**Monocyte migration explains the changes in macrophage arachidonate metabolism during the immune response**

*(thromboxane A2/Listeria/Ia expression)*

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**ABSTRACT** The profile of arachidonic acid metabolites in resident peritoneal macrophages is distinctly different from the profile of macrophages isolated after an acute bacterial infection. The latter produce decreased prostaglandins E2 and I2 and leukotriene C4 while conserving the synthesis of thromboxane A2. We show here that the initial changes in peritoneal macrophage arachidonate metabolism during the immune response appear to be the result of the large influx of blood monocytes, which have a characteristic metabolism distinct from resident macrophages. We demonstrate that the initial decrease in peritoneal macrophage arachidonate metabolism and the increase in macrophage numbers occur simultaneously after infection with *Listeria monocytogenes*. Also the macrophage arachidonate metabolism seen at the height of the peritoneal cellular influx is the same as that of purified blood monocytes. Both peritoneal macrophages and blood monocytes produce equal or greater quantities of thromboxane A2 relative to prostaglandins I2 and E2 or leukotriene C4 whereas resident cells produce 1/10 to 1/25 as much thromboxane A2 compared to the other products. Furthermore, the changes in peritoneal macrophage arachidonate metabolism in response to *Listeria* infection do not occur if the influx of blood monocytes is stopped by irradiating the mice prior to infection implying that the cellular influx is necessary to see the changes in arachidonate metabolism. Finally, activation of peritoneal macrophages, measured as an increase in Ia expression, occurs 36 hr after the influx of monocytes from the blood and the resultant shift in arachidonate metabolism during *Listeria* infection.

Immunizing animals with protein antigens or bacteria activates macrophages in vivo and results in several biochemical changes including increased expression of Ia antigens (1) and increased cytotoxicity (2) and secretion of interleukin 1 (3). Macrophages can also be activated in vitro by incubation with γ interferon (4), which results in an increased Ia expression, cytotoxicity, and interleukin 1 secretion.

Resident macrophages isolated from uninfected mice produce large quantities of arachidonic acid metabolites (5, 6). In contrast, macrophages activated in vivo by exposure to bacteria have a decreased capacity to produce prostaglandin (PG) E2, PGI2, and leukotriene (LT) C4 (5, 6) while the synthesis of thromboxane (TX) A2 is selectively conserved (7). This observation led to the hypothesis that factors generated during the immune response in vivo, such as γ interferon, may modulate the resident macrophage arachidonate metabolic enzyme activities and, hence, change the profile of arachidonate products produced. However, phospholipase activity seems to be the only arachidonate metabolic enzyme that can be modulated during in vitro activation of resident cells with various immunological factors (8, 9), whereas all of the metabolic enzymes except TX synthase are altered during in vivo activation (7). Thus an alternate explanation seems necessary to explain the changed macrophage arachidonate metabolism during in vivo activation.

Since human blood monocytes synthesize equal quantities of TXA2 compared to the other arachidonate metabolites (10, 11) and these cells accumulate at the site of an infection, we examined the possibility that the migration of blood monocytes to the site of infection during the immune response in vivo is the mechanism by which changes in macrophage arachidonate metabolism, including the selective conservation of tissue macrophage TXA2 production, occur.

**METHODS**

Macrophages were obtained from the peritoneal cavity of adult B10.A/SgSnJ mice (The Jackson Laboratory) that were either uninfected (resident macrophages) or infected intraperitoneally with *Listeria monocytogenes* (*Listeria* macrophages). Mice were infected with live bacteria as described in each of the figure legends.

Prior to irradiation (see Table 2), mice were given chlorotetracyclin (1 g/liter) for 2 days in the drinking water. Some mice were irradiated with 900 R at a rate of 133 R/min from a 137Cs source. (1 R = 0.258 mC/kg.) Forty-eight hours after irradiation, the mice were infected with 105 live *Listeria*. The peritoneal exudate cells (PEC) were collected 24 hr later. PEC were obtained by lavaging with PBS (7). PEC (1–2 × 106 cells) were allowed to adhere to 35-mm tissue culture dishes (7). The nonadherent cells were removed by washing with PBS.

**Isolation and Culture of Mouse Blood Monocytes.** Blood monocytes were isolated by Isopaque/Ficoll centrifugation and separated from platelets as described (11). The monocytes were further purified by a 4-hr adherence step as described above, followed by extensive washing to remove nonadherent cells.

**Arachidonic Acid Metabolism.** The adherent cells were incubated with Zymosan (300 μg/ml, Sigma; ref. 12), thrombin (1 unit/ml), or arachidonic acid (10 μΜ, Nu Chek Prep) (7). Arachidonic acid metabolites were determined by radioimmunoassay (RIA) (13, 14). The medium was also acidified, extracted with chloroform/ethanol (2), and analyzed for hydroxyeicosatetraenoic acid by HPLC with comigration of authentic standard.

**Expression.** Surface Ia expression was determined using an RIA (15). Briefly, anti-I-A* antibody (10-2.16, ref. 16) was iodinated by chloramine-T oxidation (17) to a specific activity between 5 and 15 μCi/μg of protein. (1 Ci = 37 GBq.) To determine Ia expression, 125I-labeled anti-I-A* antibody was

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Table 1. Comparison of arachidonic acid metabolism by various monocyte-macrophage populations

<table>
<thead>
<tr>
<th>Cells</th>
<th>Agonist</th>
<th>Metabolite, pg/μg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6kPGF1α</td>
<td>PGE2</td>
</tr>
<tr>
<td>Resident peritoneal macrophages</td>
<td>None</td>
<td>222 ± 64</td>
</tr>
<tr>
<td></td>
<td>Zymosan</td>
<td>8106 ± 1814</td>
</tr>
<tr>
<td></td>
<td>Thrombin</td>
<td>142 ± 37</td>
</tr>
<tr>
<td>Blood monocytes</td>
<td>None</td>
<td>9 ± 2</td>
</tr>
<tr>
<td></td>
<td>Zymosan</td>
<td>1149 ± 254</td>
</tr>
<tr>
<td></td>
<td>Thrombin</td>
<td>7 ± 3</td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid</td>
<td>14699 ± 5484</td>
</tr>
</tbody>
</table>

Results of either resident or Listeria peritoneal macrophages and monocytes isolated from blood were stimulated with agonists. The arachidonate metabolites secreted into the media were measured by RIA. The data represent the mean ± SEM of three different cell preparations. N.D., not detectable. 6kPGF1α, 6-keto-PGF1α. The absence (−) or presence (+, ++, +++) of hydroxysicosatetraenoic acid (12-HETE) was determined by HPLC. Zymosan, 300 μg/ml. Thrombin, 1 unit/ml. Arachidonic acid, 10 μM.

* Mice were injected i.p. with 10⁵ live Listeria on day 0 and boosted with 10⁴ live Listeria on day 7, and peritoneal macrophages were isolated on day 10.

RESULTS

Forty percent of the total arachidonate metabolites produced by purified mouse blood monocytes in response to zymosan or exogenous arachidonic acid (Table 1) is TXA₂ (measured as TXB₂), similar to results shown for human blood monocytes (11). Data (7) for resident and Listeria peritoneal macrophage populations are also shown for comparison. Although blood monocytes synthesize the same arachidonate products as both tissue macrophage populations, the quantity and profile of metabolites produced resembles that seen by the activated Listeria population more closely than that of the resident cells. Blood monocytes and Listeria macrophages synthesize similar amounts of TXB₂ relative to the production of PGI₂ (measured as 6-keto-PGF1α), PGE₂, and LTC₄. For example, the ratio (μg/μg) of the production of 6-keto-PGF1α to TXB₂ is 1:1 for Listeria peritoneal macrophages and blood monocytes whereas this ratio is 20:1 for the resident macrophages (Table 1). The TXB₂ production is not due to
platelet contamination and stimulation since zymosan is not an agonist for platelet arachidonate metabolism, and thrombin, the platelet agonist, does not stimulate these macrophage cultures. The monocytes produce only 27% and 18% of the total zymosan and exogenous arachidonate-stimulated metabolites, respectively, synthesized by the resident macrophages. In comparison to the Listeria cells, the blood monocytes produced 6 times more endogenous metabolites in response to zymosan but similar levels of products (1.5 times more) in response to exogenous arachidonate. Since blood monocytes migrate into the peritoneal cavity during this infection (18), this data suggests that a precursor relationship between the blood monocytes and the Listeria macrophages may explain the shift in macrophage arachidonate metabolism during the immune response. Thus, after infection with Listeria we simultaneously monitored the migration of cells into the peritoneal cavity, the arachidonate metabolism, and activation expressed as Ia expression of peritoneal macrophages.

As reported (18), macrophages and polymorphonuclear leukocytes (PMNs) represent the predominant cells migrating into the peritoneal cavity in response to Listeria infection (Fig. 1A). There are very few lymphocytes and mast cells, which comprise only 6% and 2%, respectively, of the resident population, and decrease to less than 1% after Listeria infection. The peak of the cellular influx occurs by 8 hr, after which time the numbers of PMNs decrease while the numbers of macrophages remain elevated. If macrophage activation is monitored with time after Listeria infection, the expression of Ia cannot be detected until 48 hr after injection of bacteria (Fig. 1B). Thus increases in peritoneal cell numbers occurs prior to the stimulation of Ia, although macrophage Ia expression occurs quite rapidly.

In contrast to Ia expression, the changes occurring in arachidonate metabolism are much quicker. With time after Listeria infection in vivo, there is a dramatic decrease in the ability to synthesize 6-keto-PGF\(_{1\alpha}\), PGE\(_2\), and LTC\(_4\) (Fig. 2). This decrease in macrophage arachidonate metabolism corresponds exactly with the increase in peritoneal macrophage numbers (Fig. 1), displaying the maximal changes in each by 8–12 hrs after Listeria infection. Production of 6-keto-PGF\(_{1\alpha}\), PGE\(_2\), and LTC\(_4\) decreased by 89%, 73%, and 89%, respectively, in response to zymosan and 84%, 72%, and 79%, respectively, in response to exogenous arachidonic acid. In sharp contrast, the production of peritoneal macrophage TXB\(_2\) (a major product of the blood monocytes) remains unchanged in response to zymosan and exogenous arachidonate during the entire time course of infection. Furthermore, the decreased levels of each arachidonate metabolite produced by peritoneal macrophages by the peak of cellular migration at 8–12 hr of infection (Fig. 2) is the same as the levels produced by purified blood monocytes (Table 1). This data suggests that the initial rapid changes in macrophage metabolism occurring during Listeria infection is due to the migration of blood monocytes into the peritoneal cavity, bringing their own distinctive arachidonate metabolic profile. Migration of cells from the circulation to the peritoneal cavity can be suppressed by irradiating the mice (19) prior to infection with Listeria (Table 2). Irradiation has no effect on the resident macrophage numbers nor on their arachidonate acid metabolism (Table 2). However, irradiating the animals

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**Table 2.** Irradiation blocks the changes in macrophage arachidonate metabolism during Listeria infection

<table>
<thead>
<tr>
<th>In vivo treatment</th>
<th>PEC, (\times 10^{-6})</th>
<th>Agonist</th>
<th>Metabolite, pg/µg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.9–2.0</td>
<td>None</td>
<td>6kPGF(_{1\alpha}) PGE(_2) TXB(_2) LTC(_4)</td>
</tr>
<tr>
<td>Irradiation</td>
<td>1.2–1.3</td>
<td>Arachidonic acid</td>
<td>110 ± 10 42 ± 6 10 ± 4 5 ± 1</td>
</tr>
<tr>
<td>Listeria</td>
<td>5.8–6.8</td>
<td>None</td>
<td>6641 ± 2036 1313 ± 198 807 ± 228 1418 ± 521</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zymosan</td>
<td>14709 ± 4295 4112 ± 1699 1272 ± 380 128 ± 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arachidonic acid</td>
<td>467 ± 111 148 ± 5 91 ± 40 11 ± 4</td>
</tr>
<tr>
<td>Listeria/irradiation</td>
<td>0.7–0.8</td>
<td>None</td>
<td>11628 ± 3354 1797 ± 662 1205 ± 708 3072 ± 610</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zymosan</td>
<td>17978 ± 4198 8582 ± 1087 1300 ± 666 125 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arachidonic acid</td>
<td>40 ± 25 16 ± 3 10 ± 3 3 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zymosan</td>
<td>268 ± 92 222 ± 32 566 ± 303 67 ± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arachidonic acid</td>
<td>248 ± 6 887 ± 150 929 ± 231 7 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>345 ± 141 78 ± 14 18 ± 8 7 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zymosan</td>
<td>7147 ± 311 1544 ± 387 817 ± 464 823 ± 134</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arachidonic acid</td>
<td>8075 ± 3366 6529 ± 1154 794 ± 224 45 ± 3</td>
</tr>
</tbody>
</table>

Mice were treated as described. 6kPGF\(_{1\alpha}\), 6-keto-PGF\(_{1\alpha}\). Macrophage cultures were stimulated with agonists and the metabolites secreted into the media were measured by RIA. The data represent the mean ± SEM of three different cell cultures.
blocks both the increase in PEC (in part by blocking the influx of cells from the blood) and the shift in macrophage arachidonate metabolism in response to Listeria infection (Table 2). Thus the initial changes in peritoneal macrophage arachidonate metabolism do not occur in the absence of a cellular influx. These data further support the hypothesis that the migration of blood monocytes into the peritoneal cavity during Listeria infection is responsible for the major shift in macrophage arachidonate metabolism.

The migration of blood monocytes is, however, not the only mechanism by which altered macrophage arachidonate metabolism can occur. For example, interferons α, β, and γ decrease zymosan-stimulated metabolism without affecting exogenous arachidonate conversion in resident macrophages in vitro (8, 9), indicative of a decrease in phospholipase activity. Since α, β, and γ interferons are produced in vivo during the immune response, there may be a decrease in phospholipase activity occurring in vivo similar to that described in vitro. Peritoneal macrophages isolated after Listeria infection (Tables 1 and 2) have decreased levels of metabolites compared to purified blood monocytes (Table 1) when stimulated with zymosan but not in response to exogenous arachidonic acid. This data indicates that the metabolism of the monocytes that have migrated into the peritoneal cavity during infection and perhaps that of the resident cells may be further decreased by an inhibition of phospholipase activity.

**DISCUSSION**

The migration of monocytes from the blood to the site of an infection can explain the initial shift in peritoneal macrophage metabolism (Fig. 3A). The selective conservation of macrophage TXA2 synthesis during the immune response in vivo appears to be the result of this metabolite being a major product of the blood monocyte. The monocytes, which have decreased PGI2, PGE2, and LTc4 synthesis compared to resident macrophages, migrate to the site of infection and quickly become activated, exemplified by the expression of Ia antigens (19). The migration of monocytes ensures the early, rapid removal of PGI2 and PGE2, which inhibits both macrophage (15, 20, 21) and lymphocyte (22, 23) activation.

It remains unclear whether, during the immune response, the resident peritoneal macrophages can become activated and shift their arachidonate metabolism. In vivo lymphokine (source of γ interferon) treatment for three days after Listeria infection on resident macrophages isolated from mice irradiated prior to Listeria infection fails to induce Ia. Thus lymphokine is only effective on blood-derived monocytes that have recently arrived at the peritoneum (19). Likewise, in response to γ interferon (24), in vitro-cultured monocytes express Ia in 3–4 days while resident macrophages do not develop Ia in vitro until 6–8 days. Thus, the resident cells may be capable of expressing Ia in vivo but not as quickly as the migrating blood monocytes. With respect to arachidonate metabolism, α, β, and γ interferons can cause decreased phospholipase activity in cultured resident macrophages (8, 9) and may also be causing the same effect in vivo on the resident macrophages and the migrating monocytes. However, the effects of the interferons on phospholipase do not explain the early initial changes in macrophage arachidonate metabolism, during which time TXA2 synthesis is conserved. Clearly the data support the importance of the monocyte influx during the immune response for the quick removal of the inhibitory immunomodulators PGI2 and PGE2 as well as a source of macrophages that can be rapidly activated.

In the absence of an immune response, resident peritoneal macrophages can originate from the following two sources (Fig. 3A): (i) blood monocytes, which means that the arachidonate metabolic enzymes will have to be induced to explain the resident phenotype, and/or (ii) a dividing precursor population residing in the peritoneal cavity having a high rate of arachidonate metabolism. The contribution of these two mechanisms may be elucidated more clearly by examining the resolution of the infection after the bacteria have been eliminated. The induction of macrophage PGI2 and PGE2 synthesis may be important at this time to inhibit the immune response so the resolution can begin. Factors that induce monocyte arachidonate metabolism or increase macrophage proliferation may be increased once the bacteria have been eliminated.

Finally, the stimulus for Ia induction at 48 hr after Listeria infection precedes any measurable T-cell activity (Fig. 3B). Bancroft et al. (25) have demonstrated that macrophages isolated from CB-17 scid mice, which lack B and T lymphocytes (26), can have induction of Ia via a mechanism independent of mature T cells. Thus these data raise the possibility that under normal circumstances, the very early
Induction of Ia and perhaps other parameters of macrophage activation may be occurring by a T-cell-independent pathway. Whether γ interferon can be synthesized by some other cell type (PMNs or natural killer cells) or whether some other factor, possibly a Listeria product, also stimulates macrophages activation (exemplified by Ia expression) early in the immune response in vivo remains unclear.

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