Hemophagocytic lymphohistiocytosis is a life-threatening systemic hyperinflammatory disorder with primary and secondary forms. Primary hemophagocytic lymphohistiocytosis is associated with inherited defects in various genes that affect the immunological cytolytic pathway. Secondary hemophagocytic lymphohistiocytosis is not inherited, but complicates various medical conditions including infections, autoimmune/autoimmune diseases, and malignancies. When senescence-accelerated mice (SAMP1/TA-1) with latent deterioration of immunological function and senescence-resistant control mice (SAMR1) were treated repeatedly with lipopolysaccharide, SAMP1/TA-1 mice displayed the clinicopathological features of hemophagocytic lymphohistiocytosis such as hepatosplenomegaly, pancytopenia, hypofibrinogenemia, hyperferritinemia, and hemophagocytosis. SAMR1 mice showed no features of hemophagocytic lymphohistiocytosis. Lipopolysaccharide induced upregulation of proinflammatory cytokines such as interleukin-1β, interleukin-6, tumor necrosis factor-α, and interferon-γ, and interferon-γ-inducible chemokines such as c-x-c motif chemokine ligands 9 and 10 in the liver and spleen in both SAMP1/TA-1 and SAMR1 mice. However, upregulation of proinflammatory cytokines and interferon-γ-inducible chemokines in the liver persisted for longer in SAMP1/TA-1 mice than in SAMR1 mice. In addition, the magnitude of upregulation of interferon-γ in the liver and spleen after lipopolysaccharide treatment was greater in SAMP1/TA-1 mice than in SAMR1 mice. Furthermore, lipopolysaccharide treatment led to a prolonged increase in the proportion of peritoneal M1 macrophages and simultaneously to a decrease in the proportion of M2 macrophages in SAMP1/TA-1 mice compared with SAMR1 mice. Lipopolysaccharide appeared to induce a hyperinflammatory reaction and prolonged inflammation in SAMP1/TA-1 mice, resulting in features of secondary hemophagocytic lymphohistiocytosis. Thus, SAMP1/TA-1 mice represent a useful mouse model to investigate the pathogenesis of bacterial infection-associated secondary hemophagocytic lymphohistiocytosis.
hepatosplenomegaly, pancytopenia, and coagulopathy. The clinical entity of HLH is different from that of sepsis or inflammatory response syndrome. However, these diseases show a common immunopathological state referred to as a cytokine storm.

Senescence-accelerated mice, senescence-prone (SAMP) show early onset of a decline in immune function such as decreased natural killer-cell and T-cell activities and are susceptible to infection. Thus, we investigated whether repeated lipopolysaccharide (LPS) treatment induces hyperinflammation by deterioration of the function of the immune system resulting in sHLH in the senescence-prone substrain, SAMP1/T-A-1 mice.

Methods

Mice

SAMP1/T-A-1 mice were bred and maintained in an experimental facility at Nihon University School of Medicine. SAMR1/Ta Slc mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). Eight- to 12-week old SAMR1 and SAMP1/T-A-1 male mice were used. All protocols involving laboratory mice were reviewed and approved by the Nihon University Animal Care and Use Committee (experiment codes AP15M038 and AP16M054). The approved experimental protocol was performed humanely in strict accordance with Nihon University rules concerning animal care and use.

Lipopolysaccharide treatment

Mice were injected intravenously with a single dose of 25 μg LPS three times at weekly intervals. The body weight of non-treated SAMR1 and SAMP1/T-A-1 mice was 29.6 ± 1.1 g and 31.2 ± 1.3 g, respectively. A control group of SAMR1 and SAMP1/T-A-1 mice was treated with the same volume of pyrogen-free saline.

Progenitor cell colony assay

Myeloid progenitor (CFU-GM) cells were assayed using MethoCult M3231 (Stem Cell Technologies Inc., Vancouver, Canada) supplemented with 10 ng/ml recombinant murine granulocyte-macrophage colony-stimulating factor. B lymphoid-progenitor (CFU-PreB) cells were assayed using MethoCult M3630 (Stem Cell Technologies Inc., Vancouver, Canada) supplemented with 10 ng/ml recombinant murine interleukin (IL)-3. Megakaryocytic progenitor (CFU-Mk) cells were assayed using MethoCult C supplemented with 50 ng/ml recombinant human thrombopoietin, 10 ng/ml recombinant murine IL-3, 20 ng/ml recombinant human IL-6, and 50 ng/ml recombinant murine IL-11. Cells were cultured in a humidified incubator at 37°C and 5% CO₂. CFU-GM, CFU-PreB, and CFU-Mk cells were counted 7 days after plating the cells. CFU-E cells were counted 10 days after plating the cells.

Gene expression assay

The levels of gene expression of cytokines were determined with real-time polymerase chain reaction (PCR) using the Applied Biosystems 7500 Fast Sequence Detection System. Briefly, total RNA from splenic and liver cells was isolated using ISOGEN reagent (Nippongene Corp., Toyama, Japan). mRNA was reverse transcribed using Superscript III (Life Technologies, Carlsbad, CA, USA) and Oligo-dT (Promega Corp., Madison, WI, USA). The levels of gene expression were determined with real-time PCR using TaqMan™ Universal Fast PCR master mix (Applied Biosystems, Foster City, CA, USA) and specific primers. Specific primers and probes for murine IL-1β, IL-6, IL-10, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, Cxcl9, Cxcl10, and GAPDH genes were purchased from Applied Biosystems as described elsewhere.

Clinical laboratory tests and enzyme-linked immunosorbent assays

Plasma fibrinogen levels were measured by ACL ELITE PRO (Instrumentation Laboratory, Bedford, MA, USA). Serum ferritin levels were evaluated with an enzyme-linked immunosorbent assay kit (Abcam Pic, Cambridge, UK).

Flow cytometry analysis for peritoneal macrophage polarization in SAMP1/T-A-1 mice

Peritoneal cells (2 x 10⁶) were labeled using fluorescein isothiocyanate-isothiocyanate-conjugated rat anti-mouse CD11b monoclonal antibody and phycoerythrin-conjugated rat anti-mouse inducible nitric oxide synthase (iNOS) monoclonal antibody, or phycoerythrin-conjugated rat anti-mouse CD11b monoclonal antibody and fluorescein isothiocyanate-conjugated rat anti-mouse CD206 monoclonal antibody. Labeled cells were analyzed by flow cytometry (Cytomics FC500, Beckman Coulter, Brea, CA, USA) for direct detection of CD11b/iNOS M1 macrophages and CD11b/CD206 M2 macrophages.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Data sets were compared using the two-tailed unpaired Student t test and two-way analysis of variance. Differences were considered statistically significant at P<0.05.

Results

Repeated lipopolysaccharide treatment induced pancytopenia in SAMP1/T-A-1 mice but not in SAMR1 mice

The numbers of peripheral white blood cells, red blood cells, and platelets were evaluated in SAMR1 and SAMP1/T-A-1 mice after repeated LPS treatment (Figure 1A). The number of white blood cells in LPS-treated SAMR1 mice was slightly decreased until day 21 after the first LPS treatment compared with that of the non-treated control group (day 0). In contrast, the number of white blood cells in LPS-treated SAMR1/T-A-1 mice was significantly decreased to 17.5% that of the non-treated control group by day 21 (SAMP1/T-A-1; day 0 vs. day 7; P<0.05, day 0 vs. day 14; P<0.005, day 0 vs. day 21; P<0.005). The number of red blood cells in LPS-treated SAMR1 mice remained unchanged compared with that in the non-treated control group. In contrast, the number of red blood cells in LPS-treated SAMR1/T-A-1 mice was significantly decreased to 44.7% that of the non-treated control group by day 21 (SAMP1/T-A-1; day 0 vs. day 7; P<0.05, day 0 vs. day 14; P<0.005, day 0 vs. day 21; P<0.005). The numbers of platelets in LPS-treated SAMR1 mice remained unchanged compared with that in the non-treated control group. In contrast, the number of red blood cells in LPS-treated SAMR1/T-A-1 mice was significantly decreased to 44.7% that in the non-treated control group by day 21 (SAMP1/T-A-1; day 0 vs. day 7; P<0.005, day 0 vs. day 14; P<0.005, day 0 vs. day 21; P<0.005). The numbers of platelets in LPS-treated SAMP1/T-A-1 mice were significantly decreased to 9.9% that in the non-treated control group by day 21 (SAMP1/T-A-1; day 0 vs. day 7; P<0.005, day 0 vs. day 14; P<0.005, day 0 vs. day 21; P<0.005).

Hemophagocytosis in hematopoietic tissues of lipopolysaccharide-treated SAMP1/T-A-1 mice

Hemophagocytosis was observed in SAMP1/T-A-1 mice
7 days after the first LPS treatment. Figure 1B shows hemophagocytic cells in peripheral blood, bone marrow, and the spleen.

**Repeated lipopolysaccharide treatment induced hepatosplenomegaly in SAMP1/TA-1 mice but not in SAMR1 mice**

The ratios of liver weight and spleen weight to whole body weight in SAMR1 and SAMP1/TA-1 mice after repeated LPS treatment were evaluated (Figure 2A). The ratio of liver weight to body weight in LPS-treated SAMR1 mice remained unchanged compared with that of the non-treated control group (day 0). In contrast, the ratio of liver weight to body weight in LPS-treated SAMP1/TA-1 mice increased continuously to 385.8% that of the non-treated control group by day 21 after the first LPS treatment (SAMP1/TA-1; day 0 vs. day 7; \( P < 0.001 \), day 0 vs. day 14; \( P < 0.001 \), day 0 vs. day 21; \( P < 0.001 \)).

The ratio of spleen weight to body weight in LPS-treated SAMR1 mice was slightly increased to 134.8% that of the non-treated control group (day 0) on day 7 after the first LPS treatment and remained unchanged thereafter. In contrast, the ratio of spleen weight to body weight in LPS-treated SAMP1/TA-1 mice was markedly increased to 548.2% that of the non-treated control group on day 7 and remained unchanged thereafter (SAMP1/TA-1; day 0 vs. day 7; \( P < 0.001 \), day 0 vs. day 14; \( P < 0.001 \), day 0 vs. day 21; \( P < 0.001 \)).

Figure 2B shows a photograph of the spleens and livers of SAMP1/TA-1 mice on day 21 after the first saline or LPS treatment. Repeated LPS treatment induced marked hepatosplenomegaly in SAMP1/TA-1 mice.

**Liver and spleen histology in SAMP1/TA-1 mice after repeated lipopolysaccharide treatment**

Figure 3A shows liver histology of SAMP1/TA-1 mice 21 days after the first treatment with saline or LPS. Figure 3B shows high-power liver histology of SAMP1/TA-1 mice 21 days after the first treatment with LPS. Congestion (Figure 3Ba) and microthrombi (Figure 3Bb) in the liver were observed in LPS-treated SAMP1/TA-1 mice.

Figure 4 shows splenic histology of SAMP1/TA-1 mice 21 days after the first treatment with saline or LPS. The red pulp region was expanded, and the structure of splenic pulp appeared somewhat chaotic in LPS-treated SAMP1/TA-1 mice compared with that in saline-treated SAMP1/TA-1 mice (Figure 4A vs. Figure 4B). Furthermore, decreased tissue staining of hemosiderin (ferric iron) was observed in LPS-treated SAMP1/TA-1 mice compared with that in saline-treated SAMP1/TA-1 mice (Figure 4C vs. Figure 4D).

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**Figure 1.** Repeated lipopolysaccharide treatment induced pancytopenia and hemophagocytosis in SAMP/TA-1 mice. (A) Numerical changes in white blood cell (WBC), red blood cell (RBC), and platelet counts in SAMR1 and SAMP1/TA-1 mice after treatment with lipopolysaccharide (LPS). Numerical changes in the counts of WBC (a), RBC (b), and platelets (c) in SAMR1 and SAMP1/TA-1 mice after repeated LPS treatment are shown. The samples of peripheral blood cells were obtained from non-treated control mice (day 0) and mice 7, 14, and 21 days after the first treatment with 25 µg LPS. Each bar represents the mean ± standard deviation obtained from three mice. (B) Hemophagocytosis in hematopoietic tissues of SAMP1/TA-1 mice after LPS treatment. A peripheral blood smear (a) and touch preparations of bone marrow (b) and spleen (c) were made on day 7 after the first treatment with 25 µg LPS. Cells were stained with Wright-Giemsa, and hemophagocytosis was evaluated.
Lipopolysaccharide treatment induced hypofibrinogenemia and hyperferritinemia in SAMP1/TA-1 mice

The levels of fibrinogen in plasma in SAMP1/TA-1 mice 7 days after the first treatment with saline or LPS were 241 ± 55 mg/dL and 103 ± 13 mg/dL, respectively (Figure 5A). The levels of fibrinogen in the plasma of other strains of mice range from 200 to 400 mg/dL.25 The levels of ferritin in serum in SAMP1/TA-1 mice 21 days after the first treatment with saline or LPS were 1680 ± 138 ng/mL and 3080 ± 1126 ng/mL, respectively (Figure 5B). The levels of ferritin in the serum of other strains of mice range from 750 to 1000 ng/mL.26,27

Numerical changes in hematopoietic progenitor cells in bone marrow from SAMR1 and SAMP1/TA-1 mice after repeated lipopolysaccharide treatment

The numbers of hematopoietic progenitor cells in femoral bone marrow were evaluated in SAMR1 and SAMP1/TA-1 mice after repeated LPS treatment (Figure 6).

The numbers of myeloid progenitor (CFU-GM) cells in both SAMR1 and SAMP1/TA-1 mice on days 7, 14, and 21 after the first LPS treatment were similar to those in the non-treated control group (day 0).

The numbers of B-cell progenitor (CFU-preB) cells in SAMR1 mice on days 7, 14, and 21 after the first LPS treatment were slightly decreased compared with those of the non-treated control group. In contrast, the numbers of CFU-preB cells in SAMP1/TA-1 mice on days 7, 14, and 21 after the first LPS treatment were significantly decreased compared with those of the non-treated control group.

The numbers of erythroid progenitor (BFU-E) cells in both SAMR1 and SAMP1/TA-1 mice on days 7, 14, and 21 after the first LPS treatment were decreased compared with those of the non-treated control group. The magnitude of the decrease in the number of BFU-E cells was greater in SAMP1/TA-1 mice than in SAMR1 mice (day 7; SAMR1 vs. SAMP1/TA-1 P < 0.05, day 14; SAMR1 vs. SAMP1/TA-1 P < 0.05, day 21; SAMR1 vs. SAMP1/TA-1 P < 0.05).

The numbers of megakaryocytic progenitor (CFU-Mk) cells in SAMP1/TA-1 mice on days 7, 14, and 21 after the first LPS treatment were decreased compared with those of the non-treated control group, whereas the numbers of CFU-Mk cells in SAMR1 mice on days 7, 14, and 21 after the first LPS treatment were increased.

Changes in the levels of gene expression of cytokines and chemokines in the liver and spleen in SAMR1 and SAMP1/TA-1 mice after the first lipopolysaccharide treatment

Levels of gene expression of inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and IFN-γ, anti-inflammatory
cytokines such as IL-10, and IFN-γ-induced chemokines such as Cxcl9 and Cxcl10 in the liver and spleen of SAMR1 and SAMP1/TA-1 mice after the first LPS treatment were evaluated (Figure 7).

Figure 7A shows the changes in levels of gene expression for cytokines in the liver in SAMR1 and SAMP1/TA-1 mice after the first LPS treatment. The levels of gene expression of IL-1β, IL-6, TNF-α, IFN-γ, IL-10, Cxcl9, and Cxcl10 in the liver of non-treated SAMP1/TA-1 mice were 401%, 253%, 146%, 580%, 694%, 353%, and 220% those of non-treated SAMR1 mice, respectively. The levels of gene expression of IL-1β, IL-6, TNF-α, IFN-γ, IL-10, Cxcl9, and Cxcl10 in the liver of both SAMR1 and SAMP1/TA-1 mice after the first LPS treatment were markedly upregulated during the first 6 h. Thereafter, the levels of gene expression of the cytokines and chemokines in the liver of SAMP1/TA-1 mice remained upregulated, whereas the levels of the cytokines and chemokines in the liver of SAMR1 mice promptly returned to pretreatment levels. Furthermore, the level of IFN-γ gene expression in the liver of SAMP1/TA-1 mice during the first 6 h after the first LPS treatment was markedly higher than that in SAMR1 mice.

Figure 7B shows the changes in levels of gene expression of cytokines in the spleen in SAMR1 and SAMP1/TA-1 mice after the first LPS treatment. The levels of gene expression of IL-1β, IL-6, TNF-α, IFN-γ, IL-10, Cxcl9, and Cxcl10 in the spleen of non-treated SAMP1/TA-1 mice were 135%, 142%, 102%, 187%, 81%, 68%, and 71% those of non-treated SAMR1 mice, respectively. The levels of gene expression of IL-1β, IL-6, TNF-α, IFN-γ, IL-10, Cxcl9, and Cxcl10 in the spleen of SAMR1 mice after the first LPS treatment were markedly upregulated during the first 6 h, followed by prompt downregulation. The time courses of gene expression of
IL-1β, IL-6, TNF-α, IL-10, Cxcl9, and Cxcl10, but not IFN-γ, in the spleen of SAMP1/TA-1 mice after LPS treatment were similar to those in SAMR1 mice. The level of gene expression of IFN-γ in the spleen of SAMP1/TA-1 mice during the first 6 h after LPS treatment was markedly higher than that in SAMR1 mice.

The polarization of M1/M2 peritoneal macrophages of SAMR1 and SAMP1/TA-1 mice after the first LPS treatment was evaluated. Figure 8 shows the changes in the polarization of M1/M2 peritoneal macrophages in SAMR1 and SAMP1/TA-1 mice after the first LPS treatment.

Figure 8. Changes in the polarization of M1/M2 peritoneal macrophages in SAMR1 and SAMP1/TA-1 mice after the first lipopolysaccharide treatment.

Figure 4. Changes in splenic histology in SAMP1/TA-1 mice after lipopolysaccharide treatment. (A-D) Spleens obtained from SAMP1/TA-1 mice 21 days after the first treatment with saline (A, C) or 25 μg lipopolysaccharide (LPS) (B, D) were sectioned and stained with hematoxylin & eosin (HE) (A, B) or Berlin blue to label trivalent iron (Fe) (C, D).

Figure 5. Changes in plasma fibrinogen levels and serum ferritin levels in SAMP1/TA-1 mice after lipopolysaccharide treatment. (A) Plasma fibrinogen levels were measured with ACL ELITE PRO in plasma obtained from SAMP1/TA-1 mice 21 days after the first injection of saline or 25 μg lipopolysaccharide (LPS). (B) Serum ferritin levels were evaluated with an enzyme-linked immunosorbent assay kit in serum obtained from SAMP1/TA-1 mice 7 days after the first treatment with saline or 25 μg LPS. Each bar represents the mean ± standard deviation obtained from three mice. *P<0.05 vs. saline-treated control.
proportions of M1 and M2 macrophages in SAMR1 and SAMP1/TA-1 mice after the first LPS treatment. The proportion of M2 macrophages (CD11b+/CD206+ cells) was higher than that of M1 macrophages (CD11b+/iNOS+ cells) in both non-treated SAMR1 and non-treated SAMP1/TA-1 mice. When treated with LPS, the proportions of M1 macrophages in SAMR1 and SAMP1/TA-1 mice were increased by day 2. Thereafter, although the proportions of M1 macrophages decreased in both SAMR1 and SAMP1/TA-1 mice, the magnitude of the decrease in the proportions of M1 macrophages on day 5 after LPS treatment differed between SAMR1 and SAMP1/TA-1 mice. Namely, the proportion of M1 macrophages in SAMP1/TA-1 mice remained high (8.0% of M1 cells) compared with that in SAMR1 mice (1.4% of M1 cells).

When treated with LPS, the proportions of M2 macrophages remained unchanged in both SAMR1 and SAMP1/TA-1 mice by day 2. Thereafter, the proportion of M2 macrophages in SAMP1/TA-1 mice decreased by day 5 (52.5% to 30.2%), whereas the proportion of M2 macrophages in SAMR1 mice remained high (57.2% to 58.4%).

Discussion

Several murine models of primary HLH and sHLH have been described. Murine models of primary HLH were generated by deletion of perforin and Rab27a genes, and mutation of the Unc13d gene, leading to defects in the granule exocytic pathway. In contrast, sHLH can be induced by Epstein-Barr virus infection in humanized mice transplanted with human CD34+ cells, Salmonella enteritica infection in Sv12956 mice, and cytomegalovirus infection in BALB/c mice. Furthermore, C57BL/6 mice repeatedly given the toll-like receptor 9 agonist, CpG, and IL-6 transgenic mice given LPS also develop sHLH.

Henter et al. proposed that the diagnosis of HLH is based on eight criteria, including fever, splenomegaly, bicytopenia, hypertriglyceremia and/or hypofibrinogenemia, hemophagocytosis, low/absent natural killer-cell activity, hyperferritinemia, and high levels of soluble IL-2 receptor. Five of these eight criteria must be fulfilled for a diagnosis, unless a family history is present that is consistent with sHLH. When repeatedly treated with LPS, SAMP1/TA-1 mice showed hepatosplenomegaly, pancy-
topenia, hypofibrinogenemia, hyperferritinemia, and hemophagocytosis in peripheral blood, the bone marrow, and the spleen. These features are compatible with sHLH. sHLH is a severe and potentially fatal condition that leads to overwhelming inflammation. Central to its pathogenesis is a cytokine storm with markedly increased levels of numerous proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ. The levels of gene expression of IL-1β, IL-6, TNF-α, and IFN-γ were all markedly upregulated in the liver and spleen during the first 6 h after the first LPS treatment in both SAMR1 and SAMP1/TA-1 mice (Figure 7). However, the subsequent time course of gene expression for the cytokines in the liver and spleen were different between SAMR1 and SAMP1/TA-1 mice. Namely, the upregulation of IL-1β, IL-6, TNF-α, and IFN-γ in the liver of SAMP1/TA-1 mice was prolonged compared with that in SAMR1 mice (Figure 7A). These data suggest that prolonged overwhelming inflammation occurred in SAMP1/TA-1 mice but not in SAMR1 mice.

Macrophages commonly exist in two distinct subsets, M1 and M2 macrophages, which have opposite functions. M1 macrophages are proinflammatory, and M2 macrophages are anti-inflammatory. The M1/M2 macrophage balance governs the inflammation process. LPS treatment resulted in a prolonged increase in the proportion of M1 macrophages and simultaneously a decrease in the M2 macrophage proportion in SAMP1/TA-1 mice on day 5, compared with SAMR1 mice (Figure 8). The proportions of M1 and M2 macrophages were analyzed using peritoneal macrophages, and these data also support the idea that prolonged overwhelming inflammation occurred in SAMP1/TA-1 mice.

In addition, the magnitude of the upregulation of IFN-γ in the liver and spleen was greater in SAMP1/TA-1 mice compared with SAMR1 mice.
than in SAMR1 mice (Figure 7Ad and 7Bd). The fluctuation of the expression of IFN-γ-inducible chemokine genes such as Cxcl9 and Cxcl10 in the liver and spleen of SAMP1/TA-1 mice paralleled that of IFN-γ, which indicated that functional IFN-γ was produced in the liver and spleen. In several animal models of sHLH, IFN-γ has been identified as a mediator of systemic inflammation and may play a pivotal role in the pathogenesis of sHLH, whereas the role of other cytokines is still not clear. IL-1β, IL-6, and TNF-α are cytokines downstream of IFN-γ in a mouse model of sHLH. Buatonis et al. demonstrated that in an sHLH model induced by repeated toll-like receptor 9 treatment, total IFN-γ levels produced in tissues were 500- to 2,000-fold higher than those measured in blood, and they identified the liver and spleen as major sites of IFN-γ production. IFN-γ may be a critical factor in the pathogenesis of sHLH in SAMP1/TA-1 mice. However, investigation of a therapeutic approach using antibodies for proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ in mice is necessary to clarify the central factor(s) in the pathogenesis of sHLH-like disease in LPS-treated SAMP1/TA-1 mice.

Acute systemic inflammation augments myelopoiesis but suppresses B lymphopoiesis and erythropoiesis. Furthermore, acute systemic inflammation provokes rapid consumption of platelets, resulting in transient thrombocytopenia. The numbers of peripheral white blood cells, red blood cells, and platelets in SAMP1/TA-1 mice after repeated LPS treatment decreased rapidly compared with those in SAMR1 mice (Figure 1A). When BALB/c

![Figure 8](image-url)

**Figure 8.** The proportions of M1 and M2 peritoneal macrophages in SAMR1 and SAMP1/TA-1 mice after lipopolysaccharide treatment. (A; B) The changes in the proportions of M1 cells (CD11b+/iNOS+ cells) (A) and M2 cells (CD11b+/CD206+ cells) (B) in SAMR1 and SAMP1/TA-1 mice after the first treatment with 25 μg lipopolysaccharide (LPS) were evaluated. The samples of peritoneal macrophages were obtained from non-treated control mice (day 0) and mice 2 and 5 days after the first treatment with 25 μg LPS.
mice were treated repeatedly with LPS, the numbers of white blood cells and platelets decreased rapidly after each treatment, followed by a prompt return to near or above pretreatment levels; the number of red blood cells remained unchanged. Thus, the changes in the number above pretreatment levels; the number of red blood cells each treatment, followed by a prompt return to near or above pretreatment levels. The number of peripheral lymphocytes was more significantly decreased in SAMP1/TA-1 mice than in SAMR1 mice (Figure 1A). The number of erythroid progenitor cells was more significantly decreased in LPS-treated SAMP1/TA-1 mice than in LPS-treated SAMR1 mice (Figure 6C). Furthermore, the number of megakaryocyte progenitor cells in LPS-treated SAMP1/TA-1 mice was decreased, whereas the number of megakaryocyte progenitor cells in LPS-treated SAMR1 mice was increased (Figure 6D). These results suggest that severe anemia and thrombocytopenia in LPS-treated SAMP1/TA-1 mice may be in part due to profound and prolonged suppression of erythropoiesis and thrombopoiesis in the bone marrow. Taken together, the prolonged and tremendous cytokine storm induced by LPS may have disrupted the dynamics of hematopoiesis.

When repeatedly treated with LPS, ferric iron storage in the spleen of SAMP1/TA-1 mice was markedly decreased compared with that of non-treated SAMP1/TA-1 mice (Figure 4C vs. 4D). A similar phenomenon was observed in Typhimurium-infected mice, a model of sHLH, which showed increased erythropoiesis in the spleen. When C57BL/6 mice are treated with an inflammatory compound, erythropoiesis in the spleen is accelerated, whereas erythropoiesis in the bone marrow is suppressed. Taken together, splenic erythropoiesis in LPS-treated SAMP1/TA-1 mice may be accelerated to compensate for suppression of erythropoiesis, resulting in decreased ferric-iron storage in the spleen.

In this study, we demonstrated that SAMP1/TA-1 mice treated repeatedly with LPS develop an sHLH-like disease. SAMP1/TA-1 mice are susceptible to infection due to latent deterioration of immunological function. Bacterial infections are reported in 9% of adult cases of HLH. LPS is a characteristic component of the wall of Gram-negative bacteria. Several cases of acute-onset sHLH associated with severe Gram-negative bacterial infection such as Salmonella typhi, Escherichia coli, Klebsiella pneumoniae and Haemophilus parainfluenzae have been reported. Thus, SAMP1/TA-1 mice are a useful model to investigate the pathogenesis of bacterial infection-associated sHLH.

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