CCL2 as a trigger of manifestations of compensatory anti-inflammatory response syndrome in mice with severe systemic inflammatory response syndrome

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Abstract: Patients with compensatory anti-inflammatory response syndrome (CARS) are at a higher risk for infection with various opportunistic pathogens. CARS develops commonly in association with the manifestation of systemic inflammatory response syndrome (SIRS). In the present study, the role of SIRS-associated soluble factors on the CARS development was examined in mice with pancreatitis, a carrier of typical SIRS. Following the production of SIRS-related cytokines [tumor necrosis factor α and interleukin (IL)-1], CC chemokine ligand 2 (CCL2), IL-4, and IL-10 (typical CARS cytokines) were detected in the sera of mice with pancreatitis. CCL2 has been described as an essential chemokine for the T helper cell type 2 manifestation. CARS effector cells (cells with an ability to produce IL-4 and IL-10) were not generated from normal T cells after stimulation with SIRS-related cytokines. However, these cells were generated from normal T cells after cultivation with peripheral blood neutrophils (PMN) from SIRS mice in a dual-chamber transwell. Normal T cells did not convert to CARS effector cells after transwell cultures with PMN from normal mice. CCL2 was detected in culture fluids of PMN from SIRS mice, and PMN from normal mice did not produce CCL2 into their culture fluids. CARS effector cells did not appear in PMN-depleted SIRS mice or SIRS mice treated with anti-CCL2 monoclonal antibody, and these cells were demonstrated in PMN-depleted SIRS mice after treatment with recombinant murine CCL2. These results indicate that CCL2 produced by PMN from SIRS mice is an active molecule on the SIRS-associated CARS manifestation.

Key Words: SIRS · CARS · neutrophils

INTRODUCTION

Systemic inflammatory response syndrome (SIRS) and sepsis (SIRS accompanying infection) remain the chief causes of death in intensive care patients, with mortality rates between 30% and 70% [1]. SIRS is triggered by localized or generalized infection, trauma, thermal injury, or sterile inflammation processes (i.e., acute pancreatitis) [2]. Patients are diagnosed as having SIRS when they have more than two of the following clinical parameters: body temperature higher than 38°C or lower than 36°C; heart rate higher than 90/min; hyperventilation evidenced by respiratory rate higher than 20/min or PaCO2 lower than 32 mmHg; white blood cell (WBC) count higher than 12,000/mm³ or lower than 4000/mm³ [3]. Recent reports described an increase in plasma concentrations of procalcitonin or C-reactive protein when SIRS-associated inflammatory responses are present [2, 4, 5]. Other pathophysiologic changes in patients with SIRS include progressive endothelial dysfunction and increased microvascular permeability [6, 7]. In addition, the following events are observed in SIRS patients [8–11]: platelet sludging, which blocks microcirculation, causing maldistribution of blood flow, and possibly ischemia, which in turn may cause reperfusion injury and induction of heat shock proteins. The coagulation system is activated, and the protein-C/protein-S inhibitory pathway is impaired [11]. This dysregulation of vasodilatory and vasoconstrictive mechanisms results in profound vasodilation, which exacerbates fluid translation and maldistribution of blood flow [12–15]. Often, the result is that patients develop profound shock, which further compromises blood flow to vital organs [12–15]. Organ dysfunction and ultimately failure result from these changes [16]. In some patients with SIRS, persistent and overwhelming inflammation with elevated levels of proinflammatory mediators is associated with an increased risk of death [13, 14, 16]. Conversely, an overwhelming systemic, proinflammatory reaction in SIRS is frequently followed by compensatory anti-inflammatory response syndrome (CARS) [17], which appears to regulate the inflammatory responses during SIRS. However, the excessive release of anti-inflammatory cytokines [interleukin (IL)-4, IL-10, and IL-13] from CARS effector cells and the impaired human leukocyte antigen-DR expression (less than 30% as compared with healthy controls) lead to severe immunosuppression [18–20]. The susceptibility of patients with
SIRS to infections increases greatly as a result of CARS-associated immunosuppression [21–23]. In our previous studies, patients who carried CARS effector cells were susceptible to Candida albicans infection [24]. Therefore, in this study, the mechanism of SIRS-associated CARS manifestation was investigated using a murine SIRS model of pancreatitis. This model has been well-recognized as typical SIRS in the absence of bacterial infection [25, 26]. In the results obtained, tumor necrosis factor α (TNF-α) and IL-1β were detected in the sera of SIRS mice, and these cytokines disappeared until 6 h after SIRS induction. However, IL-4 and IL-10 (representative CARS cytokines) were detected in the sera of SIRS mice after the disappearance of SIRS cytokines, IL-4- and IL-10-producing T cells were generated in dual-chamber transwells cultured with peripheral blood neutrophils (PMN) from mice 3 h after SIRS induction. However, these cells were not generated from normal T cells when transwell cultures were performed with anti-CC chemokine ligand 2 (CCL2) monoclonal antibody (mAb). Furthermore, CCL2 was detected in the sera of mice early after SIRS induction. Also, CCL2 was produced in cultures of PMN from mice early after SIRS induction. These results indicate that CCL2 produced by PMN from mice early after SIRS induction stimulates the subsequent development of CARS.

MATERIALS AND METHODS

Animals

Eight- to 9-week-old BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were used in these experiments. The Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston (IACUC Approval Number: 01-04-010) approved the experimental protocols for animal studies.

Reagents, cells, and media

mAbs for TNF-α, IL-1β, interferon-γ (IFN-γ), IL-4, IL-10, CCL2, Ly6G, CD3, CD4, CD6, and CD26 were purchased from BD Pharmingen (San Diego, CA). Recombinant (r) murine TNF-α, IL-1β, IL-2, IFN-γ, IL-4, IL-10, and CCL2 were obtained from PeproTech (Rocky Hill, NJ). Mononuclear cells were prepared from spleens of normal mice or SIRS mice using Ficoll-Hypaque sedimentation. T cells were isolated from splenic mononuclear cell preparations using murine T cell enrichment columns (R&D Systems, Minneapolis, MN) [27]. Then, this preparation was further purified using magnetic beads coated with anti-CD3 mAb (Dynal, Great Neck, NY) as described previously [24]. The purity of these cells was greater than 97% when measured by flow cytometry. PMN were isolated from heparinized peripheral whole blood using dextran sedimentation followed by Ficoll-Hypaque density gradient centrifugation [28, 29]. The erythrocytes in these specimens were eliminated by erythrocyte-lysing kit (R&D Systems). Our flow cytometry analysis showed that resident PMN expressed CD62 ligand (CD62L) antigen but not CD11b and major histocompatibility complex (MHC) class II antigens [29]. CD62L antigen is known to shed rapidly from PMN cell surface upon activation [30, 31]. In contrast, CD11b and MHC class II antigens are known to be expressed on PMN cell surface upon activation [30–33]. Therefore, our isolation procedures for PMN avoided nonspecific activation. Various cell preparations were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 2 mM L-glutamine antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; complete medium).

A mouse model of pancreatitis

Pancreatitis was produced in mice, according to protocols reported previously [25, 26]. This model has been recognized as a carrier of a typical SIRS [25, 26]. To create pancreatitis, mice were treated with cerulein (50 μg/kg, intraperitoneally (i.p.),) hourly for 6 h in combination with lipopolysaccharides (1.6 mg/kg, i.p., 5 h after the first injection of cerulein). All mice were alive more than 10 days after SIRS induction, and there was a markedly enhanced severity of pancreatic damage (edema, inflammatory cell infiltration, hemorrhage, and necrosis). In some experiment, SIRS was induced in mice depleted of PMN by treatment with anti-Ly6G mAb (100 μg/mouse, i.p., every day for 5 days). Functional PMN were not recovered from these mice 1–7 days after treatment.

Criteria of SIRS and CARS

As previously reported [34, 35], SIRS manifestation was evaluated by the following three parameters: (i) body temperature; temperature was measured using a microprobe rectal thermometer. If the body temperature of mice was <35.5°C, it was considered as abnormal. (ii) TNF-α and IL-1β levels in plasma; the amounts of TNF-α and IL-1β in the plasma of SIRS mice were measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s procedures. If TNF-α levels were >9000 pg/ml, and IL-1β levels were >2000 pg/ml, the mice were considered abnormal. (iii) WBC count; the numbers of WBCs in peripheral blood were measured. If WBC counts were <4000/mm³ or >12,000/mm³, the mice were considered abnormal. As required, multiple organ failure parameters (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and amylase) in plasma samples of SIRS mice were measured using diagnostic kits (Sigma-Aldrich, St. Louis, MO). All parameters of SIRS were present in SIRS mice, created as shown above.

The manifestation of CARS in SIRS mice was evaluated by the appearance of IL-4 and IL-10 in the sera of tested mice. In addition, CARS manifestation was evaluated by the appearance of T cells with the ability to produce IL-4 and IL-10. These cytokines have been shown to be representative cytokines in CARS [17].

Transwell assay

To determine the effect of PMN on the generation of CARS effector cells, PMN from SIRS mice were cultured with splenic T cells from normal mice (normal T cells) in a dual-chamber transwell [36]. Thus, 600 μl normal T cell suspension (3×10⁵ cells/well) was placed into the lower chamber of the transwell (0.4 μm micropores, Costar, Comin, NY). Before cells were added, lower chambers of all the transwells were coated with a mixture of anti-CD3 mAb (0.1 μg/ml) and anti-CD28 mAb (0.25 μg/ml). The cell suspension (100 μl) for PMN (1×10⁶ cells/well) was placed into the upper chamber of the transwell. Twelve hours later, the upper chamber was removed, and cells in the lower chamber were recultured for 5 days in the presence of IL-2 (10 ng/ml). In some experiments, transwell cultures were performed in the presence of 10 ng/ml anti-CCL2 mAb or isotype control antibody. Twelve hours later, cells harvested from the lower chamber were cultured for an additional 5 days. To confirm the proliferating abilities of T cells obtained at the end of cultivation, cells harvested from the cultures were adjusted to 2×10⁵ cells/ml and recultured in each well of a 96-well microtiter plate (100 μl) supplemented with CellTitre 96 A_{MTT} reagent, One Solution (Promega, Madison, WI). Four hours after the cultivation, the proliferative response of these cells was assayed according to the manufacturer’s instructions. In the results obtained, the absorbance of tested samples at optical density (OD)₄₉₀ was 0.268, and the absorbance of nonactivated samples at OD₄₉₀ was 0.030. This indicates that T cells, 5 days after cultivation with immobilized anti-CD3/CD28 mAb and IL-2, have a proliferating ability. As the above results also suggest that T cells cultured with immobilized anti-CD3/CD28 mAb are viable cells, we have additionally tested for the viability of these cells by the trypan blue dye exclusion test. In the results, T cells, 5 days after cultivation with immobilized anti-CD3/CD28 mAb plus IL-2, were shown to be viable cells (93% viability). These results indicate that T cells used in the transwell assay are viable cells with proliferative abilities.

Production and assay of cytokines

Culture fluids of cells harvested from the lower chambers of the above transwell cultures were assayed for IL-4 and IL-10 using ELISA. Also, splenic T cells (1×10⁶ cells/ml) from SIRS mice were cultured for 72 h without any stimulation, and culture fluids harvested were assayed for IL-4 and IL-10 using ELISA. To determine the abilities of cells to produce CCL2, PMN (5×10⁶)
cells/ml), prepared from mice various hours after SIRS induction, were cultured without any stimulation for 24 h. The culture fluids harvested from these cultures were assayed for CCL2 using ELISA. To determine circulating TNF-α, IL-1β, IL-4, IL-10, and CCL2 levels, serum specimens prepared from mice 3, 6, 12, and 24 h and 3, 5, and 7 days after SIRS induction were assayed using ELISA. The detection limit for these cytokines was 18–32 pg/ml in our assay systems. Each assay was performed three times.

Statistical analysis

Data are presented as mean ± SD. Comparisons between experimental and control groups were made by ANOVA, followed by Fisher’s protected least significant difference test. The result was considered significant if the P value was <0.05.

RESULTS

Kinetics of TNF-α, IL-1β, IL-4, and IL-10 production in the sera of mice various times after SIRS induction

The appearance of SIRS-related cytokines (TNF-α and IL-1β) and CARS-related cytokines (IL-4 and IL-10) was examined in the sera of mice various times after SIRS induction. Also, IFN-γ [a T helper cell type 1 (Th1) cytokine] in the sera of these mice was measured as a control. The amounts of TNF-α, IL-1β, IL-4, IL-10, and IFN-γ in sera were determined using ELISA. Three hours after SIRS induction, TNF-α, at a concentration of 9.3 ± 0.4 ng/ml, and IL-1β, at a concentration of 5.5 ± 0.3 ng/ml, were detected in the sera (Fig. 1). Subsequently, these cytokines declined to undetectable levels within 12 h of SIRS induction (Fig. 1). However, IL-4 and IL-10 were not detected in the sera of mice 3–12 h after SIRS induction. IL-4 and IL-10 were first detected in the sera of mice 3 days after SIRS induction. The production of IL-4 and IL-10 reached its peak in the fifth day after SIRS induction, and then, it gradually declined (Fig. 1). No amounts of TNF-α and IL-1β were detected in the sera of mice 1–7 days after SIRS induction. IFN-γ was not detected in the sera of SIRS mice through the experiments. When splenic T cells from mice 5 days after SIRS induction were cultured without any stimulation, IL-4 and IL-10 (but not IFN-γ) were demonstrated in their culture fluids. However, these cytokines were not detected in the cultures of the same T cell preparation from normal mice (Fig. 2). These results indicate that SIRS manifested within 3 h of SIRS induction, and CARS developed following SIRS manifestation.

CARS effector cells were generated from normal T cells in transwell cultures with PMN from SIRS mice

In the following experiments, PMN isolated from the peripheral blood of mice 3 h after SIRS induction were abbreviated as SIRS-PMN. To determine the influence of SIRS on the generation of CARS effector cells, SIRS-PMN (3 × 10⁵ cells/well, upper chamber) were cultured with normal T cells (3 × 10⁵ cells/well, lower chamber) in dual-chamber transwells. Twelve hours after cultivation, cells in the lower chamber were harvested for 5 days, and culture fluids harvested were assayed for IL-4 and IL-10 as representative CARS cytokines. As a control, IFN-γ in these culture fluids was measured. The results obtained are shown in Figure 3A. IL-4 and IL-10 were detected in the culture fluids of normal T cells cultured with SIRS-PMN, and IFN-γ was not detected in these culture fluids. Significant amounts of these cytokines were not produced by normal T cells alone or T cells cultured with normal PMN (PMN-N). These results indicate that CARS cells were generated from normal T cells stimulated with soluble factors released from the SIRS-PMN. Furthermore, after stimulation with SIRS-PMN, T cell subtypes (CD4 or CD8 T cells) converting to IL-4/IL-10-producing cells were examined using blocking antibodies (5 μg/ml) directed against CD4 and/or CD8. As compared with the control, the production of IL-4 was inhibited by 56% (anti-CD4 mAb) or 61% (anti-CD8 mAb) by blocking mAb, and IL-10 production was inhibited by 59% (anti-CD4 mAb) or 67% (anti-CD8 mAb) by these mAb. When T cells were treated with a mixture of anti-CD4 and anti-CD8 mAbs, the production of IL-4 and IL-10 was eliminated completely.

Fig. 1. Production of TNF-α, IL-1β, IL-4, IL-10, and IFN-γ in sera of mice various times after SIRS induction. Serum specimens, prepared from mice 1, 3, 6, 12, and 24 h and 3, 5, and 7 days after SIRS induction, were assayed for TNF-α (O), IL-1β (●), IL-4 (▲), IL-10 (■), or IFN-γ (▼) using ELISA. Each point is displayed as mean ± SD (n=5).

Fig. 2. Production of IL-4 and IL-10 in cultures of splenic T cells from SIRS mice. Splenic T cells (1×10⁶ cells/ml) from normal mice or SIRS mice (5 days after SIRS induction) were cultured without any stimulation. Culture fluids harvested were assayed for IL-4 and IL-10 using ELISA. As a control, the amount of IFN-γ in the same culture fluids was measured. Each result is expressed as mean ± SD (n=5). *, P < 0.001.
These results suggest that CD4\(^+\) T cells and CD8\(^+\) T cells responded to SIRS-PMN stimulation. CCL2 produced by SIRS-PMN was responsible for CARS effector cell generation. TNF-\(\alpha\) and IL-1\(\beta\) were detected in the sera of mice 3 h after SIRS induction (Fig. 1). However, in our preliminary studies, these cytokines did not stimulate normal T cells to generate IL-4- and IL-10-producing cells. In contrast, the necessity of CCL2 for the generation of Th2 cells has been demonstrated previously in CCL2 knockout mice [37]. Therefore, the ability of SIRS-PMN to produce CCL2 was examined in vitro. SIRS-PMN were cultured in complete medium at a cell density of 1 \(\times\) 10\(^6\) cells/ml for 24 h without any stimulation. The amount of CCL2 in the harvested culture fluids was measured. SIRS-PMN produced 2.7 \(\pm\) 0.3 ng/ml CCL2 into their culture fluids. In comparison, PMN from normal mice produced less than 18 pg/ml CCL2 into their culture fluids (Fig. 4). Furthermore, CCL2 was detected in the sera of mice 1–12 h after SIRS induction (Fig. 5). No amount of CCL2 was detected in sera from normal mice. In vitro and in vivo results indicate that CCL2 is produced in response to SIRS development.

Next, the role of CCL2, which appeared in the sera of mice early after SIRS induction, in CARS development was examined in vitro. In the presence of anti-CCL2 mAb (10 \(\mu\)g/ml), SIRS-PMN (upper chamber) were cultured with normal T cells (lower chamber) in dual-chamber transwells. The transwell cultures supplemented with isotype antibody served as a control. Cells harvested from the lower chamber 12 h after cultivation were recultured for 5 days, and culture fluids harvested were assayed for IL-4, IL-10, and IFN-\(\gamma\). In the results, IL-4 and IL-10 were not produced by normal T cells after transwell cultivation supplemented with anti-CCL2 mAb, and these cytokines were produced by normal T cells after transwell cultivation with isotype antibody (Fig. 6). These results indicate that CCL2 is a key chemokine when SIRS-PMN stimulate normal T cells to generate CARS cells.
To determine the role of PMN on CARS development, SIRS was induced by cerulein in mice previously treated with anti-Ly6G mAb (PMN-depleted mice). SIRS developed in these mice in the same manner demonstrated in normal mice treated with cerulein (Fig. 7, A–D). Therefore, the development of CARS in PMN-depleted mice exposed to SIRS was examined next. IL-4 and IL-10 were not produced by T cells from PMN-depleted mice 4 days after SIRS induction. However, these cytokines were produced by T cells from PMN-depleted mice when they were treated with CCL2 (Fig. 7E). Furthermore, the manifestation of CARS in SIRS mice depleted of CCL2 was examined. The elimination of CCL2 from SIRS mice was performed by the administration of anti-CCL2 mAb (100 µg/mouse, 6 and 0.5 h before SIRS induction). CARS manifestation was confirmed in SIRS mice treated with isotype antibody. However, after treatment with anti-CCL2 mAb, IL-4 and IL-10 were not detected in the sera of mice 3 and 5 days after SIRS induction (Fig. 7F). These results indicate that PMN from SIRS mice are key for SIRS-associated CARS manifestation.

CARS did not develop in PMN-depleted SIRS mice

Fig. 6. Effect of anti-CCL2 mAb on IL-4 and IL-10 production by normal T cells cultured with SIRS-PMN. In dual-chamber transwells, activated normal T cells (lower chamber, 3 × 10⁵ cells/well) were cultured with SIRS-PMN (upper chamber, 1 × 10⁵ cells/well) in the presence of anti-CCL2 mAb or isotype antibody. Also, as a control, normal T cells were cultured with PMN from normal mice in the same manner. Twelve hours after cultivation, cells in the lower chamber were recultured for 5 days. The amounts of IL-4, IL-10, and IFN-γ in their culture fluids were measured using ELISA. Each bar indicates mean ± SD (n=4–6). *, P < 0.001.

Fig. 7. SIRS and CARS parameters demonstrated in SIRS mice depleted of PMN, SIRS mice treated with anti-CCL2-neutralizing mAb, or PMN-depleted SIRS mice treated with rCCL2. (A–D) One hour to 12 h after SIRS induction, the development of SIRS was assayed in SIRS mice ( ), SIRS mice depleted of PMN ( ), SIRS mice treated with anti-CCL2 neutralizing mAb (A: 100 µg/mouse, subcutaneously (s.c.), 6 and 0.5 h before SIRS induction), and PMN-depleted SIRS mice treated with rCCL2 ( , 100 ng/mouse, s.c., 12 and 0.5 h before SIRS induction). TNF-α (A), IL-1β (B), WBC counts (C), and body temperature (BT; D) were measured as SIRS parameters. Hatched area shows normal levels of each parameter. (E) IL-4 and IL-10 production by splenic T cells from PMN-depleted SIRS mice treated with rCCL2. PMN-depleted SIRS mice were treated with rCCL2 or saline. Five days after SIRS induction, splenic T cells from these mice were cultured without any stimulation. Culture fluids harvested were assayed for IL-4, IL-10, and IFN-γ. (F) IL-4 and IL-10 production in the sera of SIRS mice treated with anti-CCL2 mAb. SIRS mice were treated with anti-CCL2 mAb or isotype antibody. Three and 5 days after SIRS induction, serum specimens obtained from these mice were assayed for IL-4, IL-10, and IFN-γ. Each result is displayed as mean ± SD (n=5).
CARS has been shown as an important regulator of SIRS severities [38–40]. CARS usually appears following SIRS manifestation [17]. However, some of the important host defenses against infections are down-regulated by CARS [41–45]. The expression of Th1 responses is depressed by IL-4 and IL-10 released from CARS effector cells [22]. Th1 responses inhibit the spreading of intracellular pathogens through inflammatory-tissue (or cell) injury [21]. In addition, IL-4 and IL-10 play a role in the subsequent differentiation of CARS cells from naive T cells [22]. Therefore, severe infections are demonstrated frequently in individuals with CARS [24, 44].

In the present study, the mechanism of SIRS-associated CARS manifestation was investigated using a murine SIRS model. IL-4 and IL-10, CARS cytokines, were detected in the sera of SIRS mice after the disappearance of SIRS cytokines (TNF-α and IL-1β). IL-4 was also produced by normal T cells (from normal mice, lower chamber) cultured with PMN from mice early after SIRS induction (SIRS-PMN, upper chamber) in dual-chamber transwells. However, these cytokines were not detected in the culture fluids when the same transwell culture was performed with anti-CCL2 mAb. CCL2 was detected in the sera of mice early after SIRS induction. Also, SIRS-PMN produced CCL2 when they were cultured in vitro without any stimulation. These results indicate that CARS manifestation is induced by CCL2 released from SIRS-related cells (SIRS-PMN). This finding is supported by other studies. In those studies, mice lacking CCL2 have the impaired ability to produce IL-4, IL-5, and IL-10 and resist Leishmania major infection [37].

More recently, we have found that in addition to PMN-N, there are at least two distinct populations of PMN [29]. These PMN were distinguishable in the following ways: PMN-I (Gr-1+ CD11b− CD49d+) produced IL-12/CCL3, expressed Toll-like receptor 2 (TLR2)/TLR4/TLR8, and directed resident macrophages (resident Mφ) to polarize classically activated Mφ (CAMφ); PMN-II (Gr-1+ CD11b+ CD49d−) produced IL-10/CCL2, expressed TLR2/TLR4/TLR7/TLR9, and directed resident Mφ to polarize alternatively activated Mφ (AAMφ); PMN-N (Gr-1+ CD11b+ CD49d+) produced no soluble factors, expressed TLR2/TLR4/TLR9, and directed resident Mφ to polarize neither CAMφ nor AAMφ. PMN-I were obtained from mice that were resistant to methicillin-resistant Staphylococcus aureus (MRSA) infection, and MRSA-sensitive hosts were a carrier of PMN-N. PMN-N were obtained from normal mice [29]. PMN with properties similar to PMN-I and PMN-II were also demonstrated in mice infected with virulent or avirulent strains of C. albicans [46]. PMN from mice infected with avirulent C. albicans produced IL-12. These PMN did not produce IL-10 and did not induce CD4+ T cell apoptosis. In contrast, PMN from mice infected with virulent C. albicans did not kill the pathogen in vitro. These PMN produced IL-10 and induced CD4+ T cell apoptosis. These facts suggest that PMN from SIRS mice might be classified as PMN-II. The characterization of PMN in SIRS mice will be required in future studies.

A wide variety of activated cells, including monocytes, PMN, fibroblasts, vascular endothelial cells, and smooth muscle cells, produce CCL2 in response to various stimuli [47–50]. Overproduction of CCL2 has been associated with a variety of human diseases, such as atherosclerosis [47], AIDS [48], and prostate adenocarcinoma [49]. CCL2-overexpressing transgenic mice were shown to be highly susceptible to infections with Listeria monocytogenes or Mycobacterium tuberculosis [50]. In our earlier studies, the severity of herpetic encephalomyelitis and cryptococcal encephalitis was shown to be accelerated in mice treated with CCL2 [51, 52]. More recently, we studied the role of CCL2 on the generation of AAMφ [34]. After treatment with various doses of rCCL2, resident Mφ were assayed for their AAMφ properties. According to previous reports [52, 53], Mφ with the ability to produce CCL17 and to express mannose receptor mRNA were considered as AAMφ. After treatment with 3–30 ng/ml rCCL2, resident Mφ acquired the ability to produce CCL17 and express mannose receptor mRNA [34]. These results indicate that CCL2 has the capability to generate AAMφ.

Many papers have described that CAMφ are important for the host's innate immunities against various infections [53, 54]. CAMφ were generated from resident Mφ in response to the engagement of TLRs or binding of IFN receptors by IFN-α/β or IFN-γ [53, 54]. CAMφ exhibit high oxygen consumption, killing activity against intracellular pathogens and cytotoxicity against tumor cells. Also, CAMφ express inducible nitric oxide synthase. In addition, CAMφ induce Th1 cells by producing IFN-γ, IL-12, and CCL3. However, CAMφ were not generated in individuals whose AAMφ predominated [55]. AAMφ have a role in the negative regulation of Th1 cells and CAMφ [53, 54]. Furthermore, naive T cells convert to Th2 cells under stimulation with IL-10 released from AAMφ [54]. The appearance of AAMφ has been reported in various patients who are susceptible to certain infections. Thus, patients with thermal injuries, malignancies, major surgery, or multiple traumas, who are susceptible to infections, are carriers of AAMφ [53–55].

In the present study, PMN from mice early after SIRS induction (SIRS-PMN) induced CARS effector cells (T cells with the ability to produce IL-4 and IL-10). CCL2 was identified as an effector molecule when Th2 cells were induced by SIRS-PMN. As infection in SIRS patients is classified as sepsis, the regulation of CCL2 (or SIRS-PMN) may be important for the control of infectious complications in patients with major surgery, trauma, and burn injury.

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