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Conditional Macrophage Ablation Demonstrates That Resident Macrophages Initiate Acute Peritoneal Inflammation

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The role played by resident macrophages (Mφ) in the initiation of peritoneal inflammation is currently unclear. We have used a conditional Mφ ablation strategy to determine the role of resident peritoneal Mφ in the regulation of neutrophil (PMN) recruitment in experimental peritonitis. We developed a novel conditional Mφ ablation transgenic mouse (designated CD11bDTR) based upon CD11b promoter-mediated expression of the human diphtheria toxin (DT) receptor. The murine DT receptor binds DT poorly such that expression of the human receptor confers toxin sensitivity. Intrapерitoneal injection of minute (nanogram) doses of DT results in rapid and marked ablation of F4/80-positive Mφ populations in the peritoneum as well as the kidney, and ovary. In experimental peritonitis, resident Mφ ablation resulted in a dramatic attenuation of PMN infiltration that was rescued by the adoptive transfer of resident nontransgenic Mφ. Attenuation of PMN infiltration was associated with diminished CXC chemokine production at 1 h. These studies indicate a key role for resident peritoneal Mφ in sensing perturbation to the peritoneal micro-environment and regulating PMN infiltration. The Journal of Immunology, 2005, 174: 2336–2342.

Macrophages (Mφ) are dispersed throughout the tissues and have an important role in innate immunity (1), apoptotic cell clearance (2), development (3), and morphogenesis (4, 5). Resident tissue Mφ and dendritic cells are regarded as sentinels of the innate immune system. Various strategies, such as Mφ depletion induced by administration of liposomal clodronate (6), have been used to examine Mφ function in vivo. However, previous work using this method to study the role of resident peritoneal Mφ in experimental peritonitis has produced conflicting results because they have suggested a key role (7), an inhibitory role (8), or no role at all (8). In the current study, we have used a conditional ablation strategy to clarify the role of resident Mφ in the sensing of peritoneal injury and the regulation of neutrophil (PMN) infiltration.

A conditional Mφ ablation strategy has advantages over the available naturally occurring and induced Mφ-deficient mutant mice because the timing of Mφ elimination can be chosen. Despite their limitations, nonconditional Mφ-deficient mice have proven valuable for analysis. For example, the Csfmop/Csfmop (osteopetrosis) mouse is a naturally occurring mutant of the CSF-1 gene and exhibits a Mφ deficiency at a level that permits viability in homozygotes (9). These mice have provided insight into Mφ function during development (3). In addition, mice targeted at the PU.1 locus exhibit multiple defects in development of hematopoietic lineages including a complete absence of tissue Mφ (10, 11). Although PU.1 mutation results in perinatal lethality, these mice have been used to demonstrate that mesenchymal cells are able to clear apoptotic cells during embryonic regression of interdigital tissues (12).

Previous strategies used to eliminate specific cell types in a living organism include the generation of transgenic lines that express diphtheria toxin A-chain (13, 14) or the ricin polypeptide (15). However, even low levels of unanticipated transgene expression can give unpredictable consequences (13). The alternative ablation strategy of killing thymidine kinase-expressing cells with gancyclovir (16, 17) only permits the elimination of proliferating cells. More recently, conditional Mφ ablation has been achieved using transgenic expression of Fas under the control of the c-fms promoter coupled with drug-inducible Fas dimerization to induce cell death (18). Identification of the human receptor for diphtheria toxin (DT) (also known as heparin-binding epidermal growth factor (hbEGF) (19)) created an opportunity for a unique ablation strategy. The murine form of hbEGF binds DT poorly, but mouse cells can be rendered sensitive through transgenic expression of human hbEGF. In transgenic mice expressing human hbEGF lineage specifically, cell ablation results following toxin injection. In addition, DT is a protein synthesis inhibitor and kills both mitotic and terminally differentiated cells. This strategy has recently been used to generate transgenic mice in which hepatocytes (20) or dendritic cells (21) may be conditionally ablated. In the current...
report, we describe how we have used this strategy to generate a conditional Mφ ablation mouse to assess the role of resident peritoneal Mφ in the initiation of acute inflammation in experimental sterile peritonitis induced by Brewer’s thioglycollate (BTG). We demonstrate that resident peritoneal Mφ are essential for PMN recruitment through Mφ-dependent CXC chemokine production.

Materials and Methods

Transgenic construct

The CD11b promoter from coordinates −1704 to +83 (22) was used to drive expression of the human hEGF cDNA (19). Splicing and polyadenylation signals were provided by a region of the human growth hormone gene that had previously worked effectively with the CD11b promoter (22). The fusion protein between hEGF and GFP was generated by continuing the open reading frame from the final residue of hEGF with the first residue of GFP.

Transgenic mice

The CD11b-DTR construct was used to generate transgenic mice on the FVB/N background (23) using conventional techniques; transgene expression was detected using an RT-PCR assay. The primer sequences used were 5′-AAGATCCCGCAACAATCG for the forward primer and 5′-GCACGTCAAGTGGATTCTG for the reverse primer. Because the reverse primer sequence was taken from the base pairs flanking intron III of hGH, no PCR product could be amplified from genomic DNA.

Flow cytometry analysis

Bone marrow-derived Mφ were prepared as previously described (4). Resident peritoneal cells were isolated by peritoneal lavage. Elicited peritoneal Mφ were lavaged from the peritoneal cavity 4 days after i.p. injection of 1 ml of 3% BTG (Difco). For flow cytometric analysis, and PE conjugates (Caltag and Serotec). Annexin V-FITC conjugate (US Biochemicals), anti-GR1 PE (eBiosciences), anti-B220 PE-CY5 (eBiosciences), anti-CD11b FITC (Caltag Laboratories), anti-CD11c FITC (Caltag Laboratories), and anti-CD11b PE-CY5 (eBiosciences) were used. The Ab to the MC marker c-kit (CD117) to stain Mφ or mast cells (MC) was also determined in vitro. Peritoneal cells were incubated with PE-conjugated anti-F4/80 Ab or an Ab to the MC marker c-kit (CD117) to stain Mφ or MC, respectively. Peritoneal cells were then incubated with anti-PE-conjugated MACS magnetic beads, and Mφ or MC were removed by passing the cells over a magnetic MACS column (Miltenyi Biotec). As a control, total peritoneal cells were incubated with an isotype control Ab followed by magnetic beads and subsequently passed over the magnetic MACS column. This method resulted in >97% depletion of Mφ or MC. Control peritoneal cells and Mφ- or MC-depleted peritoneal cells were then plated in 48-well plates (5 × 10⁴ cells per well) and exposed to 1% BTG for 3 h. Peritoneal cell-conditioned supernatants were harvested, spun, and stored at −80°C until analyzed by specific ELISA for MIP-2 and keratinocyte-derived chemokine (KC) (R&D Systems). Chemokine production by peritoneal cell populations that had been depleted of either Mφ or mast cells (MC) was also determined in vitro. Peritoneal cells were incubated with PE-conjugated anti-F4/80 Ab or an Ab to the MC marker c-kit (CD117) to stain Mφ or MC, respectively. Peritoneal cells were then incubated with anti-PE-conjugated MACS magnetic beads, and Mφ or MC were removed by passing the cells over a magnetic MACS column (Miltenyi Biotec). As a control, total peritoneal cells were incubated with an isotype control Ab followed by magnetic beads and subsequently passed over the magnetic MACS column. This method resulted in >97% depletion of Mφ or MC. Control peritoneal cells and Mφ- or MC-depleted peritoneal cells were then plated in 48-well plates (5 × 10⁴ cells per well) and exposed to 1% BTG for 3 h. Peritoneal cell-conditioned supernatants were harvested, spun, and stored at −80°C until analyzed by specific ELISA for MIP-2 and KC (R&D Systems).

Statistical analysis

The Student t test with a tailed distribution or ANOVA was used to analyze data. A value of p < 0.05 was deemed statistically significant. Data are presented as mean ± SE.

Results

Generation of transgenic mice

Conditional ablation transgenic mice were generated using an established strategy (21) and a construct (designated CD11b-DTR) that used the CD11b promoter (22) to provide Mφ expression specificity. CD11b-DTR expresses the DT receptor (alternatively named hEGF (19)) as a GFP fusion protein. The hEGF-GFP construct conferred sensitivity to DT in transiently transfected murine cells indicating that it was functional (data not shown).

Six transgenic lines were produced with the CD11b-DTR construct. Although the fluorescence signal from the hEGF-GFP fusion protein was insufficient to permit detection of transgene expression by FACS analysis, a RT-PCR assay indicated that four lines exhibited detectable transgene expression in peritoneal cells and spleen with line 34 exhibiting high expression (Fig. 1, B and C). WT and CD11b-DTR-34 bone marrow–derived Mφ expressed the Mφ-specific gene F4/80 as expected (Fig. 1D), indicating that expression of the DT receptor was unlikely to have disrupted normal Mφ differentiation. Transgene expression was also observed in day 4 and 8 CD11b-DTR-34 bone marrow–derived Mφ (Fig. 1E). Because line CD11b-DTR line 34 showed the highest levels of transgene expression, further analysis was restricted to this line.

Transgenic Mφ are killed by DT in vitro and in vivo

Treatment of BTG-elicited peritoneal Mφ from CD11b-DTR-34 mice with concentrations of DT between 1 ng/ml to 1 mg/ml over...
A period of 48 h induced cell death at concentrations as low as 25 ng/ml. In contrast, Mφ from WT mice or other transgenic lines were resistant (data not shown). We then asked whether i.p. injection of DT ablated resident peritoneal Mφ in vivo. DT was injected at 25 ng/g mouse weight, and FACS analysis of peritoneal cells was performed 24 h later. Normal numbers of resident peritoneal Mφ (F4/80 positive, CD11b positive, and Ly6C/G negative) were evident in WT mice receiving DT (Fig. 2A) as well as CD11b-DTR-34 transgenic mice injected with either PBS (Fig. 2B) or the inactive form of the toxin DTmut (Fig. 2C). However, CD11b-DTR-34 transgenic mice showed an almost complete absence of F4/80-positive peritoneal Mφ after a single dose of DT (Fig. 2D). Administration of DT doses of 6.25 and 12.5 ng/g mouse weight resulted in Mφ ablation of ~72 and 82%, respectively, whereas lower doses resulted in <50% ablation. We therefore chose to use a dose of 25 ng/g body weight for the in vivo studies.

Time course of Mφ elimination in vivo

We then examined the kinetics of Mφ ablation in the peritoneal cavity following administration of a single dose of DT (25 ng/g body weight). The appearance of apoptotic and necrotic cells was monitored using Annexin V FITC and PI staining, respectively. Peritoneal lavages and flow cytometric analyses were conducted on a series of mice 4, 6, 8, and 12 h after DT injection (Fig. 3). After 6 h, 65% of the peritoneal population was annexin V positive, indicating a dramatic increase in early-stage apoptotic cells (Fig. 3A). The maximal numbers of PI-positive cells occurred ~2 h later at 8 h after DT injection and represented ~20% of the total peritoneal cells (Fig. 3B). The number of F4/80-positive cells was nearly zero at 12 h (Fig. 3C), and this corresponded to very low levels of PI-positive and annexin V-positive cells (A and B). These data suggest that DT induces sensitive cells to undergo apoptosis and that some of these dying cells then undergo secondary necrosis. All F4/80-positive cells were cleared by 12 h. Restoration of the peritoneal Mφ population occurs by day 4 following a single i.p. dose of DT (3.94 × 10^5 ± 1.7 × 10^5 Mφ vs 5.8 × 10^5 ± 1.3 × 10^5; day 4 following DT treatment vs day 1 following PBS treatment; n = 5 per group, p > 0.05).

Specificity of Mφ elimination in vivo

To test the specificity of Mφ elimination, we examined CD3^+ T cells in spleen and peritoneal cavity and B220^+ B cells in the spleen. In this case, we injected two doses of DT at 25 ng/g at 48 h intervals and assessed ablation 24 h later. The F4/80-positive peritoneal population was unaffected in WT mice (Fig. 2, E and F, and F4/80 upper-left quadrants). The relative increase in CD3^-positive and double-negative cells in the DT-treated CD11b-DTR-34 animals is due to the plotting of equal numbers of detection events in the FACS analyses. B220^- and CD3^- populations in spleen were unaffected in either WT or CD11b-DTR-34 mice receiving two doses of DT (data not shown).

Because CD11b is expressed on both granulocytes and Mφ, we asked whether both of these cell types were sensitive to DT. CD11b-DTR-34 mice were injected with BTG. DT (25 ng/g body weight) or PBS was injected 8 h after initiation of peritonitis with peritoneal lavage being performed 12 h later. Despite 90% Mφ ablation (0.5 × 10^6 ± 0.15 × 10^6 vs 4.8 × 10^6 ± 0.23 × 10^6; DT injection vs PBS; p < 0.005), there was no difference in PMN number (2.3 × 10^6 ± 0.22 × 10^6 vs 1.6 × 10^6 ± 0.39 × 10^6; DT injection vs PBS; p > 0.05). In addition, flow cytometric analysis of whole blood performed 24 h following DT administration indicated that circulating PMN numbers were unaffected by DT administration (1.02 × 10^6 ± 0.18 × 10^6 PMNs/ml whole blood vs

FIGURE 1. Structure and expression of the CD11b-DTR transgene. A, Schematic of the CD11b-DTR construct. The transcription start is indicated by the right-facing arrow and exons by shaded boxes. The DTR-eGFP fusion cDNA is inserted between the human CD11b promoter and the human growth hormone (hGH) sequences that provides splicing and polyadenylation signals. Oligonucleotides eGFP and hGH used for RT-PCR transcript detection are indicated by small arrows. B–E, RT-PCR expression analysis performed on BTG-elicited peritoneal cells (B), spleen cells (C), and bone-marrow derived Mφ (D and E). Transgene mRNA amplification products were not evident in samples from WT mice or when reverse transcriptase was omitted but was detected in line 34 peritoneal and spleen cells (B and C). Line 34 bone-marrow derived Mφ also exhibited normal expression of the Mφ marker F4/80 (D) and persistent transgene expression (E).

FIGURE 2. Flow cytometric analysis of peritoneal cell Mφ killing by DT. A–D, Cells were removed from the peritoneal cavity by peritoneal lavage and labeled with PE-conjugated F4/80 Ab, and flow cytometric analysis was performed. A, WT mice injected with DT (25 ng/g body weight) show a normal percentage of peritoneal Mφ. B and C, CD11b-DTR-34 mice receiving either PBS (B) or DTmut (C) have normal F4/80 profiles. D, In contrast, CD11b-DTR-34 mice treated with active DT show complete absence of F4/80-positive cells. E, In WT mice, injection of DT at 25 ng/g mouse weight does not affect either the small population of CD3^- T cells (upper-left quadrant) or the larger population of F4/80^- Mφ (lower-right quadrant) in the peritoneal cavity. F, CD11b-DTR-34 mice receiving DT exhibit elimination of the F4/80^- population, whereas the CD3^- cells remain unaffected.
0.97 \times 10^6 \pm 0.22 \times 10^6; DT injection vs PBS; p > 0.05). In contrast, DT administration induced significant depletion of circulating monocytes (0.117 \times 10^6 \pm 0.059 \times 10^6 monocytes/ml whole blood vs 0.52 \times 10^6 \pm 0.073 \times 10^6; DT injection vs PBS; p < 0.05).

**FIGURE 3.** Time course of peritoneal Mφ depletion. Cells were removed from the peritoneal cavity by peritoneal lavage. Mice were either injected with a single dose of DT (gray lines) or DTmut (black lines) at 25 ng/g mouse weight i.p., and the resident peritoneal population was assessed for the appearance of annexin V-positive cells (A), for labeling of cells with PI (B), and for presence of the Mφ marker F4/80 (C). Flow cytometric analysis identified the labeled proportion of total cells over a 12-h time course following DT injection.

**Differential deletion of Mφ populations**

We also asked whether DT injection could eliminate Mφ in distant organs. Two doses of DT (25 ng/g) were administered IP at 48-h intervals, and the presence of F4/80-positive Mφ in kidney, liver, and lung was analyzed 24 h later and in the ovary 16 h later (Fig. 4). The ovary was examined at 16 h, because there was evidence of some patchy ovarian necrosis present at 20 h. WT mice injected with DT and CD11b-DTR-34 homozygote mice injected with DTmut were unaffected (Fig. 4, top two rows). Both kidney and ovary of CD11b-DTR-34 homozygote mice injected with DT exhibited an absence of F4/80+ cells. In the kidney, mesangial and interstitial Mφ were ablated in the absence of overt renal injury. However, hepatic sinusoidal Mφ and alveolar Mφ were unaffected, indicating that not all populations of tissue Mφ were susceptible. However, the rapid elimination of Mφ populations in the peritoneal cavity and kidney while leaving other cell populations intact establishes the basic validity of this approach to conditional cell ablation.

**Resident Mφ ablation reduces PMN influx and CXC chemokine responses during experimental peritonitis**

We used the conditional ablation strategy to investigate the role of resident tissue peritoneal Mφ in sensing perturbation of the microenvironment and subsequent initiation of acute peritoneal inflammation and PMN recruitment in experimental peritonitis. Resident Mφ ablation markedly attenuated PMN infiltration following the administration of 3% BTG (Fig. 5A). We also performed Mφ repletion studies with either Mφ-rich or Mφ-depleted peritoneal cells derived from WT mice. Reconstitution of DT-treated CD11b-DTR-34 homozygote mice with Mφ-rich peritoneal cells4hb e - before BTG treatment resulted in complete restoration of peak PMN infiltration at 8 h. In contrast, the administration of Mφ-depleted peritoneal cells was ineffective (Fig. 5B). Previous work suggested that the nature of the inflammatory stimulus may determine the involvement of Mφ in experimental peritonitis (8), and we therefore performed Mφ depletion studies in zymosan peritonitis. We also found that depletion of resident peritoneal Mφ resulted in a significant reduction in PMN infiltration 8 h following the induction of zymosan peritonitis (2.6 \times 10^6 \pm 8.8 \times 10^5 PMNs vs 5.4 \times 10^5 \pm 3.7 \times 10^5; DTR plus DT vs FVB/N WT controls plus DT; n = 6 per group; p < 0.05).

In this model, we found peak levels of the PMN CXC chemokines MIP-2 and KC at the 1-h time point. Resident peritoneal Mφ
in vivo is predominantly Mφ at 3 h (204 pg/ml). Much lower levels of MIP-2 between DT-treated and control mice were noted, with a slight, albeit statistically significant, difference in the CXC chemokine responses being Mφ dependent in vitro. Peritoneal cells were depleted of either Mφ or MC by incubation with PE-conjugated anti-F4/80 or anti-c-kit (CD117) followed by incubation with anti-PE-conjugated magnetic beads and passage over a magnetic column (>97% depletion of Mφ or MC). Incubation of total peritoneal cells with an isotype control Ab followed by magnetic beads and passage over the magnetic column served as control. Cells were then plated in 48-well plates (5 × 10^5 cells per well) and exposed to 1% BTG for 3 h. Peritoneal cell-conditioned supernatants were harvested, spun, and analyzed by specific ELISA for MIP-2 and KC.

\[ p < 0.005. \]

FIGURE 6. CXC chemokine production in response to BTG stimulation is Mφ dependent and MC independent in vitro. Peritoneal cells were depleted of either Mφ or MC by incubation with PE-conjugated anti-F4/80 or anti-c-kit (CD117) followed by incubation with anti-PE-conjugated magnetic beads and passage over a magnetic column (>97% depletion of Mφ or MC). Incubation of total peritoneal cells with an isotype control Ab followed by magnetic beads and passage over the magnetic column served as control. Cells were then plated in 48-well plates (5 × 10^5 cells per well) and exposed to 1% BTG for 3 h. Peritoneal cell-conditioned supernatants were harvested, spun, and analyzed by specific ELISA for MIP-2 and KC.

\[ p < 0.005. \]

Discussion

Previous analyses (20, 21) and the experiments we describe here show that expression of human hbEGF (19) in mouse cells can confer sensitivity to DT in vivo, and that, as a consequence, injection of DT will kill cells that express hbEGF. Our data indicate that Mφ populations in the peritoneal cavity and kidney can be rapidly killed or eliminated while leaving other cell populations intact, and this establishes the basic validity of this approach to conditional cell ablation. We noted that hepatic and alveolar Mφ populations were unaffected, and it may be the case that a higher dose of DT may have ablated these cells. However, we found that mice could become unwell with doses of DT >25 ng/g body weight, and we therefore did not use doses >25 ng/g body weight in this study. It is pertinent that, despite PMN expression of MIP-2, the administration of DT did not induce the death of recruited or circulating PMNs, indicating that PMNs are insensitive to DT, potentially as a result of their lower level of protein synthesis.

We used the conditional ablation strategy to investigate the role of resident peritoneal Mφ in the initiation of acute peritoneal inflammation following the administration of BTG. Previous work has indicated that leukotrienes derived from resident peritoneal Mφ are involved in the development of early vascular permeability in sterile peritonitis (25). Although early work in rat models of peritonitis implicated the resident peritoneal Mφ in the orchestration of PMN recruitment (26–28), more recent studies have produced conflicting results (7, 8). Indeed, studies by Ajuebor et al. (8) suggest that resident Mφ depletion inhibits PMN influx in LPS-induced inflammation, has no effect in BTG peritonitis, and augments PMN influx in zymosan peritonitis. In the latter model, it is proposed that Mφ-derived IL-10 inhibits PMN recruitment. Conversely, work by Knudsen et al. (7) using clodronate-induced depletion of peritoneal Mφ in a rat model of sterile peritonitis demonstrated that PMN infiltration was Mφ dependent.

In this study, administration of DT resulted in a dramatic 98% Mφ ablation that markedly blunted PMN infiltration, thereby indicating a key role for the resident Mφ in the orchestration of acute peritoneal inflammation in this experimental model. It is important to note that the reduced PMN infiltration in DT-treated mice was not attributable to a systemic neutropenia, because PMNs were not sensitive to DT and the number of circulating PMNs in DT-treated mice were stained for the PMN marker Gr1. Resident peritoneal Mφ ablation induced a marked blunting of PMN infiltration of the peritoneal cavity. B. WT and three groups of CD11b-DTR mice were injected i.p. with DT (25 ng/g body weight). Four hours before i.p. injection of 3% BTG, two groups of Mφ-depleted CD11b-DTR mice were reconstituted with either Mφ-rich peritoneal cells (MφR) or Mφ-depleted peritoneal cells (MφD). Administration of PBS served as control to the remaining groups. Mice underwent peritoneal lavage 8 h following administration of 3% BTG, and peritoneal cells were stained for Gr1. * \( p < 0.05 \).

ablation before the initiation of BTG peritonitis markedly reduced the elevation in MIP-2 levels (148.5 ± 34.8 vs 1762.1 ± 153.5 pg/ml; Mφ-depleted mice vs nondepleted mice; \( p < 0.00001 \)). There was a slight, albeit statistically significant, difference in the much lower levels of MIP-2 between DT-treated and control mice at 3 h (204 ± 54 vs 74 ± 8 pg/ml; Mφ-depleted mice vs nondepleted mice; \( p < 0.05 \)). This suggests that the production of MIP-2 in vivo is predominantly Mφ dependent. In addition, a 50% reduction in the level of KC was evident in Mφ-depleted mice at the 1-h time point (1408.2 ± 322.5 vs 2467.5 ± 264.9 pg/ml; Mφ-depleted mice vs nondepleted mice; \( p < 0.05 \)). Interestingly, the levels of KC levels at 3 h are higher in Mφ-depleted mice compared with control mice (1477 ± 400 vs 74 ± 8 pg/ml; Mφ-depleted mice vs nondepleted mice; \( p < 0.01 \)), thereby suggesting a source of KC other than resident Mφ.

CXC chemokine responses are Mφ dependent in vitro

Previous studies of peritoneal and dermal inflammation have implicated the MC as playing an important role in the initiation of PMN infiltration (8, 24). We therefore performed in vitro studies to determine the production of PMN chemokines by BTG-stimulated peritoneal cell populations that had been depleted of Mφ or MC. Control peritoneal cells produced significant levels of MIP-2 and KC, which was not affected by MC depletion (Fig. 6). However, chemokine levels were dramatically reduced following the depletion of Mφ, thereby indicating that chemokine production was completely Mφ dependent with no involvement of MC (Fig. 6).
mice was comparable with that of PBS-treated mice at 24 h. The importance of the resident Mφ was reinforced by experiments involving the adoptive transfer of nontransgenic peritoneal Mφ following DT-mediated Mφ ablation and before the initiation of peritonitis. The presence or absence of Mφ in the transferred peritoneal cell population directly correlated with the restoration of the PMN influx, thereby suggesting that the Mφ exerts a critical role in this process. In addition, we found that depletion of resident Mφ also significantly reduced PMN infiltration in zymosan peritonitis, thereby suggesting that the sensing function of the resident Mφ may be stimulus independent.

The magnitude of the Mφ depletion may explain the apparent discrepancy between these results and the study by Ajuebor et al. (8). Administration of a single dose of DT induced 98% Mφ ablation, whereas three doses of liposomal clodronate resulted in >85% Mφ depletion in the study by Ajuebor et al. (8). Mφ are a potent source of chemokines and cytokines, and it may be the case that, in certain circumstances, a relatively small population of residual Mφ may exert significant biological effects. Although administration of liposomal clodronate may exert marked biological effects despite depletion of only ~80% of Mφ (29), it may be necessary to deplete almost all peritoneal Mφ to delineate their roles as sentinel cells.

Although peritoneal Mφ may produce myriad mediators capable of recruiting PMNs (30, 31), we examined the effect of Mφ ablation upon the level of CXC chemokines in this model. Our data suggest that the initiation of PMN infiltration is mediated by resident peritoneal Mφ-dependent production of chemokines previously documented to play a role in orchestrating PMN recruitment in BTG peritonitis (32, 33) and in other inflammatory situations (34–36). MC are also a rich source of proinflammatory and vasoactive mediators and have been documented to play an important role in PMN recruitment during inflammation of the peritoneum (8) as well as other sites such as the skin (24).

We found that resident Mφ ablation markedly reduced the peak level of MIP-2 and significantly blunted the level of KC at 1 h. However, partial inhibition (50%) of KC production at 1 h and the persistent elevation of KC at 3 h in Mφ-depleted mice suggest that KC may be produced by other cells within the peritoneum. The persistent elevation of KC also suggests that Mφ may play a role in the negative regulation of KC production by non-Mφ cells. In this context, it is pertinent that our in vitro data indicate that both KC and MIP-2 production by peritoneal cells obtained by peritoneal lavage is almost entirely dependent upon Mφ, because peritoneal cells depleted of Mφ produced minimal levels of chemokines. These findings suggest that peritoneal cells retrievable by peritoneal lavage are not the source of KC detected in our in vivo study. This interpretation of the data is consistent with recent work in a wound model (37) demonstrating MIP-2 expression by inflammatory cells and KC expression by resident tissue cells such as endothelial cells and fibroblasts. Peritoneal mesothelial cells undoubtedly participate in peritoneal inflammation and can produce chemokines and cytokines (38), and it may be the case that mesothelial cells contribute to the KC production evident in this study. Despite this, however, we found that PMN infiltration is still markedly blunted despite the persistent presence of KC at 3 h.

We also examined the potential interaction between Mφ and MC by performing in vitro studies of peritoneal cells that had been depleted of Mφ or MC before stimulation with BTG. Interestingly, depletion of MC had no significant effect upon the production of KC and MIP-2 following BTG stimulation, suggesting that chemokine production in this model was Mφ dependent and MC independent.

In conclusion, this work has used a novel model of conditional Mφ ablation to dissect the role of resident peritoneal Mφ in the initiation of acute peritoneal inflammation. Our data indicate a key role for the resident Mφ in sensing peritoneal irritation and orchestrating PMN infiltration in BTG and zymosan peritonitis. This proinflammatory function is predominantly mediated by production of the potent PMN CXC chemokine MIP-2 and, to a lesser extent, KC. Although previous work has implicated the involvement of other cells such as MC, our study suggests that resident Mφ are critically important producers of PMN chemokines and act to orchestrate PMN recruitment in murine BTG peritonitis. We also anticipate that CD11b-DTR transgenic mice will be valuable for studying other Mφ functions in vivo in a variety of different biological contexts. The option of being able to choose the time and, with local toxin injection, perhaps the locality of ablation offers a number of advantages over other cell ablation systems.

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References


