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Signaling Lymphocyte Activation Molecule-Associated Protein Is a Negative Regulator of the CD8 T Cell Response in Mice

Gang Chen,2* Albert K. Tai,2* Miao Lin,* Francesca Chang,* Cox Terhorst,† and Brigitte T. Huber3*

The primary manifestation of X-linked lymphoproliferative syndrome, caused by a dysfunctional adapter protein, signaling lymphocyte activation molecule-associated protein (SAP), is an excessive T cell response upon EBV infection. Using the SAP−/− mouse as a model system for the human disease, we compared the response of CD8+ T cells from wild-type (wt) and mutant mice to various stimuli. First, we observed that CD8+ T cells from SAP−/− mice proliferate more vigorously than those from wt mice upon CD3/CD28 cross-linking in vitro. Second, we analyzed the consequence of SAP deficiency on CTL effector function and homeostasis. For this purpose, SAP−/− and wt mice were infected with the murine γ-herpesvirus 68 (MHV-68). At 2 wk postinfection, the level of viral-specific CTL was much higher in mutant than in wt mice, measured both ex vivo and in vivo. In addition, we showed that the SLAM-SAP-FynT pathway is essential for virus-specific CD8+ T cell expansion triggered by MHV-68 infection is also enhanced and prolonged in SAP−/− mice. Taken together, our data indicate that SAP functions as a negative regulator of CD8+ T cell activation. The Journal of Immunology, 2005, 175: 2212–2218.

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3 Address correspondence and reprint requests to Dr. Brigitte T. Huber, Tufts University School of Medicine, 150 Harrison Avenue, Boston, MA 02111. E-mail address: Brigitte.huber@tufts.edu
4 Abbreviations used in this paper: XLP, X-linked lymphoproliferative syndrome; ICC, intracellular cytokine; IM, infectious mononucleosis; LD, limiting dilution; MHV-68, murine γ-herpesvirus 68; SAP, signaling lymphocyte activation molecule-associated protein; SH2, Src homology 2; SLAM, signaling lymphocyte activation molecule; wt, wild type.
of wild-type (wt) cells. In addition, long-term infections of SAP−/− and wt C57BL/6 (B6) mice with MHV-68 were used to examine how SAP controls the cellular immune response to this virus. To this end, virus-specific CTLs were compared in both ex vivo and in vivo assays. We consistently observed stronger MHV-68-specific CTL activity in SAP−/− than in wt mice 2 wk postinfection. In addition, the frequency of virus-specific IFN-γ-producing PBL was higher in SAP−/− mice during the entire course of infection. Consequently, the frequency of splenocytes that harbor the MHV-68 genome, an indicator of latency establishment, was significantly lower in SAP−/− mice, demonstrating that MHV-68-specific CTLs effectively control viral infection in these mice. Finally, we observed that another aspect of the T cell response to MHV-68 infection, namely the Vβ4-specific CD8+ T cell expansion (22, 24, 26), was significantly enhanced and prolonged in SAP−/− mice, as compared with wt mice.

Materials and Methods

Virus and cell lines

MHV-68 (WUMS, ATCC VR1465) was purchased from American Type Culture Collection. The EL4 cell line was a kind gift from Dr. Manjunath at CBRS Institute for Biomedical Research (Harvard Medical School, Boston, MA). It was maintained in RPMI 1640, supplemented with 10% FCS, penicillin (100 U/ml), HEPES (10 mM, pH 7.4), sodium pyruvate (1 mM), 2-ME (55 mM), and streptomycin (100 μg/ml). BALB/3T12-3 cell line was purchased from American Type Culture Collection (ATCC CCL-164) and maintained in DMEM with 4.5 g/L glucose, supplemented with 10% FCS penicillin (100 U/ml) and streptomycin (100 μg/ml).

Mice and viral infection

The wt B6 mice were purchased from The Jackson Laboratory. SAP−/− mice on the B6 background (18) were bred in the animal facility at Tufts University School of Medicine. All mice were housed in a specific pathogen-free barrier facility at Tufts-New England Medical Center. Age- and sex-matched naive B6 mice were labeled with 100, 10, 1 μM CFSE (Molecular Probes), and the latter two populations were used for the MHV-68-specific CTL and intracellular cytokine (ICC) staining assays. The Institutional Animal Care and Use Committee of Tufts University approved all procedures.

Ex vivo cytotoxicity assay

MHV-68-specific CTL against two major viral epitopes, p56 (AGPHNDMEI) and p79 (TSINFVKI), was examined in a modified 6-h 51Cr release assay against peptide-loaded EL4 targets. Basically, 1 × 104 EL4 cells were loaded with the corresponding peptide (10 μg/ml) in 1 ml of RPMI 1640 with 10% FCS for 2 h at 37°C. Then the cells were labeled with 100 μCi of 51Cr (New England Biolabs) for 1 h at 37°C. Later, 1000 target cells/well in 96-well plates were incubated with effector cells at different E/T ratios at 37°C for 6 h before 50 μl of supernatant was harvested in LumaPlate-96. The radioactivity was measured with a Topcount microplate reader (Packard Instrument). The percentage of specific killing was calculated as follows: percentage of specific killing = (experimental release – SR/RT – SR) × 100%. SR, spontaneous release; TR, total release; and the SR should always be <5% of the TR.

In vivo CTL assay

Splenocytes from sex-matched naive B6 mice were labeled with 100, 10, or 1 μM CFSE (Molecular Probes), and the latter two populations were load-pulsed with p56 and p79 peptides (1 μM), respectively. After washing with Dulbecco’s PBS, the labeled spleen cells were mixed and injected i.v. as target cells into wt and SAP−/− mice that had been infected 14 days previously with MHV-68. MHV-68-infected or uninfected control mice were given 2 × 105 target cells by i.v. injection and sacrificed 4 h later, and their spleen cells were analyzed by FACS. Percent specific lysis of targets was calculated as number of unspurred targets × A/ number of peptide-pulsed targets (number of unspurred targets). Percent specific lysis of targets was calculated as

ICC staining assay

Spleen cells and PBL from MHV-68-infected mice were incubated with either peptide p56 (AGPHNDMEI) or p79 (TSINFVKI) (1 μg/ml) for 6–12 h in the presence of 10 μg/ml brefeldin A (Sigma-Aldrich). Then the cells were stained with anti-mouse CD8α PE-Cy5 (BD Pharmingen), fixed with 1% paraformaldehyde in PBS, permeabilized with 0.5% saponin, and stained with anti-mouse IFN-γ PE (BD Pharmingen). PMA- and ionomycin (Sigma-Aldrich)-activated cells were used as positive control for this assay, and nonstimulated cells were used as negative control. For the kinetics studies, viral-specific IFN-γ-producing PBL were analyzed by FACSCalibur (BD Biosciences) with CellQuest software. Data analysis was performed with Cytomation Summit software.

Limiting dilution (LD) nested PCR detection of MHV-68 genome-positive cells

The frequency of spleen cells harboring the MHV-68 genome was measured with a previously described, single copy-sensitive, nested PCR assay to detect the ORF50 gene of MHV-68 (28) with modifications. The outer PCR primers were 5′-AACTGGAACCTTCTGTTGCG-3′ and 5′-GC CGGACAGCTTATGAC-3′, which amplify a 586-bp product. The inner PCR primers were 5′-CCCCCATGTTCATAAGTGG-3′ and 5′-AT CAGCAGGCTCATACATC-3′, which amplify a 382-bp product. Splenocytes from three to five wt or SAP−/− mice at each time point were pooled, resuspended in isotonic buffer, and plated in serial 3-fold dilutions on a background of 104 uninfected BALB3T12-3 cells in 96-well PCR plates (USA Scientific). Cells were lysed with proteinase K lysis buffer at 56°C, and proteinase K was inactivated for 15 min at 95°C. Then the cell lysate was mixed with 2× round 1 PCR mix (25 mM KCl, 10 mM Tris- HCl (pH 9.0), 0.5% Triton X-100, 1.5 mM MgCl2, 0.4 mM nucleotides, each primer at 0.5 μM, and 1 U of Taq polymerase (Promega)). The first round of PCR was performed with 45 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by extension at 72°C for 5 min. Then 2 μl of round 2 PCR mix (22.5 μl of Platinum PCR SuperMix (Invitrogen Life Technologies), 0.5 μl of inner primers at 0.5 μM, and 1.0 μl of PCR grade H2O) was added to a 96-well PCR plate, and 1 μl of round 1 PCR product was used as template. The conditions for the second round of PCR were identical, except that the reaction was amplified for 25 cycles. Products were run on a 2% agarose gel and analyzed by ethidium bromide staining. Twelve PCRs were performed for each cell dilution, and at least six dilutions of each sample were analyzed, starting at 106 cells/well for days 19 and 75, respectively, and 3 × 105 cells/well for the later time points, e.g., days 100 and 210, respectively. Control reactions (104 uncontaminated BALB3T12-3 cells) were included in each experiment.

Flow cytometry analysis of Vβ4 subset in PBL

Blood samples from MHV-68-infected wt and SAP−/− mice were collected by tail bleeding. RBC were lysed using Pharm Lyse (BD Biosciences), as instructed by manufacturer. The PBLs were then stained by allophycocyanin-conjugated anti-CD4, PE/Cy5-conjugated anti-CD8α, and FITC-conjugated anti-Vβ4 Abs (BD Biosciences) on ice for 30 min, and washed and analyzed on a FACSCalibur.

In vitro proliferation assay

Splenocytes were harvested from wt and SAP−/− mice. RBC were lysed with Pharm Lyse (BD Biosciences). Whole splenocytes were stained with anti-mouse CD4-FITC and Thy-1.2 allophycocyanin (BD Biosciences), and the CD8+ T cells were sorted by MoFlo cell sorter (DakoCytomation) by gating on the Thy-1.2 CD4+ population. The purified CD8+ T cells were then stimulated with plate-bound anti-mouse CD3/CD28 (1, 0.1, or 0.01 μg/ml). On day 1–5, 1 μl of [3H]thymidine/well was used to pulse the cells for 16 h, before the plates were harvested with the TOMTEC cell harvester and 1H incorporation was measured by TRILUX Microbeta counter (PerkinElmer Life Science).

Statistical analysis

All data were analyzed with GraphPad Prism software (GraphPad). Frequencies of viral genome-positive cells were obtained from the cell number at which 63% of the wells scored positive for presence of the viral genome, based on the Poisson distribution; data were subjected to nonlinear regression analysis to obtain the single-cell frequency for each LD analysis.
Results

Enhanced proliferative response to CD3/CD28 cross-linking in SAP−/− CD8+ T cells

To characterize the CD8+ T cell response in the absence of SAP, we first activated these cells in vitro by CD3/CD28 cross-linking, which stimulates all T cells, regardless of specificity. For this purpose, CD8+ T cells were sorted by FACS from spleen cells of wt and SAP−/− mice and stimulated in vitro with various concentrations of plate-bound anti-CD3/CD28 mAbs. Proliferation was measured by incorporation of [3H]thymidine. As can be seen in Fig. 1, a significantly stronger proliferative response was observed in SAP−/− CD8+ T cells on days 2, 3, and 4 after activation.

Increased CTL responses in SAP−/− mice infected with MHV-68

To elucidate the role of SAP in the cellular immune reaction to viruses, we compared CTL responses in SAP−/− and wt mice that had been infected with MHV-68. We first determined whether SAP−/− and wt mice displayed distinct CTL responses to two MHV-68 lytic-cycle H-2b-restricted T cell epitopes, p56/D9 (AG PHNDMEI) from the immediate early gene (ORF6) and p79/Kb (TSINFVKI) from an early gene (ORF61) (29–31). Two weeks postinfection, significantly stronger ex vivo CTL activity, directed at the p56 peptide epitope, was observed in SAP−/− mice (Fig. 2A, p < 0.05 for 2 of 3 E:T ratios). Although the CTL response to the p79 peptide epitope is also higher in the mutant mice, the difference is only statistically significant at the highest E:T ratio (Fig. 2B, p < 0.05 for 1:200 E:T ratio, unpaired Student's t test, one-tailed). This is probably due to less efficient killing of target cells ex vivo. Next, MHV-68-specific CTL responses were determined in vivo by using p56 or p79 peptide-loaded naive splenocytes as target cells. Two weeks postinfection with MHV-68, peptide-loaded and CSFE-labeled target cells were injected i.v. into both infected and uninfected mice. After 4 h, all mice were sacrificed, and their spleen cells were analyzed by FACS for the number of remaining CSFE+ targets. Consistent with the ex vivo CTL assays, significantly stronger in vivo CTL responses to both p56 and p79 epitopes were observed in SAP−/− mice (Fig. 2, C and D; * p < 0.05; ** p < 0.01, unpaired Student's t test, one-tailed).

Higher frequency of Ag-specific CD8+ T cells generated in SAP−/− mice

To address the possibility that SAP−/− mice generate more Ag-specific T cells, the frequency of MHV-68 p56- and p79-specific CD8+ T cells was determined, based on their capacity to produce INF-γ upon stimulation by cognate peptides. First, we tested PBL and splenocytes at the peak of their CTL response on day 14 postinfection. As expected, we observed a significantly higher frequency of viral epitope-specific CD8+ T cells in SAP−/− compared with wt mice (Fig. 3A, ** p < 0.01 for p79-specific CD8+ T cells; Fig. 3B, * p < 0.05 for p56-specific CD8+ T cells and p = 0.054 for p79-specific CD8+ T cells, unpaired Student’s t test, one-tailed). Second, the frequency of epitope-specific PBL was determined between days 10 and 45 postinfection, to compare the kinetics of the antiviral response in SAP−/− and wt mice. As can be seen in Fig. 3, C and D, the overall frequency of virus-specific CD8+ T cells was significantly higher in SAP−/− than in wt mice (paired Student’s t test, p < 0.05 for both p56- and p79-specific response). Thus, the frequency of MHV-68-specific CD8+ T cells is much higher in the blood of SAP−/− mice throughout the course of infection. This holds true in the spleen at the peak of the CTL response, providing an explanation for the stronger cytotoxicity observed in the spleens of these mice in both ex vivo (Fig. 2) and in vivo (Fig. 3, A and B) assays. These data indicate that SAP plays an important regulatory role in the homeostasis of CD8+ T cells during infection with a herpesvirus.

Reduced frequency of latently infected splenocytes in SAP−/− mice

MHV-68 latently infects splenic B cells after the acute phase of infection, which is controlled by virus-specific CTL. Based upon our observations, we expected to detect lower levels of latency in the spleens of SAP−/− mice due to the hyperproliferation of CD8+ T cells and enhanced CTL function in these mice. The frequency of splenocytes harboring the MHV-68 genome was determined by LD-PCR, targeting the ORF50 sequence, followed by nonlinear regression analysis. The absolute frequency of viral genome-positive cells within the total splenocyte pool was calculated and compared between the two groups. We found that the frequency of MHV-68 genome-positive cells in SAP−/− mice was significantly lower than in wt mice at three of four time points tested (Fig. 4A, day 19 p.i., p = 0.022; day 75 p.i., p = 0.035; day 100 p.i., p = 0.049; day 210 p.i., p = 0.067), and the kinetics of splenic latency in SAP−/− mice were different from those of wt mice (Fig. 4B, p < 0.05). The most dramatic difference was seen at the earliest time point, in which 1 of 4570 SAP−/− splenocytes was found to harbor the MHV-68 genome, as compared with 1 of 194 cells in wt mice. Because MHV-68 latency is reported to be predominantly in the surface IgD-negative memory B cell subset in the spleen (32), we expect a smaller proportion of these cells to be infected with MHV-68 in SAP−/− mice due to hyperproliferation of CD8+ T cells present in these mice. This is consistent with the data presented in Fig. 4, in which we observed a significantly lower frequency of SAP−/− mice infected with MHV-68 compared with wt mice (Fig. 4C, * p < 0.05 for p56-specific CD8+ T cells, unpaired Student’s t test, one-tailed).

FIGURE 1. Enhanced proliferation of SAP−/− CD8+ T cells upon CD3/CD28 cross-linking. Splenocytes from naive SAP−/− and wt mice were collected, and CD8+ T cells were sorted by FACS. Then 1 x 10^4 cells/well were placed in 96-well plates that had been coated with anti-mouse CD3 and CD28 mAbs. On days 1–5, the cells were pulsed with 1 μCi of [3H]thymidine/well for 16 h, followed by harvesting with the TOMTEC cell harvester and measuring 3H incorporation into DNA by TRILUX Microbeta counter.
were mixed and injected i.v. as target cells (2) infection and used as target cells in a 51Cr release assay. EL4 cells, loaded with either p56 or p79 peptide, were used as targets, and unloaded EL4 cells as negative control. Percent specific killing = experimental release − SR/(TR − SR) × 100 (SR, spontaneous release; TR, total release of the target). Numbers in A and B are mean ± SD of five mice per group. A, p56-specific cytotoxicity; unpaired Student’s t test (one-tailed) p values for each E:T ratio: 1:200 (p < 0.05), 1:80 (p < 0.01), and 1:20 (p = 0.007). B, p79-specific cytotoxicity; unpaired Student’s t test p values for each E:T ratio: 1:200 (p < 0.05), 1:80 (p = 0.0693), and 1:20 (p = 0.123). •, Represent killing by SAP−/− cells; ▲, represent killing by wt cells; ◇ and △ represent background killing of either SAP−/− or wt cells. C, and D, MHV-68-specific in vivo CTL assay. Splenocytes from naïve B6 mice were labeled with 100, 10, or 1 μM CSFE, and the latter two populations were loaded with p79 or p56 peptide (1 μM/ml), respectively. The cells were mixed and injected i.v. as target cells (2 × 10⁷/mouse) into both wt and SAP−/− MHV-68-infected mice. Four hours later, recipient spleens were harvested and analyzed by FACS. CSFE+ cells were gated and are shown in histograms. Percent specific killing (numbers on top of each peak) = (number of unpulsed targets × A − number of peptide-pulsed targets/number of unpulsed targets × A) × 100, where A = number of peptide-pulsed targets/number of unpulsed targets in unpulsed recipient mice. C, Representative histograms; D, representative data from one in vivo CTL assay. □, Represents SAP−/−; ■, represents wt mice (SAP−/−, n = 5; wt, n = 5). *, Unpaired Student’s t test (one-tailed), p < 0.05 for p56-specific response; **, p < 0.01 for the p79-specific response.

Further test the frequency of virus genome-positive cells within B220+ IgD− cells. We found that the MHV-68 viral burden is significantly lower in the SAP−/− memory B cell subset on days 15 and 45 postinfection, and the absolute frequency of viral genome-positive cells in the SAP−/− memory B cell compartment is 7.5- to 90-fold lower than that in wt cells during this time period (data not shown).

Enhanced and prolonged Vß4-specific CD8+ T cell expansion upon MHV-68 infection in SAP−/− mice

In this study, the Vß4-specific CD8+ T cell expansion was followed by sampling blood at multiple time points post-MHV-68 infection by flow cytometry. As expected, the expansion phase of Vß4 CD8+ T cells had comparable kinetics and magnitude in wt and SAP−/− mice. This population gradually declined in wt mice, as had been reported previously (33). In contrast, the number of Vß4+ CD8+ T cells remained elevated or expanded even further in SAP−/− mice (p < 0.001) (Fig. 5A). These cells had no CTL function against either the MHV-68 p56 or p79 epitope (Fig. 5B). In contrast, only a minor increase in the Vß4+ CD4+ population was seen in SAP−/− vs wt mice (data not shown).

Discussion

Contradictory results have been published to date on the fate of CTL in XLP patients and SAP−/− mice (16, 18, 19). To shed light on this seemingly controversial matter, we conducted a detailed comparison of CD8+ T cell reactivity of mutant and wt mice in response to various stimuli. Particularly, the proliferative response of these cells had not been previously analyzed. Interestingly, we...
observed that cross-linking CD3 and CD28 in vitro led to significantly higher proliferation in SAP−/− CD8+ T cells when compared with that of wt mice. These data suggest that TCR-induced proliferation of CD8+ cells is enhanced in the absence of SAP.

Next, we used a more physiological system, in vivo MHV-68 infection, to compare the CTL response of mutant and wt mice. MHV-68 is a type 2 γ-herpesvirus that is genetically homologous to EBV (34, 35). It can cause IM-like symptoms (33, 36) and establishes long-term latency in memory B cells and lung epithelial cells (37–39). Thus, it is considered a good model for studying herpesvirus infection in mice. CD8+ CTL have been shown to be fully capable of controlling the acute phase of MHV-68 infection in wt B6 mice (40, 41). In both ex vivo and in vivo CTL assays, we consistently observed greater cytotoxicity in MHV-68-infected SAP−/− compared with wt mice. These results demonstrate that the Ag-specific CTLs generated in the absence of SAP are fully functional. Moreover, they indicate that the cellular immune effector function of SAP−/− mice is stronger than that generated by the wt counterparts. Our study is consistent with previous reports that SAP−/− mice generated more CTLs upon lymphocytic choriomeningitis virus and T. gondii infection than wt mice (19).

Because bulk splenocytes were used as effectors in the CTL assays, there are at least two explanations for the greater cytotoxicity seen in SAP−/− mice: 1) the mutant mice generate more Ag-specific CTLs than the wt mice; or 2) the cytotoxic function of SAP−/− CTLs is stronger at the single cell level than that of wt mice. It has been shown that the MHV-68-specific CTL response in B6 mice starts at day 7 postinfection, peaks at day 14, and subsides after day 20 postinfection (29, 42). Thus, we examined the frequency of viral Ag-specific IFN-γ-producing CD8+ cells as a readout for CTL activity at multiple time points postinfection. Our study provides strong evidence that the frequency of MHV-68-specific CD8+ cells in peripheral blood is much higher in SAP−/− mice throughout the course of infection. Similarly, the frequency of p56-specific CTL is significantly higher in spleen, providing an explanation for the stronger cytotoxicity observed in these mice in both ex vivo and in vivo assays. These results suggest that SAP plays an important regulatory function for the homeostasis of the CD8+ T cell response during herpesvirus infection. According to this model, the loss of SAP would lead to an uncontrolled CTL response to the pathogen. Our finding is consistent with human studies, in which XLP patients have excessive...

FIGURE 4. The number of splenocytes that harbor the MHV-68 viral genome is lower in SAP−/− than in wt mice. A, Pooled splenocytes from MHV-68-infected SAP−/− or wt mice were analyzed by LD-PCR at various time points postinfection. Shown are the percentages of PCR that scored positive as a function of the number of cells analyzed. For each cell dilution, 12 PCR were analyzed. The dotted line indicates 63.2%, which was used to calculate the frequency of virus genome-positive cells. Data represent single experiments with a pool of splenocytes from three to five mice. In each graph, triangles represent SAP−/− mice, and rectangles represent wt mice. Values of *p* of paired Student’s *t* test for each time point are listed in the charts. B, Absolute frequency of viral genome-positive cells, calculated from the LD-PCR shown in A (paired Student’s *t* test, *p* < 0.05). Dotted line represents SAP−/− mice, and solid line represents wt mice.
enhanced CTL function in these mice. We found that the frequency of SAP 

B

0.001).

CTL responses in SAP

cell line. This may be due to the difference in the systems used.
defective cytotoxic function against autologous lymphoblastoid

cells. The wt and SAP

mice were infected intranasally with MHV-68 at day 0. The mice were bled at indicated time points, followed by FACS analyses. All data points were gated on live CD8

lymphocytes (p < 0.001). B, Vβ4

CD8

T cells are not specific for MHV-68 epitopes. Peripheral blood from MHV-68-infected SAP

and wt mice was collected on day 14 postinfection. Then MHV-68 p56- and p79-specific IFN-γ production was tested, as outlined in Fig. 2. All data shown were gated on live CD8

lymphocytes.

FIGURE 5. Enhanced and prolonged Vβ4 CD8 T cell expansion in SAP

mice upon MHV-68 infection. A, Percentage of Vβ4

CD8

T cells. The wt and SAP

mice were infected intranasally with MHV-68 at day 0. The mice were bled at indicated time points, followed by FACS analyses. All data points were gated on live CD8

lymphocytes (p < 0.001). B, Vβ4

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and wt mice was collected on day 14 postinfection. Then MHV-68 p56- and p79-specific IFN-γ production was tested, as outlined in Fig. 2. All data shown were gated on live CD8

lymphocytes.

accumulation of activated CD8

T cells (43). It has been reported that most of the XLP patients who experienced fulminant IM after EBV infection died of bone marrow failure or liver necrosis due to excessive lymphocyte infiltration (44–47). The exaggerated CTL response demonstrated in our murine model indicates that the EBV-specific CTL in XLP patients might be functional.

There is a major discrepancy between our findings of stronger CTL effector function in SAP

mice and those reported by Sharifi et al. (16) that XLP EBV-CTL lines generated in vitro have defective cytotoxic function against autologous lymphoblastoid cell line. This may be due to the difference in the systems used. Although we examined both primary and secondary viral-specific CTL responses in SAP

mice, Sharifi et al. analyzed the memory response to EBV.

Because MHV-68 can establish latent infection in lung epithelial cells and splenic B cells (32, 48) after the acute phase of infection is controlled by virus-specific CTLs, among which both CD8

and CD4

T cells contribute (49), we expected to see much lower level of latency in the spleens of SAP

mice due to enhanced CTL function in these mice. We found that the frequency of MHV-68 genome-positive cells in the SAP

mice was significantly lower than that of wt mice. The most dramatic difference was seen on day 19 postinfection: while 1 in 194 wt splenocytes harbored the MHV-68 genome, only 1 in 4570 SAP

splenocytes was positive, which is ~20-fold lower. One explanation is that the stronger viral-specific CTL generated in the SAP

mice controls MHV-68 infection much better, because virus-specific CTL can eliminate lytically infected splenocytes as well as any latently infected B cells that are reverting to the lytic phase. In contrast, it is also possible that the virus has difficulty getting into the latency state in the SAP

memory B cell compartment, which has been established to be the major reservoir for MHV-68 latent infection (32); thus, the virus-specific CTL might eliminate any lytically infected cells. Furthermore, we did observe an at least 7.5-fold lower frequency of viral genome-positive cells within the SAP

memory B cell subset than in wt memory B cells (data not shown). In addition, it has been shown that the induction of endogenous IL-10 is an important factor for the dysregulation of the MHV-68 host immune response, because IL-10

mice have a significantly decreased viral burden as compared with wt B6 mice (50). This may be due to the limitation of IL-12 expression and dampening of the Th1 response by IL-10. SAP

mice have been shown to have a defect in IL-10 production (9), which provides yet another explanation for the decreased viral burden that we observed in these mice. Finally, because MHV-68-infected germinal center B cells, a population that is defective in SAP

mice (51), can also be latently infected with MHV-68, this provides an alternative explanation for the decreased latency establishment in SAP

mice.

Another characteristic of MHV-68 infection in mice is the expansion of the Vβ4 CD8

T cell population, which does not react with any known viral Ag (24–26). This provided an opportunity to study yet another aspect of CD8

T cell activation in SAP

mice that is not driven by conventional viral Ag. Contrary to a previously published report using a similar experimental system (20), we observed an enhanced and prolonged expansion of Vβ4

CD8

T cells in SAP

mice, although these cells did not recognize either the p56 or p79 epitope. It has been reported that the kinetics and magnitude of the CD8

Vβ4 T cell expansion vary between different mouse strains. Although B6 mice mount the most vigorous response among all the strains tested, BALB/c mice make a more moderate response (25). When we infected SAP

mice on the BALB/c background, we observed that the Vβ4 expansion in SAP

mice reached an average of 48.3 and 55.9% of the total CD8

T cell population on days 25 and 41, respectively, as compared with 25.5 and 24.7% observed in wt mice. The CD8

Vβ4 T cell population remained above 50% until day 100 in MHV-68-infected SAP

mice, as compared with 17.5% in the wt mice.

We conclude from our studies that SAP is a general negative regulator of the CD8

T cell response in mice. The exaggerated T cell cytotoxicity seen in SAP

mice strongly indicates that these mice may be missing an essential negative regulatory mechanism that operates through one or more SLAM family receptors. In addition, the elevated SAP

CD8

T cell response to anti-CD3/CD28 cross-linking specifies that SAP is indispensable for the homeostasis of CD8

T cells. SAP deficiency results in increased numbers of IFN-γ-expressing CD8

T cells and a defective Th2 cytokine production (8, 9), as well as defects in B lymphocyte functions (51). The general insights provided by this study, namely that CTL responses to viruses are augmented in the absence of SAP, are consistent with observations in XLP patients, in which enhanced cellular responses to EBV infection result in excessive production of IFN-γ by CD8

and NK cells, which lead to liver and bone marrow failure (52).
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Disclosures

The authors have no financial conflict of interest.

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