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CCL17 and IL-10 as Effectors That Enable Alternatively Activated Macrophages to Inhibit the Generation of Classically Activated Macrophages

Tatsushi Katakura,* Masaru Miyazaki,† Makiko Kobayashi,*‡ David N. Herndon,‡ and Fujio Suzuki2*‡

Classically activated macrophages (CAMφ) have long been recognized as heterogeneous (1). The heterogeneity of Mφ almost certainly reflects their plasticity and versatility in response to microenvironmental signals. Recent studies have suggested five (1) or three (2) pathways of Mφ response to microenvironmental signals. Recent studies have supplemented with CpG DNA. In addition, RM/H9278/Fujio Suzuki 2* ‡/H9278/Fujio Suzuki 2* ‡ study, the mechanism by which AAM activated macrophages (CAMφ) have been described as a major effector cell on the host’s innate immunities. However, CAMφ have generally been divided into two populations, classically activated macrophages (CAMφ) and alternatively activated macrophages (AAMφ), based on their immunobiological properties (3–5). CAMφ play an important role in the antimicrobial innate immunities of the host (6–8). CAMφ are generated from RMφ stimulated with a microbe, microbial product (CpG DNA, poly(I:C), and LPS) or various biological response modifiers (muramyl dipeptide and poly(styrene-maleic acid) conjugated with neocarzinostatin) (5, 9–12). CAMφ exhibit 1) high oxygen consumption, 2) the ability to kill cells infected with intracellular pathogens, 3) cytotoxicity against tumor cells, 4) the ability to express inducible NO synthase (iNOS), and 5) the ability to secrete NO, proinflammatory cytokines (IL-1, IL-6, and TNF-α) and Th1 response-associated cytokines (IFN-γ, IL-12, IL-18, CCL3, CXCL9, and CXCL10) (4, 5). In addition, CAMφ induce Th1 cells by producing IL-12, CCL3, and IFN-γ. In contrast, AAMφ play a role in the negative regulation of both CAMφ and Th1 cell generation (4, 5, 13–16). AAMφ preferentially express receptors for foreign Ags, such as mannose receptor, β-glucan receptors, and scavenger receptors (17, 18). AAMφ have been described as a producer cell for IL-1R antagonist, IL-10, CCL17, CCL22, and arginase (4, 5). They also inhibit IFN-γ production and developmental Th1 responses (15, 16).

Numerous studies have shown that CAMφ generation is required for a host’s defense against infections with various intracellular pathogens or the growth of tumors (1, 5). In fact, CAMφ that appeared in mice infected with Mycobacterium bovis bacillus Calmette-Guérin and Listeria monocytogenes were shown to be powerful killer cells for these pathogens, tumor cells, and cells infected with various bacteria and viruses (1). The ability of CAMφ to kill these targets was displayed nonspecifically (1). In other words, the host resistance of individuals exposed to foreign invasions may be effectively improved if CAMφ can be induced. However, CAMφ were not generated in individuals whose AAMφ predominate, because AAMφ inhibited CAMφ generation (19, 20). Hosts susceptible to foreign invasions are generally recognized as individuals with a predominance of AAMφ.

In the present study, CCL17 and IL-10 released from AAMφ were shown to be effector molecules that inhibit CAMφ generation from RMφ stimulated with CpG DNA. The regulation of the production of these cytokines may improve the resistance to various opportunistic infections in patients whose AAMφ predominate.

Materials and Methods

Animals

Eight- to 11-wk-old, pathogen-free, male BALB/c mice purchased from The Jackson Laboratory (Bar Harbor, ME) were used in this study. The Institutional Animal Care and Use Committee of the University of Texas

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Medical Branch approved all procedures performed in these animal experiments (Institutional Animal Care and Use Committee approval no. 02-04-024).

Reagents and medium

rIL-1β, IL-2, IL-4, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and CCL3 were purchased from PeproTech (Rocky Hill, NJ). TGF-β and mAbs for IL-1β, IL-6, IL-10, IFN-γ, TGF-β, TNF-α, CCL3, and CCL2 were purchased from BD Pharmingen (San Diego, CA). PGE2, ELISA kit and CCL17 as well as mAbs for CCL3 and CCL17 were purchased from R&D Systems (Minneapolis, MN). CpG DNA (5′-TTCATGACGTTCCTGACGT-3′) was purchased from Sigma-Genosys (Woodlands, TX). CpG DNA was used to induce CAMφ generation, as previously described (21). Mφ were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 2 mM l-glutamine, and antibiotics (complete medium).

Burn injury

Thermally injured mice, BALB/c mice exposed to flame-burn injuries (22), were prepared as follows. Mice were anesthetized with pentobarbital (40 mg/kg i.p.) and electric clippers were used to shave the hair on the back of each mouse from groin to axilla. The mice were then exposed to a gas flame for 9 s by pressing the window of the custom-made insulated mold (with a 3.5-cm window) firmly against the shaved back. A Bunsen burned equipped with a flame-dispersing cap was used as a source of the gas flame. This procedure consistently produced a third-degree burn on ~15% of total body surface area (TBSA) for a 26-g mouse (22). Immediately after thermal injury, physiologic saline (3 ml/mouse i.p.) was administered for fluid resuscitation. Animals were then housed until use for experiments. Control mice (not burned) had their back shaved but were not exposed to the gas flame. They also received physiologic saline (3 ml/mouse i.p.).

Preparation of RMφ, AAMφ, and CAMφ

For the Mφ isolation, 4–10 × 10^6 cells of peritoneal exudates from mice were centrifuged. The cell pellets were suspended in 2 ml of RPMI 1640 medium supplemented with 2% FBS (maintenance medium) and cultured in ibonectin-coated petri dishes (60 × 15 mm) for 15 min at 37°C (23). At the end of culture, the dishes were washed twice with maintenance medium warmed to 37°C. Adherent cells (Mφ populations) were recovered from the dishes using a rubber policeman. Mφ purity was measured as 92% or more when tested by FACS Vantage (BD Biosciences, Mountain View, CA). RMφ were Mφ freshly isolated from peritoneal exudates of normal mice. As described previously (24), AAMφ were isolated from peritoneal exudates of mice 2 days after severe burn injuries (third-degree flame burn, >15% TBSA). As previously described (4), standard AAMφ were induced in cultures of RMφ treated with a mixture of IL-4 and IL-13 (10 ng/ml each) for 48 h. CAMφ were generated in vitro from RMφ after stimulation with CpG DNA (24 h stimulation, 10 μg/ml) (21).

Criteria of CAMφ and AAMφ

Mφ were identified as CAMφ when the following typical properties were demonstrated: production of CCL3 and IL-10 (25, 26), expression of mannose receptor mRNA (27), induction of TH1 responses (25), and expression of killing activity against bacteria (6). Mφ were identified as AAMφ when the following criteria were demonstrated: production of CCL17 and IL-10 (3, 28), expression of mannose receptor mRNA (29), and induction of TH2 responses (30). For the production of cytokines, various Mφ populations (1 × 10^6 cells/ml) were cultured for 24 h without any stimulation. Then, the culture fluids harvested were assayed for the cytokines using ELISA. The detection limits of CCL3, IL-10, TNF-α, and IL-12 (25, 26) were performed as described previously (32). A 96-well, Millititer HA plate (Millipore, Bedford, MA) was coated with 2 μg/ml anti-mouse CCL3 mAb or anti-mouse CCL17 mAb in 0.1 M carbonate buffer (pH 9.6) overnight at 4°C. The plates were then blocked with complete medium for 1 h. Mφ suspensions diluted serially with complete medium were incubated in the plates for 24 h at 37°C. Next, the plates were washed with PBS containing 0.05% Tween 20 and overlaid with biotinylated anti-mouse CCL3 mAb or anti-mouse CCL17 mAb. After washing steps, the plates were washed and treated with a 1/2000 dilution of avidin-conjugated alkaline phosphatase (Sigma-Aldrich, St. Louis, MO). CCL3 or CCL17, secreted by single cells, was visualized by adding a solution of 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (Sigma-Aldrich). The colorimetric reaction was halted after 30 min by washing the plates with water, and the spots were enumerated under ×40 magnification. The dilution of the cells producing spots was used to calculate the total numbers of CCL3- or IL-10 releasing AAMφ.

Inhibition of CAMφ generation from RMφ stimulated with CpG DNA

The inhibitory effect of AAMφ on CAMφ generation was analyzed using the following procedures: 1) RMφ-stimulated with CpG DNA were cocultured with antibody-coupled AAMφ, 2) RMφ were cultured with AAM0 in a dual-chamber Transwell supplemented with CpG DNA, and 3) RMφ stimulated with CpG DNA were cultured with the culture fluids of AAMφ (AAMφ Culture-Sup). After cultivation by these different methods, the RMφ were harvested and cultured for an additional 24 h to produce CCL3. CCL3 was chosen as a parameter of CAMφ generation, because it has been described as such in previous studies (4, 5). Cocultivation experiments (above, procedure 1) were performed as follows. In the presence of 10 μg/ml CpG DNA, RMφ were cocultured with AAMφ at a ratio of 1:100 to 64:100 in 96-well microtiter plates. Twenty-four hours after cultivation, the cells were washed three times with complete medium and cultured for an additional 24 h with complete medium. The culture fluids harvested were assayed for CCL3. A Transwell culture (above, procedure 2) was performed as described below. RMφ were cultured with AAMφ in a dual-chamber Transwell supplemented with CpG DNA. Six hundred microliters of RMφ suspensions (1 × 10^6 cells/ml) was placed into the lower chamber of the Transwell (0.4-μm pore size; Costar, Corning, NY). One hundred microliters of AAMφ suspension (10^6 cells/ml) was placed into the upper chamber of the Transwell. Twenty-four hours after cultivation, Mφ harvested from the lower chamber were recultured for 24 h. The resulting culture fluids were assayed for CCL3. RMφ were also cultured with the AAMφ Culture-Sup (above, procedure 3). AAMφ Culture-Sup was the culture fluids of AAMφ (1 × 10^6 cells/ml) cultured for 24 h after cultivation with CpG DNA were cultured with complete medium supplemented with AAMφ Culture-Sup (15% v/v) for 24 h. The cells harvested were washed three times with complete medium and cultured for an additional 24 h. The resulting culture fluids were assayed for CCL3.

Determination of soluble factors from AAMφ

To determine the active components in AAMφ Culture-Sup, AAMφ were cultured without any stimulation for 24–48 h, and the culture fluids harvested were assayed for IL-1β, IL-6, IL-10, TNF-α, TGF-β, CCL17, and PGE2 using ELISA. These soluble factors have been well described as the products of Mφ or Mφ-related cells (4, 5, 16). The detection limits of IL-1β, IL-6, IL-10, TNF-α, TGF-β, PGE2, and CCL17 were 20, 12, 8, 20, 22, 18, and 16 pg/ml, respectively. Then, the recombinant cytokines, specifically detected in the culture fluids of AAMφ, were assayed for their ability to inhibit CAMφ generation. Thus, in the presence of CPG DNA, RMφ (1 × 10^6 cells/ml) were cultured with various doses of recombinant cytokines. Twenty-four hours after the cultivation, the cells were washed three times and then cultured for an additional 24 h. The culture fluids harvested were assayed for CCL3. After certain cytokines were determined to inhibit CAMφ generation, the AAMφ Culture-Sup was treated with mAbs directed against the appropriate cytokines and applied to the assay of CAMφ generated from RMφ stimulated with CpG DNA. Amounts of mAbs (clone 110904; clone JESS-2A5) used in the experiments were determined according to the manufacturer’s protocol.

ELISPOT assay

To determine the percentage of AAMφ in the CAMφ preparation or the percentage of CAMφ in the AAMφ preparation, an ELISPOT assay was performed as previously described (32). A 96-well, Millititer HA plate (Millipore, Bedford, MA) was coated with 2 μg/ml anti-mouse CCL3 mAb or anti-mouse CCL17 mAb in 0.1 M carbonate buffer (pH 9.6) overnight at 4°C. The plates were then blocked with complete medium for 1 h. Mφ suspensions diluted serially with complete medium were incubated in the plates for 24 h at 37°C. Next, the plates were washed with PBS containing 0.05% Tween 20 and overlaid with biotinylated anti-mouse CCL3 mAb or anti-mouse CCL17 mAb. After washing steps, the plates were washed and treated with a 1/2000 dilution of avidin-conjugated alkaline phosphatase (Sigma-Aldrich, St. Louis, MO). CCL3 or CCL17, secreted by single cells, was visualized by adding a solution of 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (Sigma-Aldrich). The colorimetric reaction was halted after 30 min by washing the plates with water, and the spots were enumerated under ×40 magnification. The dilution of the cells producing spots was used to calculate the total numbers of CCL3- or IL-10 releasing AAMφ.
CCL17-producing Mϕ per sample. The results were displayed by the ratio between CCL3-producing Mϕ and CCL17-producing Mϕ.

**Statistical analysis**

Data are presented as mean ± SEM. Comparisons of the results between the experimental and control groups were made by ANOVA. Analysis was accomplished using Statview 4.5 (Abacus Concepts, Berkeley, CA). If a p value was <0.05, the result was considered to be significant.

**Results**

**Inhibition of CAMϕ generation by AAMϕ or AAMϕ products**

The inhibitory effect of AAMϕ on CAMϕ generation was examined. Three kinds of Mϕ populations were used in these experiments: RMϕ, freshly isolated peritoneal Mϕ from normal mice; CAMϕ, RMϕ stimulated with CpG DNA; AAMϕ, peritoneal Mϕ from thermally injured mice. As shown in Table I, three Mϕ populations used in this study exhibited typical properties for RMϕ, CAMϕ, and AAMϕ, respectively. In the following studies, Mϕ with the ability to produce CCL3 are considered CAMϕ because CCL3 has been described as a typical chemokine specifically produced by CAMϕ (4, 5). As shown in Table II, CAMϕ were not found in cultures of RMϕ stimulated with CpG DNA when they were cocultured with AAMϕ at percentages of 39% or more. In addition, no CAMϕ were generated when RMϕ (lower chamber) were cultured with AAMϕ (upper chamber) in a dual-chamber Transwell supplemented with CpG DNA (Fig. 1A). In the presence of CpG DNA, conversion of RMϕ to CAMϕ drastically decreased when the cultivation was performed with AAMϕ Culture-Sup (culture supernatants of 1 × 10^6 cells/ml AAMϕ 24 h after cultivation, 15%, v/v; Fig. 1B). Similar results were obtained when standard AAMϕ induced by a mixture of IL-4 and IL-13 or culture fluids of these Mϕ were subjected to the same test (Fig. 1). The results shown in Table II and Fig. 1 indicate that the ability of AAMϕ to inhibit CAMϕ generation from RMϕ stimulated with CpG DNA is evidenced by the soluble factors released from AAMϕ.

**Determination of soluble inhibitory factors released from AAMϕ**

To determine which components inhibited CAMϕ generation, AAMϕ were cultured at the cell density of 1 × 10^6 cells/ml for 24 h in the presence or absence of CpG DNA (10 μg/ml). RMϕ (6 × 10^5 cells/well, lower chamber) were used as CAMϕ from burn mice (5 × 10^5 cells/well, upper chamber) in a dual-chamber Transwell. As a control, AAMϕ induced by a mixture of IL-4 and IL-13 were added to the same assay system. Twenty-four hours after the initial cultivation, Mϕ in the lower chamber were harvested and cultured for an additional 24 h. The culture fluids were assayed for CCL3 using ELISA. B, AAMϕ Culture-Sup: in the presence of CpG DNA, RMϕ (1 × 10^6 cells/ml) were cultured with AAMϕ Culture-Sup (15%, v/v) for 24 h. AAMϕ Culture-Sup was composed of culture fluids of 1 × 10^6 cells/ml burn-associated AAMϕ 24 h after cultivation. As a control, culture fluids of standard AAMϕ (1 × 10^6 cells/ml, 24 h) were added to the same assay system. Mϕ were washed and cultured for an additional 24 h. The culture fluids harvested were assayed for CCL3. *p < 0.001 compared with RMϕ cultured with CpG DNA.

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**Table I. The properties of Mϕ populations used in this study**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Assay Procedures</th>
<th>RMϕ</th>
<th>AAMϕ</th>
<th>CAMϕ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose receptor mRNA expression</td>
<td>RT-PCR</td>
<td>Trace</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>iNOS mRNA expression</td>
<td>PT-PCR</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Th1/Th2 polarization</td>
<td>Transwell culture</td>
<td>No</td>
<td>Th2</td>
<td>Th1</td>
</tr>
<tr>
<td>Killing against MRSA</td>
<td>CFU</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cytokine production</td>
<td>ELISA</td>
<td>Trace</td>
<td>IL-10</td>
<td>IL-12</td>
</tr>
<tr>
<td>Chemokine production</td>
<td>ELISA</td>
<td>Trace</td>
<td>CCL17</td>
<td>CCL3</td>
</tr>
<tr>
<td>CCL3-producing Mϕ/CCL17-producing Mϕ</td>
<td>ELISPOT</td>
<td>1:0.7</td>
<td>1:122</td>
<td>149:1</td>
</tr>
</tbody>
</table>

* Peritoneal Mϕ freshly isolated from normal BALB/c mice were used as RMϕ (5). Peritoneal Mϕ from mice 2 days after third-degree 15% TBSA burn were used as AAMϕ (24). RMϕ stimulated with CpG DNA (10 μg/ml, 24 h) were used as CAMϕ (21). The number of CCL3- or CCL17-producing Mϕ was determined using the ELISPOT assay as described in the text. The results are displayed as the ratio between CCL3-producing Mϕ and CCL17-producing Mϕ.

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**Table II. Inhibitory effect of AAMϕ on the CAMϕ generation in cocultivation**

<table>
<thead>
<tr>
<th>No. of AAMϕ/ No. of RMϕ × 10^5</th>
<th>CCL3 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Media</td>
</tr>
<tr>
<td>1</td>
<td>&lt;18</td>
</tr>
<tr>
<td>4</td>
<td>&lt;18</td>
</tr>
<tr>
<td>16</td>
<td>&lt;18</td>
</tr>
<tr>
<td>64</td>
<td>&lt;18</td>
</tr>
</tbody>
</table>

* In the presence or absence of CpG DNA (10 μg/ml), RMϕ were cocultured with AAMϕ at a ratio of 1:100 to 64:100. Twenty-four hours after cultivation, cells harvested were washed three times and recultured for 24 h. Culture fluids obtained were assayed for CCL3 by ELISA.

* One × 10^6 cells/well RMϕ were mixed with 1–64 × 10^3 cells/well AAMϕ.

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**FIGURE 1.** Effect of AAMϕ or AAMϕ culture fluids on CAMϕ generation from RMϕ stimulated with CpG DNA. A, Transwell cultures: in the presence of CpG DNA (10 μg/ml), RMϕ (6 × 10^5 cells/well, lower chamber) were cultured with AAMϕ from burn mice (5 × 10^5 cells/well, upper chamber) in a dual-chamber Transwell. As a control, AAMϕ induced by a mixture of IL-4 and IL-13 were added to the same assay system. Twenty-four hours after the initial cultivation, Mϕ in the lower chamber were harvested and cultured for an additional 24 h. The culture fluids were assayed for CCL3 using ELISA. B, AAMϕ Culture-Sup: in the presence of CpG DNA, RMϕ (1 × 10^6 cells/ml) were cultured with AAMϕ Culture-Sup (15%, v/v) for 24 h. AAMϕ Culture-Sup was composed of culture fluids of 1 × 10^6 cells/ml burn-associated AAMϕ 24 h after cultivation. As a control, culture fluids of standard AAMϕ (1 × 10^6 cells/ml, 24 h) were added to the same assay system. Mϕ were washed and cultured for an additional 24 h. The culture fluids harvested were assayed for CCL3. *p < 0.001 compared with RMϕ cultured with CpG DNA.
or 48 h. The culture fluids were assayed for various cytokines and PGE₂, which have been described as typical products of Mø or Mø-related cells (4, 5, 16). As a control, the culture fluids of RMφ were used for the experiments. The results are shown in Fig. 2. PGE₂, CCL17, IL-6, IL-10, and TGF-β were all found in AAMφ culture fluids. In addition, almost the same amount of PGE₂ and TGF-β was detected in the culture fluids of RMφ. These results suggest that CCL17, IL-6, and IL-10 all may inhibit the generation of CAMφ from RMφ stimulated with CpG DNA.

Therefore, the next series of experiments tested the ability of cytokines to inhibit the generation of CAMφ from RMφ stimulated with CpG DNA. In these experiments, various doses of murine rCCL17, rIL-10, and rIL-6 were individually added to cultures of RMφ stimulated with CpG DNA. rIL-10 at a dose of 100 pg/ml did not inhibit CAMφ generation. However, rIL-6 at doses ranging from 20 to 1000 pg/ml did not inhibit CAMφ generation. Since 500–600 pg/ml IL-10 and CCL17 were detected in AAMφ-Culture Sup (culture fluids of AAMφ 24 h after cultivation, Fig. 2) and 15% (v/v) of AAMφ-Culture-Sup was added to RMφ cultures (Fig. 1B), the individual amounts (75–90 pg/ml) of IL-10 or CCL17 contained in this assay system were not enough to completely inhibit CAMφ generation.

When a mixture of rIL-10 and rCCL17 (100 pg/ml each) was added to cultures of RMφ stimulated with CpG DNA, CAMφ generation was completely inhibited (Fig. 3). These results suggest that both IL-10 and CCL17 contained in AAMφ Culture-Sup play a cooperative role in inhibiting CAMφ generation. These results were reproduced when the experiment was performed with AAMφ Culture-Sup and mAbs directed against CCL17 and IL-10. As shown in Fig. 4, AAMφ Culture-Sup inhibited CAMφ generation from RMφ (89% inhibition). This activity of AAMφ Culture-Sup was not completely abrogated when it was treated with anti-IL-10 mAb (clone 110904) and anti-CCL17 mAb (clone JES5-2A5) individually. Anti-IL-10 mAb treatment eliminated the activity of AAMφ Culture-Sup by 15%. Anti-CCL17 mAb treatment eliminated the activity by 50%. AAMφ Culture-Sup treated with isotype control Ab inhibited CAMφ generation from RMφ (90% inhibition). When AAMφ Culture-Sup was treated with a mixture of mAbs for IL-10 and CCL17, the ability of AAMφ Culture-Sup to inhibit CAMφ generation was eliminated by 78%. These results indicate that IL-10 and CCL17 may cooperatively inhibit CAMφ generation. Data shown in Figs. 3 and 4 suggest that small amounts of CCL17 released from AAMφ early after cultivation may have an influence on IL-10 production from AAMφ or induce expanded expression of IL-10R on RMφ.

**Appearance of CAMφ or AAMφ in various RMφ cultures**

In the presence or absence of CpG DNA, RMφ (lower chamber) were cultured with AAMφ (upper chamber) in a dual-chamber system.
Transwell supplemented with or without a mixture of mAbs for IL-10 (50 ng/ml) and CCL17 (500 ng/ml). Mφ harvested from the lower chamber 24 h after cultivation were assayed using ELISPOT to determine the number of CCL3-producing Mφ or CCL17-producing Mφ. As shown in Fig. 4, CCL3-producing Mφ were not found in the lower chamber of the Transwell (RMφ stimulated with CpG DNA) 24 h after cultivation with AAMφ (upper chamber). A majority of cells in the lower chamber was shown to be CCL17-producing Mφ. However, a majority of cells in the lower chamber (RMφ stimulated with CpG DNA) converted to CCL3-producing Mφ when Transwell cultures were performed with AAMφ (upper chamber) in the presence of a mixture of mAbs directed against IL-10 and CCL17 when they were cultured with culture fluids of RMφ, recombinant murine preparations of these cytokines were tested for their ability to inhibit the generation of CAMφ from RMφ. The various immunosuppressive activities of TGF-β have already been documented (34, 35). NO production and arginase induction by Mφ were inhibited when >1 ng/ml TGF-β was added to the cultures (36). In these results, however, the amount of TGF-β detected in the culture fluids of AAMφ was <100 pg/ml. This amount of TGF-β may not represent inhibitory activity on CAMφ generation. Together, all of these results indicate that AAMφ inhibit the generation of CAMφ from RMφ by producing IL-10 and CCL17.

Recently, suppressor of cytokine signaling (SOCS) 1 and SOCS3 were described as key inhibitors of Mφ activation (37, 38). Especially, IL-6 has been described as an activator of SOCS3, which inhibits the Mφ activation stimulated by LPS (39). In addition, IL-6 has been identified as an activator of SOCS3 (37, 38). In this study, however, IL-6 did not inhibit CAMφ generation. These findings appear to stem from the different sensitivities of IL-10- and IL-6-induced signal transduction toward inhibitory mechanisms. SOCS3 inhibits IL-6-induced signal transduction; however, IL-10-induced signal transduction is not inhibited by SOCS3 (38, 40). The role played by CCL17 in the induction of CAMφ when they were cultured with culture fluids of AAMφ (AAMφ Culture-Sup). Since IL-6, IL-10, and CCL17 were specifically detected in AAMφ Culture-Sup, recombinant murine preparations of these cytokines were tested for their ability to inhibit CAMφ generation. The results showed that only IL-10 and/or CCL17 were shown to inhibit CAMφ generation from RMφstimulated with CpG DNA, while IL-6 did not. TGF-β was found in the culture fluids of RMφ and AAMφ. The various immunosuppressive activities of TGF-β have already been documented (34, 35). NO production and arginase induction by Mφ were inhibited when >1 ng/ml TGF-β was added to the cultures (36). In these results, however, the amount of TGF-β detected in the culture fluids of AAMφ was <100 pg/ml. This amount of TGF-β may not represent inhibitory activity on CAMφ generation. Together, all of these results indicate that AAMφ inhibit the generation of CAMφ from RMφ by producing IL-10 and CCL17.

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SOCS3 is not known. The effect of this chemokine on the various functions of MΦ is also unknown. However, the results of this study show that CCL17 may inhibit the immunobiological functions expressed by CAMΦ. CCL17 has been suggested as an effector molecule on Th2 responses associated with AAMΦ. CCL17 stimulates these responses enhanced by AAMΦ. Further studies for CCL22 will be required.

CCL3 production as a representative maker of CAMΦ function. Th2 cytokines (IL-4 and IL-13) in bronchoalveolar lavage fluids were measured as possible factors released from RMΦ or MΦ, because TNF-α, IL-1β, IL-6, PGE₂, IL-10, CCL17, and TGF-β were measured as possible soluble factors released from CAMΦ. TNF-α, IL-1β, IL-6, and IL-12 were described as products of MΦ or AAMΦ-related cells. PGE₂, IL-10, and TGF-β have also been described as MΦ products with immunosuppressive activities. Therefore, we tested these soluble factors as possible effector molecules of AAMΦ. Also, we tested CCL17 as a possible effector molecule of AAMΦ. CCL17 and CCL22 have been described as chemokines equally associated with the induction of Th2 responses (1). This means there is a possibility that these two chemokines may be equally produced by AAMΦ and equally cooperate with IL-10 on inhibiting CAMΦ generation. However, due to availability, in this study we only measured CCL17. Further studies for CCL22 will be required.

In conclusion, CCL17 and IL-10 released from AAMΦ were shown to inhibit CAMΦ generation from RMΦ stimulated with CpG DNA. The impairment of the host’s innate immune associated with the function of CAMΦ may be remedied by controlling AAMΦ or AAMΦ products (CCL17 and IL-10).

References


