, F. Marangoni, L.G. Guidotti, et al., Defective Th1 Cytokine Gene Transcription in CD4 < sup > + < /sup > and CD8 < sup > + < /sup > T Cells from Wiskott-Aldrich Syndrome Patients, The Journal of Immunology. 177 (10) (2006) 7451–7461.
[53] Dupré Lc, A. Aiuti, S. Trifari, S. Martino, P. Saracco, C. Bordignon, et al., Wiskott-Aldrich syndrome protein regulates lipid raft dynamics during immunological synapse formation, Immunity. 17 (2) (2002) 157–166.
[54] I. Molina, J. Sancho, C. Terhorst, F. Rosen, E. Remold-O'Donnell, T cells of patients with the Wiskott-Aldrich syndrome have a restricted defect in proliferative responses, The Journal of Immunology. 151 (8) (1993) 4383–4390.
[55] A. Cianferoni, M. Massaad, S. Feske, M.A. de la Fuente, L. Gallego, N. Ramesh, et al., Defective nuclear translocation of nuclear factor of activated T cells and extracellular signal-regulated kinase underlies deficient IL-2 gene expression in Wiskott-Aldrich syndrome, J Allergy Clin Immunol. 116 (6) (2005 Dec) 1364–1371.
[56] J. De Meester, R. Calvez, S. Valitutti, L. Dupré, The Wiskott-Aldrich syndrome protein regulates CTL cytotoxicity and is required for efficient killing of B cell lymphoma targets, Journal of leukocyte biology. 88 (5) (2010) 1031–1040.
[57] M.D. Gallego, M. Santamaria, J. Pena, I.J. Molina, Defective actin reorganization and polymerization of Wiskott-Aldrich T cells in response to CD3-mediated stimulation, Blood. 90 (8) (1997 Oct 15) 3089–3097.
[58] R. Rengan, H.D. Ochs, L.I. Sweet, M.L. Keil, W.T. Gunning, N.A. Lachant, et al., Actin cytoskeletal function is spared, but apoptosis is increased, WAS patient hematopoietic cells. Blood. 95 (4) (2000 Feb 15) 1283–1292.
[59] S. Kothakota, T. Azuma, C. Reinhard, A. Klippel, J. Tang, K. Chu, et al., Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis, Science. 278 (5336) (1997 Oct 10) 294–298.
[60] M. Ohtsu, N. Sakai, H. Fujita, M. Kashiwagi, S. Gasa, S. Shimizu, et al., Inhibition of apoptosis by the actin-regulatory protein gelsolin, EMBO J. 16 (15) (1997 Aug 01) 4650–4656.
[61] M. van Engeland, H.J. Kuijpers, F.C. Ramaekers, C.P. Reutelingsperger, B. Schutte, Plasma membrane alterations and cytoskeletal changes in apoptosis, Exp Cell Res. 235 (2) (1997 Sep 15) 421–430.
[62] D. Kenney, L. Cairns, E. Remold-O'Donnell, J. Peterson, F.S. Rosen, R. Parkman, Morphological abnormalities in the lymphocytes of patients with the Wiskott-Aldrich syndrome, Blood. 68 (6) (1986 Dec) 1329–1332.
[63] I.J. Molina, D.M. Kenney, F.S. Rosen, E. Remold-O'Donnell, T cell lines characterize events in the pathogenesis of the Wiskott-Aldrich syndrome, J Exp Med. 176 (3) (1992 Sep 01) 867–874.
[64] M.J. Massaad, N. Ramesh, S. Le Bras, S. Giliani, L.D. Notarangelo, W. Al-Herz, et al., A peptide derived from the Wiskott-Aldrich syndrome (WAS) protein-interacting protein (WIP) restores WAS protein level and actin cytoskeleton reorganization in lymphocytes from patients with WAS mutations that disrupt WIP binding, J Allergy Clin Immunol. 127 (4) (2011) 998–1005.e1–2.