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

(thromboxane A_2/*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 E_2 and I_2 and leukotriene C_4 while conserving the synthesis of thromboxane A_2. 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 *Listeria* peritoneal macrophages and blood monocytes produce equal or greater quantities of thromboxane A_2 relative to prostaglandins I_2 and E_2 or leukotriene C_4 whereas resident cells produce 1/10 to 1/25 as much thromboxane A_2 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). Resident 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) E_2, PGI_2, and leukotriene (LT) C_4 (5, 6) while the synthesis of thromboxane (TX) A_2 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 TXA_2 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 arachidonic acid metabolism, including the selective conservation of tissue macrophage TXA_2 production, occur.

METHODS

Macrophages were obtained from the peritoneal cavity of adult B10.A/Sg SnJ 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 chlortetracyclin (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 ^{137}Cs source. (1 R = 0.258 mC/kg.) Forty-eight hours after irradiation, the mice were infected with 10^5 live *Listeria*. The peritoneal exudate cells (PEC) were collected 24 hr later. PEC were obtained by lavaging with PBS (7). PEC (1–2 × 10^6 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 μM, Nu Chek Prep) (7). Arachidonate 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.

Ia Expression. Surface Ia expression was determined using an RIA (15). Briefly, anti-I-A^k 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, ^{125}I-labeled-anti-I-A^k antibody was

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Abbreviations: PG, prostaglandin; LT, leukotriene; TX, thromboxane; PEC, peritoneal exudate cells; PMN, polymorphonuclear leukocyte.

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

Cells	Agonist	Metabolite, pg/μg of protein				
		$6kPGF_{1\alpha}$	PGE_2	TXB_2	LTC_4	12-HETE
Resident peritoneal macrophages	None	222 ± 64	49 ± 21	13 ± 4	N.D.	−
	Zymosan	8106 ± 1814	1702 ± 264	326 ± 53	1152 ± 370	−
	Thrombin	142 ± 37	27 ± 16	14 ± 6	N.D.	−
	Arachidonic acid	14699 ± 5484	2614 ± 326	842 ± 162	62 ± 19	+
Blood monocytes	None	9 ± 2	30 ± 6	69 ± 15	17 ± 8	−
	Zymosan	1149 ± 254	520 ± 109	1668 ± 302	603 ± 34	+
	Thrombin	7 ± 3	26 ± 7	70 ± 20	11 ± 5	−
	Arachidonic acid	1213 ± 304	698 ± 80	1346 ± 155	142 ± 62	++
Listeria peritoneal macrophages*	None	21 ± 9	13 ± 6	11 ± 2	6 ± 2	−
	Zymosan	171 ± 49	175 ± 51	233 ± 45	47 ± 32	−
	Thrombin	18 ± 9	8 ± 1	6 ± 2	N.D.	−
	Arachidonic acid	812 ± 412	736 ± 164	743 ± 93	26 ± 16	−

Cultures 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. $6kPGF_{1\alpha}$, 6-keto-$PGF_{1\alpha}$. The absence (−) or presence (+, ++) of hydroxyicosatetraenoic 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 10^4 live *Listeria* on day 0 and boosted with 10^5 live *Listeria* on day 7, and peritoneal macrophages were isolated on day 10.

incubated with macrophages (1.5×10^5 cells per 7 mm) at 0°C for 2 hr, and then the unbound antibody was removed by washing the cells with PBS at 0°C. The ^{125}I-labeled cells were solubilized with 0.62 M NaOH, and the radioactivity was quantitated using a γ counter while the cellular protein was determined using the fluorescamine assay. The nonspecific binding, determined using the negative haplotype macrophages B10.D2 was comparable to binding found in the presence of 100-fold excess unlabeled antibody.

The percentage of Ia-positive macrophages was determined with indirect immunofluorescence (1). Adherent macrophages ($1-2 \times 10^5$ cells) were fixed with 1% paraformaldehyde, then incubated with the anti-I-A^k antibody 10-2.16, followed by a fluorescein-conjugated $(Fab)_2$ rabbit anti-mouse immunoglobulin. The cells were examined using a standard fluorescence microscope set up with phase-contrast optics and fluorescence epillumination. The specificity controls have been reported (1).

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 TXA_2 (measured as TXB_2), 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 TXB_2 relative to the production of PGI_2 (measured as 6-keto-$PGF_{1\alpha}$), PGE_2, and LTC_4. For example, the ratio (μg/pg) of the production of 6-keto-$PGF_{1\alpha}$ to TXB_2 is 1:1 for *Listeria* peritoneal macrophages and blood monocytes whereas this ratio is 20:1 for the resident macrophages (Table 1). The TXB_2 production is not due to

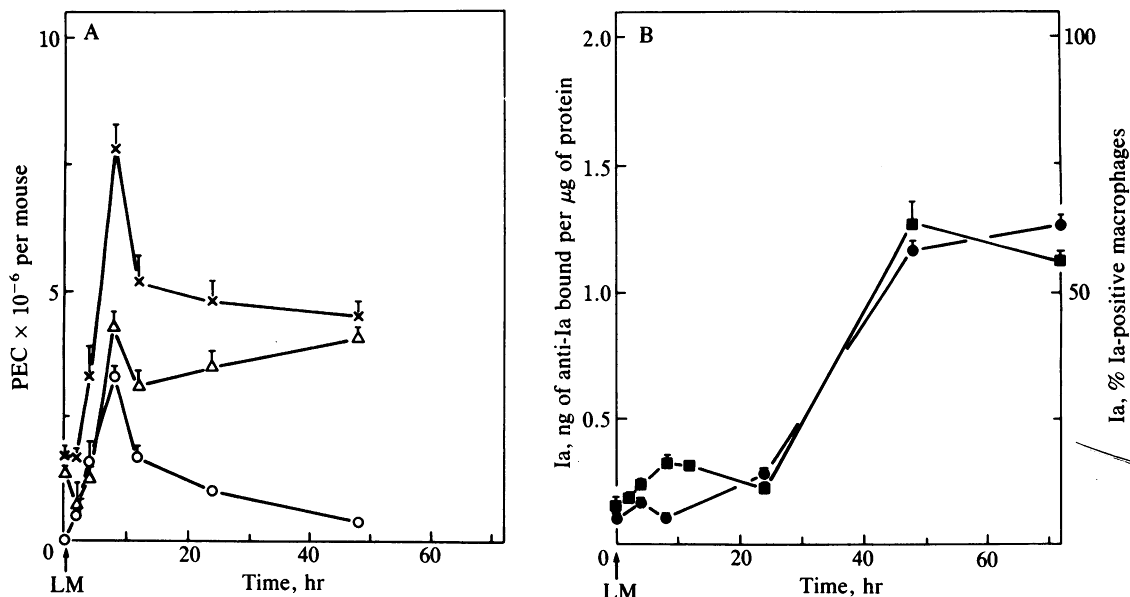

FIG. 1. Composition of the cellular influx and macrophage Ia expression after infection with *Listeria*. Mice were injected with 10^5 live *Listeria* (LM) at time 0, and the PEC were isolated at various times after infection. (*A*) The total number of PEC (X), macrophages (△), and PMN (○) were determined after Giemsa staining. The data represent the mean ± SEM of three separate experiments. (*B*) The quantity of Ia per μg of protein (■) as well as the percentage of Ia-positive cells (●) were determined. The data represent the mean ± SEM of a single experiment. The protocol was repeated three times.

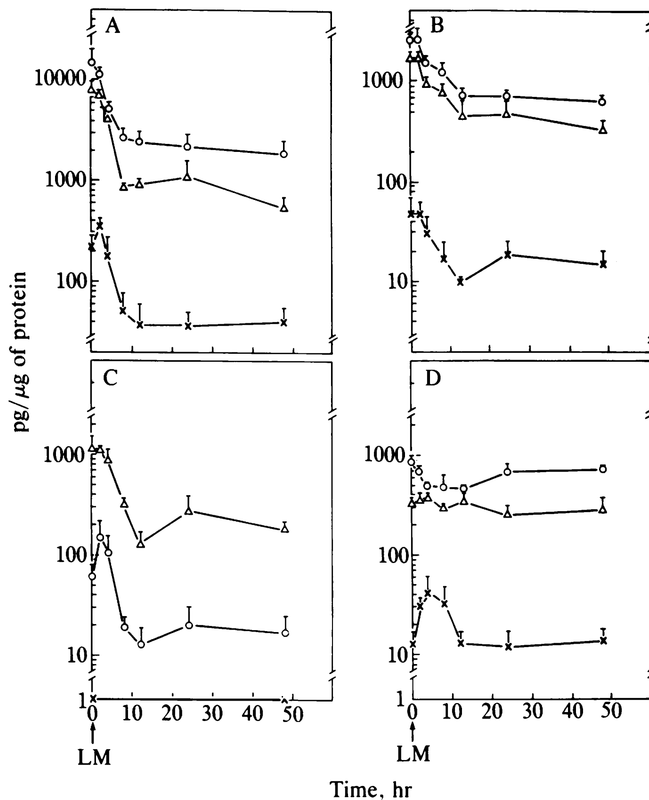

FIG. 2. Changes in arachidonic acid metabolism by peritoneal macrophages after *Listeria* infection *in vivo*. Mice were injected with 10^5 live *Listeria* (LM) at time 0, and the PEC were isolated at various times after the infection. The macrophages were then incubated *in vitro* in the absence (x) and presence of zymosan at 300 μg/ml (△) or 10 μM arachidonic acid (○). The productions of 6-keto-$PGF_{1\alpha}$ (*A*), PGE_2 (*B*), LTC_4 (*C*), and TXB_2 (*D*) were measured by RIA. The data represent the mean ± SEM of three separate experiments.

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. 1*A*). 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. 1*B*). 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.

The migration of cells from the blood into 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 arachidonic acid metabolism (Table 2). However, irradiating the animals

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

In vivo treatment	PEC, no. × 10^{-6}	Agonist	Metabolite, pg/μg of protein			
			$6kPGF_{1\alpha}$	PGE_2	TXB_2	LTC_4
None	1.9–2.0	None	110 ± 10	42 ± 6	10 ± 4	5 ± 1
		Zymosan	6641 ± 2036	1313 ± 198	807 ± 228	1418 ± 521
		Arachidonic acid	14709 ± 4295	4112 ± 1699	1272 ± 380	128 ± 43
Irradiation	1.2–1.3	None	467 ± 111	148 ± 5	91 ± 40	11 ± 4
		Zymosan	11628 ± 3354	1797 ± 662	1205 ± 708	3072 ± 610
		Arachidonic acid	17978 ± 4198	8582 ± 1087	1300 ± 666	125 ± 15
Listeria	5.8–6.8	None	40 ± 25	16 ± 3	10 ± 3	3 ± 1
		Zymosan	268 ± 92	222 ± 32	566 ± 303	67 ± 13
		Arachidonic acid	248 ± 63	887 ± 150	929 ± 231	7 ± 3
Listeria/irradiation	0.7–0.8	None	345 ± 141	78 ± 14	18 ± 8	7 ± 1
		Zymosan	7147 ± 311	1544 ± 387	817 ± 464	823 ± 134
		Arachidonic acid	8075 ± 3366	6529 ± 1154	794 ± 224	45 ± 3

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. 3*A*). The selective conservation of macrophage TXA_2 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 PGI_2, PGE_2, and LTC_4 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 PGI_2 and PGE_2, 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* injection 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 TXA_2 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 PGI_2 and PGE_2 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. 3*A*): (*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 PGI_2 and PGE_2 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.

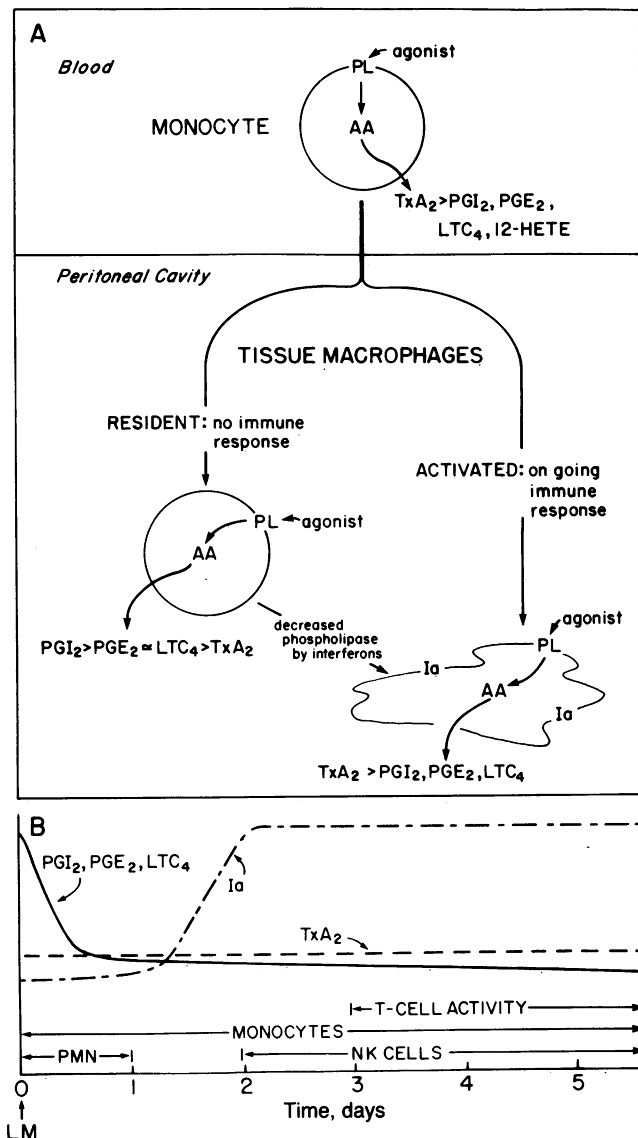

FIG. 3. A hypothetical model of the mechanism for explaining the changes in arachidonic acid metabolism during an immune response. (*A*) During an immune response, blood monocytes, having their own distinct arachidonate metabolism, migrate to the site of infection and rapidly become activated. The resident cells may also become activated and shift their arachidonate profile in response to interferons generated during the immune response. (*B*) Cell types other than the lymphocytes may also regulate the activation of macrophages during an immune response.

Finally, the stimulus for Ia induction at 48 hr after *Listeria* infection precedes any measurable T-cell activity (Fig. 3*B*). 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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