Parasitology International 66 (2017) 227–235

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Activation and exhaustion of antigen-specific CD8$^+$ T cells occur in different splenic compartments during infection with *Plasmodium berghei*

Ganchimeg Bayarsaikhan [a,b], Mana Miyakoda [a], Kazuo Yamamoto [c], Daisuke Kimura [a], Masoud Akbari [a], Masao Yuda [d], Katsuyuki Yui [a,b,*]

[a] Division of Immunology, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4, Sakamoto, Nagasaki 852-8523, Japan
[b] Program for Nurturing Global Leaders in Tropical and Emerging Infectious Diseases, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4, Sakamoto, Nagasaki 852-8523, Japan
[c] Division of Cell Function Research Support, Biomedical Research Support Center, School of Medicine, Nagasaki University, 1-12-4, Sakamoto, Nagasaki 852-8523, Japan
[d] Department of Medical Zoology, School of Medicine, Mie University, 2-174, Edobashi, Tsu 514-8507, Japan

ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form 12 December 2016
Accepted 16 January 2017
Available online 03 February 2017

Keywords:
CD8$^+$ T cells
Infectious diseases
Inhibitory receptors
Malaria
Spleen

ABSTRACT

The spleen is the major organ in which T cells are primed during infection with malaria parasites. However, little is known regarding the dynamics of the immune responses and their localization within the splenic tissue during malaria infection. We examined murine CD8$^+$ T cell responses during infection with *Plasmodium berghei* using recombinant parasites expressing a model antigen ovalbumin (OVA) protein and compared the responses with those elicited by *Listeria monocytogenes* expressing the same antigen. OVA-specific CD8$^+$ T cells were mainly activated in the white pulp of the spleen during malaria infection, as similarly observed during Listeria infection. However, the fates of these activated CD8$^+$ T cells were distinct. During infection with malaria parasites, activated CD8$^+$ T cells preferentially accumulated in the red pulp and/or marginal zone, where cytokine production of OVA-specific CD8$^+$ T cells decreased, and the expression of multiple inhibitory receptors increased. These cells preferentially underwent apoptosis, suggesting that T cell exhaustion mainly occurred in the red pulp and/or marginal zone. However, during Listeria infection, OVA-specific CD8$^+$ T cells only transiently expressed inhibitory receptors in the white pulp and maintained their ability to produce cytokines and become memory cells. These results highlighted the distinct fates of CD8$^+$ T cells during infection with *Plasmodium* parasites and Listeria, and suggested that activation and exhaustion of specific CD8$^+$ T cells occurred in distinct spleen compartments during infection with malaria parasites.

1. Introduction

Malaria, caused by infection with *Plasmodium* species parasites, is one of the most prevalent infectious diseases in the world [1]. During the blood-stage of *Plasmodium* infection, both CD4$^+$ and CD8$^+$ T cells are activated and these T cells play critical roles in both malaria pathogenesis and protection against malaria. CD4$^+$ T cells help B cells produce anti-parasite antibodies, activate macrophages to engulf parasites, and play pivotal roles in protecting against parasites [2,3]. CD8$^+$ T cells play major roles in malaria pathogenesis, as observed with experimental cerebral malaria [4,5]. These parasite-specific CD8$^+$ T cells were generated by transporter of antigen presentation (TAP)-dependent cross-presentation of malaria antigens by CD8α$^+$ dendritic cells (DCs) during blood-stage infection [6,7]. Effector CD8$^+$ T cells also appeared to play protective roles against the blood-stage of malaria infection, as shown by the transfer of protective immunity by CD8$^+$ T cells from the repeatedly immunized mice [8]. However, CD8$^+$ T cells that are activated by infection with malaria parasites express multiple inhibitory molecules, including programmed cell death protein-1 (PD-1) and lymphocyte activated gene-3 (LAG-3), and are functionally disabled during chronic infection, reflecting a state of exhaustion [9,10]. In a model of experimental cerebral malaria, it was also shown that the activation and pathogenesis of T cells were inhibited by PD-1 in resistant BALB/c mice during acute infection with *P. berghei* ANKA, although T cells exhibited pathogenesis in susceptible C57BL/6 stain despite high levels of PD-1 expression [11].

Abbreviations: CFSE, 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester; DCs, dendritic cells; LAG-3, lymphocyte activated gene 3; LCMV, lymphocytic choriomeningitis virus; LM-OVA, *Listeria monocytogenes* expressing OVA; mAb, monoclonal antibody; MFI, mean fluorescent intensity; MMM, marginal zone metallophilic macrophages; MZ, marginal zone; MZM, marginal zone macrophages; OVA, ovalbumin; PbA-OVA, *Plasmodium berghei* ANKA expressing OVA; PD-1, programmed cell death protein; RBCs, red blood cells; RP, red pulp; SLEC, short-lived effector; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; WP, white pulp.

* Corresponding author at: Division of Immunology, Department of Molecular Microbiology and Immunology, Graduate School of Biomedical Sciences, 1-12-4, Sakamoto 852-8523, Japan.
E-mail address: katsu@nagasaki-u.ac.jp (K. Yui).

http://dx.doi.org/10.1016/j.parint.2017.01.022
1383-5769/

The spleen is the main organ to filter red blood cells (RBCs) and elicits immune responses to blood-borne pathogens [12]. It is composed of 3 main compartments, the white pulp (WP), where most T and B cells resides and immune responses are initiated, the red pulp (RP), where RBCs are filtered, and the marginal zone (MZ) that separates the WP and RP and contains MZ B cells and macrophages [12]. The MZ is the site of antigen trapping in the spleen and contains 2 types of macrophages; MZ metallophilic macrophages (MMMs), which are located in the inner MZ near the WP, and MZ macrophages (MZMs) localized in the outer MZ towards the RP [13,14]. $CD8^+$ T-cell responses in the spleen have been characterized using infection models with microbes, including intracellular bacterium *Listeria monocytogenes* and lymphocytic choriomeningitis virus (LCMV). The spleen contains 2 main DC subsets, $CD8\alpha^+$ DCs, which reside mainly in the T cell zone, and $CD8\alpha^-$ DCs, which are localized primarily in the RP and MZ [15,16]. $CD8\alpha^+$ DCs are directly infected with *L. monocytogenes* or capture microbial antigens, after which they can move from the MZ to enter the WP, where they present antigens to $CD8\alpha^+$ T cells [17–19]. The initial activation of antigen-specific $CD8^+$ T cells occurs at the borders of the B and T cell zones in the WP, followed by cluster formation with DCs in the WP [20]. After activation, these $CD8^+$ T cells proliferate, forming effector and memory cells, and exit to the RP through bridging channels. Studies using the LCMV model showed that memory precursor cells mainly localized in the T cell zone of the WP, whereas terminal effector cells localized exclusively to the RP of the spleen. Upon re-challenge, memory or memory precursor $CD8^+$ T cells expand and are redistributed in the RP [21–23].

Results from previous studies showed the dynamics of splenic lymphocyte responses during infection with *Plasmodium* parasites [24,25]. Furthermore, the spleen architecture is altered, and MZMs and MMMs are both lost during infection with *P. chabaudi*, with the loss of MMMs being dependent on $CD8^+$ T cells [26]. However, the localization and dynamics of antigen-specific $CD8^+$ T cells in the spleen during malaria infection are not clearly understood. The model antigen ovalbumin (OVA) protein has been used to study antigen-specific immune responses during infection with recombinant pathogen expressing OVA, such as *L. monocytogenes* expressing OVA (LM-OVA) and T-cell receptor-transgenic OT-1 mice [20]. We developed a model of $CD8^+$ T cell activation during malaria infection using *Plasmodium berghei* ANKA that express OVA (PbA-OVA) [6]. In this study, we used this model system to study specific $CD8^+$ T-cell responses in different splenic tissue compartments. Using OVA as a common model antigen, we compared the responses of specific $CD8^+$ T cells during infection with *Plasmodium* parasites and Listeria.

2. Materials and methods

2.1. Mice

OT-I transgenic mice expressing the T cell receptor specific for $OVA_{257–264}/K^b$ were provided by Dr. H. Kosaka (Osaka University, Osaka, Japan) [27]. C57BL/6 mice were purchased from SLC (Shizuoka, Japan). B6.SJL and OT-I mice were bred, and the offspring were intercrossed to obtain $CD45.1^+$ OT-I mice. Mice were maintained in the Laboratory Animal Center for Animal Research at Nagasaki University, and both male and female mice were used at 7–10 weeks of age. The animal experiments were approved by the Institutional Animal Care and Use Committee of Nagasaki University and were conducted according to the guidelines for Animal Experimentation of Nagasaki University.

2.2. Infections and adoptive transfer

The recombinant parasite PbA-OVA, which constitutively expresses OVA under the control of the hsp70 promotor, was maintained as described previously [6]. Mice were infected with RBCs (5×10^4) infected with PbA-OVA or PbA by intraperitoneal injection. LM-OVA [28] was provided by Dr. Y. Yoshikai (Kyushu University) and Dr. H. Shen (University of Pennsylvania). Mice were infected with LM-OVA (10^6 colony-forming units, 0.1 LD_{50}) by intraperitoneal injection. $CD8^+$ (>95%) were prepared from the spleen, brachial, and inguinal lymph nodes using *anti*-CD8 IMag (BD Biosciences, San Diego, CA, USA) and labeled with CFSE (15 μM, Molecular Probes), as described previously [6]. C57BL/6 mice were adoptively transferred intravenously (i.v.) with $CD8^+$ OT-I cells (1×10^6) prepared from age- and sex-matched OT-I mice and were infected with PbA-OVA or LM-OVA on the following day.

2.3. Immunohistochemistry

Spleen tissues were prepared for immunohistochemistry, as previously described [29]. Briefly, fresh frozen spleens were embedded in Tissue-Tek OCT compound (Sakura Finetek, Tokyo, Japan), cut into 5-μm sections with a cryomicrotome, and fixed with acetone for 15 min at room temperature. Samples were blocked by Blocking One Histo (Nacalai, Kyoto, Japan) for 1 h in a humid chamber at room temperature. Sections were stained for CD45.1 and CD169 (BioLegend, San Diego, CA, USA) overnight at 4 °C and mounted in DAKO fluorescent mounting medium (Agilent Technologies, Santa Clara, CA, USA). Images were acquired by fluorescence microscopy (Olympus, Tokyo, Japan) and merged using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The number of OT-I cells was counted in a 0.5-mm^2 area in a microscopic field using ImageJ software. Statistical analysis was performed on 15 different areas from 1 spleen, and 3 different mice were examined.

2.4. Flow cytometry

To stain cells in the RP, mice received PE-*anti*-CD8α mAb (3 μg, clone 53–6.7) via i.v. injection and were euthanized 3 min later, as previously described [30]. The spleens were harvested, and RBCs were lysed using Gey's solution. Lymphocytes were stained with mAbs or their isotype controls. All mAbs were purchased from eBioscience (San Diego, CA, USA), BioLegend, BD Pharmingen (Franklin Lakes, NJ, USA), or Tonbo (San Diego, CA, USA). Staining for annexin V was performed in annexin V binding buffer composed of HEPES (100 mM), $CaCl_2$ (25 mM), and NaCl (1.4 M, pH 7.5), according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). The compound 7-aminoactinomycin D (7AAD) was added to exclude dead cells from the analysis. For intracellular staining, $CD8^+$ T cells were purified using magnetic-activated cell sorting (MACS) microbeads and AutoMACS (Miltenyi Biotec, Gladbach, Germany) and were stimulated for 4 h with DCs (3×10^4) pulsed with the $OVA_{257–264}$ peptide (1 μg/mL) in 24-well plates. Cells were stained for the appropriate surface markers, fixed, permeabilized using Cytofix/Cytoperm buffer (BD Bioscience), stained with *anti*-cytokine mAbs, and analyzed using a FACS Canto II instrument (BD Biosciences). The number of cellular subsets was determined by multiplying the total number of spleen cells by the proportion of each subset in the spleen, as determined by flow cytometry.

2.5. Statistical analysis

Results are shown as the mean ± standard deviations (SD). Data were analyzed using GraphPad Prism software, version 6 (GraphPad, San Diego, CA, USA). An overall difference between groups was determined by 2-way analysis of variance (ANOVA). If the 2-way ANOVA revealed a significant difference, then differences between individual groups were estimated using the Tukey's multiple comparison test. $p < 0.05$ was considered significant.