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Resident Pleural Macrophages Are Key Orchestrators of Neutrophil Recruitment in Pleural Inflammation

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Rationale: The role played by resident pleural macrophages in the initiation of pleural inflammation is currently unclear. Objective: To evaluate the role of resident pleural macrophages in the initiation of inflammation. Methods: We have used a conditional macrophage ablation strategy to determine the role of resident pleural macrophages in the regulation of neutrophil recruitment in a murine model of experimental pleurisy induced by the administration of carrageenan and formalin-fixed Staphylococcus aureus.

Measurements and Main Results: Conditional macrophage ablation mice express the human diphtheria toxin receptor under the control of the CD11b promoter such that the administration of diphtheria toxin induces ablation of nearly 97% of resident macrophages. Ablation of resident pleural macrophages before the administration of carrageenan or S. aureus dramatically reduced neutrophil influx into the pleural cavity. In the carrageenan model, the reduction in neutrophil infiltration was associated with marked early reduction in the level of macrophage inflammatory protein 2 as well as reduced levels of various cytokines, including tumor necrosis factor α, interleukin 6, and interleukin 10. Adoptive transfer of nontransgenic macrophages partially restored neutrophil infiltration. We also stimulated macrophage-depleted and nondepleted pleural cell populations with carrageenan in vitro and determined the production of chemokines and cytokines. Chemokine and cytokine production was markedly reduced by macrophage depletion, reinforcing the role of resident pleural macrophages in the generation of mediators that initiate acute inflammation.

Conclusion: These studies indicate a critical role for resident pleural macrophages in sensing perturbation to the local microenvironment and orchestrating subsequent neutrophil infiltration.

Keywords: inflammation; macrophage; pleural diseases

The pleural membranes and associated cells are important because they are metabolically active and act as a barrier to invading pathogens by generating an innate and adaptive immunologic response. The pleural cavity is lined with mesothelium and contains resident macrophages (Mφ), mast cells, and lymphocytes (1, 2). During pleural inflammation, it has been reported that mesothelial cells are predominantly responsible for the secretion of C-X-C chemokines, such as interleukin 8 (IL-8), and C-C chemokines, such as macrophage inflammatory protein 1α (MIP-1α) and macrophage chemotactic protein 1 (MCP-1), which act to recruit neutrophils (polymorphonuclear leukocytes [PMNs]) and mononuclear cells (3–6). In addition, a recent study demonstrated that activated pleural fibroblasts may also be a source of C-X-C and C-C chemokine production (7).

Previous work suggested that the initiation of inflammation is dependent on endogenous IL-6 secretion that subsequently stimulates the additional production of tumor necrosis factor α (TNF-α) and IL-1β from resident pleural cells (8). In contrast, increased IL-1β levels have been reported to precede elevated IL-6 levels (9), thereby suggesting that IL-1β might induce IL-6 production. There is no doubt that TNF-α and IL-1β are key cytokines in the development of pleural inflammation because they act to enhance IL-8 and MCP-1 production from mesothelial cells (3, 5, 10–12). In addition, studies using function-blocking antibodies suggest that activated resident Mφ could be responsible for this TNF-α and IL-1β secretion (10, 12).

Carrageenan-induced pleurisy is a well-established model of acute inflammation (13) and is characterized by a rapid influx of PMNs followed by mononuclear cell infiltration (14, 15). This model is often used to assess the anti-inflammatory effects of pharmaceutical agents (16–20) and to assess the in vivo importance of established inflammatory mediators (21–23). Although the neutrophil influx evident in this model is generally used as an experimental readout of acute inflammation, there are data indicating that neutrophils are involved in the release of injurious enzymes and modulation of vascular permeability in carrageenan-mediated pleural inflammation (24, 25).

To date, there has been little study of the role of the resident pleural Mφ in the initiation of inflammation and orchestration of PMN recruitment. Previous work demonstrated a reduced eosinophil influx after administration of LPS to mice that had been previously treated with diphosphonate-containing liposomes to deplete resident pleural Mφ (26). Although this suggests that resident pleural Mφ may play a key role in the initiation of pleural inflammatory responses, there are no definitive data available for PMN infiltration and proinflammatory cytokine production.

This study used transgenic mice expressing the human diphtheria toxin receptor (DTR) under the CD11b promoter (designated CD11b-DTR mice) (27) to examine the role of resident pleural Mφ in carrageenan-induced pleurisy. Administration of diphtheria toxin (DT) to CD11b-DTR mice results in rapid depletion of resident pleural Mφ. Our data indicate that ablation of resident pleural Mφ markedly blunted both PMN recruitment and the levels of key chemokines and cytokines. In addition, resident Mφ ablation markedly reduced the acute PMN infiltration that followed the instillation of fixed, killed Staphylococcus aureus. This study demonstrates that resident pleural Mφ play an essential role in the orchestration of pleural PMN recruitment.
in pleural inflammation induced by carrageenan and fixed, killed *S. aureus*.

**METHODS**

**Macrophage Ablation and Pleurisy Induction**

Mice were housed in the University of Edinburgh animal facilities and experiments were performed in accordance with institutional and U.K. Home Office guidelines. CD11b-DTR transgenic mice were generated as previously described and were on an FVB/N background (27). Resident pleural Mφ were ablated in homozygous CD11b-DTR mice by intraperitoneal injection of DT (25 ng/g mouse body weight) 24 h before the administration of carrageenan. DT-treated FVB/N wild-type (WT) mice served as control animals. Carrageenan-induced pleurisy was induced as described previously (28). X-Carrageenan (0.1 ml of a 1% solution) was injected into the pleural cavity. Animals were culled 4 h later.

**Cell Processing and Analysis**

Pleural cavities were washed with 1 ml of 3.15% (weight/volume) sodium citrate (Sigma, Dorset, UK) in saline. We performed flow cytometric analysis of pleural lavage and circulating blood as described previously (27). The antibodies used were anti-CD11b fluorescein isothiocyanate, anti-GR1 phycoerythrin (PE) and anti–c-kit PE (all from Caltag, Botolph Claydon, UK). PMN infiltration in CD11b-DTR mice did reach approximately 24 h demonstrated a dramatic difference between groups. Although numbers seen after the administration of DT, indicating an absence of any role of resident pleural Mφ in initiating PMN recruitment after the administration of carrageenan. PMN infiltration after the administration of DT, resulting in more than 98% Mφ depletion. Control pleural cells and Mφ-depleted pleural cells were plated in 48-well plates and exposed to 0.25% carrageenan for 6 h. In control experiments, cell preparations were exposed to medium alone. Pleural cell-conditioned supernatants were analyzed as above. No bioassays were undertaken.

**Statistical Analysis**

One-way analysis of variance with a Bonferroni multiple comparison post hoc test, with a 95% confidence interval, or a Student’s t test was used as appropriate. Statistical analysis including correlation analysis was performed using GraphPad Prism software (San Diego, CA). The significance level was set at *p* < 0.05. Data are presented as mean ± SEM.

**RESULTS**

**Transgenic Pleural Resident Mφ Are Ablated by DT In Vivo**

There was no difference in the number of pleural Mφ, B cells, T cells, or mast cells between CD11b-DTR and FVB/N control mice (data not shown). Flow cytometric analysis of pleural cells was performed 24 h after the injection of DT (25 ng/g mouse body weight). CD11b-DTR transgenic mice exhibited almost complete ablation (96.1% ± 0.8) of F4/80-positive pleural Mφ after a single dose of DT (Figure 1). In addition, flow cytometric analysis of whole blood performed 24 h after DT administration indicated a significant 88% reduction in circulating monocyte numbers (1.07 x 10^5 ± 5.9 x 10^4 monocytes/ml whole blood vs. 5.23 x 10^5 ± 7.3 x 10^5, DT injection vs. control; *p* < 0.05). Circulating monocyte and pleural macrophage numbers remained markedly reduced for 48 h after the administration of DT with recovery of monocyte/macrophage numbers evident at 72 h (data not shown). However, no reduction in the number of circulating PMNs was evident 24 h after DT administration (10.1 x 10^5 ± 1.9 x 10^5 PMNs/ml whole blood vs. 4.7 x 10^5 ± 0.8 x 10^5, DT injection vs. control; *p* > 0.05). In addition, no difference in circulating PMN number was evident 6, 48, or 72 h after the administration of DT, indicating an absence of any initial neutropenia or delayed effects (6 h: 7.9 x 10^5 ± 1.2 x 10^5 PMNs/ml whole blood vs. 5.0 x 10^5 ± 1.4 x 10^5, DT injection vs. control; *p* > 0.05; 48 h: 4.0 x 10^5 ± 0.6 x 10^5 PMNs/ml whole blood vs. 4.9 x 10^5 ± 0.1 x 10^5, DT injection vs. control; *p* > 0.05; 72 h: 2.9 x 10^5 ± 1.4 x 10^5 PMNs/ml whole blood vs. 4.4 x 10^5 ± 0.3 x 10^5, DT injection vs. control; *p* > 0.05). We did, however, note a significant reduction in the number of B cells and mast cells within the pleural cavity 24 h after the administration of DT (although T-cell numbers were unaffected (B cells: 8.1 x 10^5 ± 5.7 x 10^4 vs. 32.9 x 10^4 ± 8.8 x 10^4, DT vs. control; *p* < 0.05; mast cells: 6.1 x 10^5 ± 0.1 x 10^5 vs. 67.8 x 10^5 ± 18.2 x 10^5, DT vs. control; *p* < 0.05). Interestingly, the depletion of pleural Mφ is almost complete at 6 h at which time no significant difference in the number of B lymphocytes or mast cells was evident. The loss of B cells and mast cells may be a consequence of the secondary necrosis of apoptotic macrophages that may occur in the absence of a population of viable macrophages to phagocyte the dying cells. Also, a subset of B lymphocytes and mast cells may express CD11b and this may account for the reduced numbers seen after the administration of DT (30–32).

**Pleural Resident Mφ Ablation Reduces PMN Influx in Carrageenan-induced Pleurisy**

We used the conditional Mφ ablation strategy to investigate the role of resident pleural Mφ in initiating PMN recruitment after the administration of carrageenan. PMN infiltration after the administration of 1% carrageenan was markedly attenuated at all experimental time points after resident Mφ ablation (Figure 2). It is particularly noteworthy that the early time points of 6 and 24 h demonstrated a dramatic difference between groups. Although PMN infiltration in CD11b-DTR mice did reach approximately...
50% of control levels at the later time points of 72 h, this was still significantly less than DT-treated nontransgenic FVB/N WT mice.

Adoptive Transfer of Nontransgenic Purified Mφ or Mφ-rich Pleural Cell Populations Partially Restores PMN Influx in Mφ-ablated CD11b-DTR Mice after Carrageenan Administration

To further analyze the role of resident pleural Mφ in the initiation of acute pleural inflammation, we also performed Mφ repletion studies using the adoptive transfer of either Mφ-rich or Mφ-depleted pleural cell populations derived from DT-insensitive nontransgenic FVB/N WT mice. In these experiments, the adoptive transfer of Mφ-rich pleural cell populations restored Mφ number to approximately 50% of the Mφ number normally present in pleural lavage fluid. However, despite the fact that Mφ reconstitution of DT-treated CD11b-DTR mice was incomplete, the administration of Mφ-rich pleural cells concurrently with carrageenan significantly increased PMN infiltration at 6 h (Figure 3). The partial restoration of peak PMN infiltration was approximately 55% of levels present in control DT-treated FVB/N WT mice at the same time point. In contrast, administration of Mφ-depleted pleural cells concurrently with carrageenan made no significant impact on PMN infiltration compared with Mφ-depleted CD11b-DTR mice (Figure 3). Interestingly, a significant correlation \( R^2 = 0.9979 \) was found between the Mφ number present in the pleural space at the initiation of inflammation and the number of infiltrating PMNs present at 6 h. We also reconstituted DT-treated CD11b-DTR mice with purified Mφ (90% pure) concurrently with the administration of carrageenan and this resulted in a comparable PMN influx to that evident after reconstitution with Mφ-rich pleural cells. It should be noted that, although DT-induced Mφ ablation is associated with a reduction of B-cell and mast cell number, the administration of Mφ-depleted pleural cells comprising B cells, mast cells, and T cells had no significant impact on PMN infiltration. Last, the adoptive transfer of a control population of Mφ-rich pleural cells or purified Mφ was noninflammatory (Figure 3).

Mφ-dependent Chemokine and Cytokine Responses during Carrageenan-induced Pleurisy

In this model, we found peak levels of the PMN C-X-C chemokines MIP-2 and KC at the 1- and 3-h time points, respectively. Ablation of resident pleural Mφ before administration of carrageenan markedly reduced MIP-2 levels at both 1 and 3 h (Figure 4A), thereby suggesting that the early production of MIP-2 in vivo is predominantly Mφ dependent. Interestingly, however, Mφ-ablated mice exhibited a delayed and significantly blunted MIP-2 response. It is of interest that very few Mφ (<30,000) are present within the pleural cavity of DT-treated CD11b-DTR mice at the 6-h time points, suggesting that the delayed MIP-2 response may be derived from production by local cells, such as mesothelial cells and others. MIP-2 levels are very low at the 24-h time point and beyond in both experimental groups. In contrast to the MIP-2 data, a very modest, albeit statistically significant, reduction in KC levels was evident in Mφ-ablated mice at the 1-, 3-, and 6-h time points (Figure 4B), but no differences were evident thereafter, suggesting that cells other than Mφ may be responsible for production of this chemokine. The fact that ablation of resident pleural Mφ dramatically

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**Figure 1.** Administration of DT 24 h before performing pleural lavage results in ablation of pleural F4/80-positive macrophages (Mφ). CD11b-DTR and FVB/N wild-type (WT) mice were treated with diphtheria toxin (DT) intraperitoneally at a dose of 25 ng/g body weight. Pleural lavage was performed 24 h later. Cells were stained for the Mφ surface marker F4/80 and analyzed by flow cytometry. (A) Representative flow cytometry dot plot indicating that over 50% of pleural cells retrievable by pleural lavage was performed 24 h after DT administration in FVB/N mice are F4/80 positive. (B) Administration of DT results in marked ablation of resident F4/80-positive pleural Mφ in CD11b-DTR mice. DT administration ablated 96.1 ± 0.8% of the resident Mφ population compared with baseline Mφ numbers (n = 9 mice, p < 0.0001). APC = allophycocyanin.

**Figure 2.** Resident Mφ ablation 24 h before administration of carrageenan blunts neutrophil (PMN) recruitment. 0.1 ml of 1% carrageenan was administered to CD11b-DTR and FVB/N WT mice 24 h after DT treatment. Pleural lavage was performed at 0, 6, 24, and 72 h after carrageenan administration. Lavaged cells were stained for GR1 and counted by flow cytometry (*p < 0.05 vs. CD11b-DTR group; n = 4–5 mice/group).

**Figure 3.** The partial restoration of peak PMN infiltration was approximately 55% of levels present in control DT-treated FVB/N WT mice at the same time point. In contrast, administration of Mφ-depleted pleural cells concurrently with carrageenan made no significant impact on PMN infiltration compared with Mφ-depleted CD11b-DTR mice (Figure 3). Interestingly, a significant correlation \( R^2 = 0.9979 \) was found between the Mφ number present in the pleural space at the initiation of inflammation and the number of infiltrating PMNs present at 6 h. We also reconstituted DT-treated CD11b-DTR mice with purified Mφ (90% pure) concurrently with the administration of carrageenan and this resulted in a comparable PMN influx to that evident after reconstitution with Mφ-rich pleural cells. It should be noted that, although DT-induced Mφ ablation is associated with a reduction of B-cell and mast cell number, the administration of Mφ-depleted pleural cells comprising B cells, mast cells, and T cells had no significant impact on PMN infiltration. Last, the adoptive transfer of a control population of Mφ-rich pleural cells or purified Mφ was noninflammatory (Figure 3).

**Figure 4.** Mφ-dependent Chemokine and Cytokine Responses during Carrageenan-induced Pleurisy

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blunted PMN infiltration suggests that early PMN influx is very dependent on resident Mφ production of MIP-2. The ablation of resident pleural Mφ did not exert marked effects on the production of MCP-1 as levels were only reduced by approximately 36% at the 3-h time point (Figure 4C), suggesting a source other than Mφ.

Analysis of the levels of cytokines in pleural lavage samples indicated a key role for resident Mφ in the early production of the cytokines TNF-α, IL-6, and IL-10. Mφ ablation resulted in greater than 90% reduction in TNF-α and IL-6 levels, with a less dramatic but significant inhibitory effect on IL-10 levels (Figure 5). IL-12 levels were also reduced with Mφ ablation at 24 h (data not shown). Despite these important differences in these cytokines, IFN-γ levels were comparable between DT-treated CD11b-DTR and FVB/N control mice at each time point (data not shown), suggesting a source other than resident Mφ.

Chemokine and Cytokine Responses of Pleural Cell Populations In Vitro Are Mφ Dependent

Because pleural mesothelial cells may be an important source of chemokines, we performed additional in vitro studies to determine the production of chemokines and cytokines by carrageenan-stimulated pleural cell populations that had been depleted of Mφ. Immunomagnetic Mφ depletion using antibodies for the Mφ specific marker F4/80 resulted in 98% depletion of Mφ from pleural cell populations, whereas B-cell and mast cell numbers were comparable between groups (data not shown). Stimulation of control Mφ-rich pleural cell populations for 6 h with carrageenan resulted in significant production of MIP-2 and KC (Figure 6). In contrast, no significant chemokine production was evident after stimulation of pleural cell populations depleted of resident Mφ but containing B cells, T cells, and mast cells, thereby indicating that production of these PMN C-X-C chemokines in vitro was completely Mφ dependent. Limited production of MCP-1 was evident in vitro but this was also significantly reduced by depletion of resident Mφ (25.3 ± 5.3 vs. 7.1 ± 4.7 pg/ml, Mφ-rich pleural cells vs. Mφ-depleted pleural cells; p < 0.05). Analysis of in vitro cytokine production demonstrated that resident Mφ were key cytokine producers, because Mφ depletion before carrageenan stimulation resulted in a reduction of 63, 67, and 92% in the production of TNF-α, IL-10, and IL-6, respectively (Figure 7).

Pleural Resident Mφ Ablation Reduces PMN Influx in Response to S. aureus

Although the carrageenan model of pleurisy is a useful model of inflammation and has been used by many investigators to dissect inflammatory pathways, we sought evidence that resident Mφ were involved in models of inflammation that were more closely related to clinical disease. We initially used the model of intrapleural LPS instillation, but this resulted in a very low level of PMN infiltration compared with carrageenan. We therefore instilled formalin-fixed, fluorescently labeled S. aureus into the pleural cavity and this induced a marked PMN infiltrate at the 4-h time point (> 1.5 × 10^6 PMNs). The ablation of resident Mφ significantly reduced PMN infiltration after the administration of S. aureus (Figure 8). We also found comparable PMN infiltration in DT-treated FVB/N WT mice and PBS-treated CD11b-DTR mice, indicating that insertion of the transgene had no significant effect on the generation of acute inflammatory responses (Figure 8A), with comparable findings evident after the administration of carrageenan (data not shown). Cytospin preparations of pleural lavage cells indicated prominent ingestion of S. aureus particles by Mφ in DT-treated FVB/N WT mice (Figure 8B) with very limited uptake by PMNs. In contrast, in the absence of Mφ, DT-treated CD11b-DTR mice exhibited marked ingestion of S. aureus particles by PMNs (Figure 8B).

DISCUSSION

We used a conditional macrophage ablation strategy to dissect the role of the resident pleural Mφ in the initiation of pleural inflammation and PMN recruitment in carrageenan-induced pleurisy. Carrageenan induces inflammatory responses that are likely to be involved in human disease such as tuberculosis, which is a cause of significant morbidity and mortality. We also examined the effect of Mφ ablation before the administration of fixed S. aureus, a model with direct clinical relevance. Although
Figure 4. Resident Mφ ablation attenuates chemokine production in carrageenan-induced pleurisy. CD11b-DTR and FVB/N WT mice were injected with DT (25 ng/g body weight) 24 h before administration of carrageenan. Pleural lavage was performed 1, 3, 6, 24, and 72 h after the induction of pleurisy. The levels of macrophage inflammatory protein 2 (MIP-2; A) and keratinocyte-derived chemokine (KC; B) were determined in the pleural lavage supernatant by specific ELISA. The level of macrophage chemoattractant protein 1 (MCP-1; C) in the pleural lavage supernatant was determined by cytometric bead array (CBA) analysis (*p < 0.05 vs. CD11b-DTR group; n = 5 mice/group).

The first major finding of this study is that the administration of DT to CD11b-DTR transgenic mice results in the rapid and effective ablation of resident pleural Mφ, with greater than 96% of resident pleural Mφ being depleted 24 h after DT treatment. This is comparable with our previous work studying peritoneal inflammation (27). Interestingly, despite PMN expression of CD11b, the administration of DT did not induce the death of circulating PMNs, indicating that PMNs are insensitive to DT, potentially as a result of their lower level of protein synthesis.

Figure 5. Resident Mφ ablation attenuates cytokine production in carrageenan-induced pleurisy. CD11b-DTR and FVB/N WT mice were injected with DT (25 ng/g body weight) 24 h before carrageenan injection. Pleural lavage was performed 1, 3, 6, 24, and 72 h after the induction of pleurisy. The level of tumor necrosis factor α (TNF-α; A) in the pleural lavage supernatant was determined by specific ELISA, whereas the levels of interleukin 6 (IL-6; B) and IL-10 (C) were determined by CBA analysis (*p < 0.05 vs. CD11b-DTR group; n = 5 mice/group).

The second major finding of this study is that resident pleural Mφ can secrete chemokines and cytokines, their role in pleurisy is currently unclear. Pleural mesothelial cells also have the capacity to secrete various chemokines (3, 6, 7, 12, 33, 34). In addition, some studies have identified resident pleural Mφ-derived proinflammatory cytokines such as TNF-α that are essential for the secretion of C-X-C and C-C chemokines from pleural mesothelial cells (3, 5, 8, 10–12, 33), suggesting important cross-talk between different pleural cells.

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number of pleural \(\text{M}^\phi\) at the initiation of disease and the number of infiltrating PMNs at 6 h after carrageenan administration strongly supports a key proinflammatory role for resident pleural \(\text{M}^\phi\). It is possible that carrageenan pleurisy may be partially dependent on the proinflammatory actions of recruited monocytes, unlike experimental peritonitis where acute PMN infiltration is monocyte independent (27). Our data indicate a profound effect of \(\text{M}^\phi\) depletion on PMN recruitment at the early time point of 6 h and because monocyte recruitment occurs significantly later in the carrageenan model it is likely that monocyte recruitment will be very limited at this early time point. Thus, a reduction in monocyte recruitment in DT-treated CD11b-DTR mice is unlikely to be involved in the early reduction in PMN infiltration in these studies, although recruited monocytes may play a role in PMN infiltration at later time points.

Although defective PMN migration consequent on exposure to DT is an alternative explanation for these findings, our previous work in experimental peritonitis indicated that reconstitution of \(\text{M}^\phi\)-depleted mice with nontransgenic \(\text{M}^\phi\) was able to fully restore PMN infiltration in response to thioglycollate (27). In addition, intrapleural administration of the chemokine MIP-2 to \(\text{M}^\phi\)-depleted CD11b-DTR mice resulted in significant PMN infiltration (3.2 \(\times\) 10^5 \(\pm\) 0.9 \(\times\) 10^5 PMNs/ml at 4 h after the intrapleural administration of 30 ng MIP-2), suggesting that PMN migration is not defective under these experimental conditions.

In these experiments, DT administration and the subsequent induction of widespread \(\text{M}^\phi\) death did affect the numbers of pleural B cells and mast cells. However, despite this potentially confounding issue, several factors support the prominent role of the pleural \(\text{M}^\phi\) in the carrageenan model. First, data from \textit{in vitro} experiments indicate a dramatic reduction in chemokine and cytokine production after \(\text{M}^\phi\) depletion from resident pleural cell populations. In these studies, pleural cells were labeled with a PE-conjugated antibody to the specific \(\text{M}^\phi\) marker F4/80 before immunomagnetic depletion, and F4/80 is not expressed by B cells or mast cells. Second, adoptive transfer of \(\text{M}^\phi\)-depleted pleural cells comprising B cells, T cells, and mast cells did not induce significant PMN recruitment after carrageenan administra-

![Figure 6](image1.png)

**Figure 6.** \textit{In vitro} production of MIP-2 and KC after carrageenan stimulation is \(\text{M}^\phi\) dependent. Resident pleural cells were harvested and immunodepleted of resident pleural \(\text{M}^\phi\) by passage over a magnetic column. Equivalent numbers of cells were plated and stimulated with 0.25% carrageenan or normal medium for 6 h. Supernatants were harvested and analyzed by specific ELISA for MIP-2 and KC (*p < 0.05 vs. all cells with medium; n = 4 wells/condition).

![Figure 7](image2.png)

**Figure 7.** \textit{In vitro} production of the cytokines TNF-\(\alpha\), IL-10, and IL-6 after carrageenan stimulation is \(\text{M}^\phi\) dependent. Resident pleural cells were harvested and immunodepleted of resident pleural \(\text{M}^\phi\) by passage over a magnetic column. Equivalent numbers of cells were stimulated with 0.25% carrageenan or normal medium for 6 h. Supernatants were harvested and analyzed by specific ELISA for TNF-\(\alpha\) and by CBA for IL-10 and IL-6 (*p < 0.05 vs. all cells vs. \(\text{M}^\phi\)-depleted for their respective condition, i.e., \(\text{M}^\phi\) with medium or \(\text{M}^\phi\) with carrageenan). n = 4 wells/condition.
and control mice with pleurisy because the preparation of pure chemotactic activity of pleural lavage fluid from M did not perform of both chemokines giving little additional effect (39, 40). We marked (characteristic lobulated or circular nuclear morphology. Note that in B, the cell indicated with an arrow is the only Mφ present in the field and exhibits a large, rounded nucleus, whereas the remaining smaller PMNs exhibit a polylobular nuclear morphology. There are no Mφ present in C and D. Prominent ingestion of S. aureus particles by Mφ is evident in control DT-treated FVB/N WT mice (examples shown with arrows in A and B), with very limited uptake by PMNs. In contrast, in the absence of Mφ, DT-treated CD11b-DTR mice exhibit marked ingestion of S. aureus particles by PMNs (examples shown with arrows in C and D).

In conclusion, this study used a transgenic model of conditional Mφ ablation to demonstrate a key role for the resident pleural Mφ in sensing pleural irritation and orchestrating PMN infiltration in carrageenan-induced pleurisy. This proinflammatory function is predominantly mediated by production of the potent PMN C-X-C chemokine MIP-2 and proinflammatory cytokines such as TNF-α and IL-6 that can promote the production of the PMN C-X-C chemokine KC by mesothelial cells. Our study suggests that resident Mφ are critically important producers of PMN chemokines and proinflammatory cytokines and act to orchestrate PMN recruitment in murine carrageenan-induced pleurisy.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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