

Absence of T Lymphocyte-Derived Cytokines Fails to Diminish Macrophage 12/15-Lipoxygenase Expression In Vivo¹

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IL-4 and IL-13 are the only known activators of 15-lipoxygenase (LO) expression in cultured macrophages. To determine whether these lymphocyte-derived cytokines regulate 15-LO expression in vivo, the abundance of the murine homologue (12/15-LO) was assessed in peritoneal macrophages from immune-deficient strains of mice. Macrophages were harvested from recombinase activator gene (RAG)-2^{-/-} mice that do not develop mature lymphocytes and cannot secrete activation-dependent cytokines. Unexpectedly, 12/15-LO protein and activity were significantly increased in peritoneal macrophages from RAG-2^{-/-} mice compared with strain-matched controls. This increase was related to phenotypic differences between cells from RAG-2^{+/+} and RAG-2^{-/-} mice. After 3 h in culture, RAG-2^{+/+} macrophages were of two distinct sizes, with only the larger cells immunostaining for 12/15-LO. However, all RAG-2^{-/-} cells were distributed in the large size range, and all were immunoreactive for the enzyme. The activation of 12/15-LO expression appears to be related to prolonged residence within the peritoneum, since there were fewer resident peritoneal macrophages in RAG-2^{-/-} than in RAG-2^{+/+} mice, and newly recruited macrophages elicited by the administration of Sephacryl to RAG-2^{-/-} mice did not immunostain for 12/15-LO. To determine whether 12/15-LO expression was due to IL-4 or IL-13 from nonlymphoid cells, the abundance of the enzyme was quantified in peritoneal macrophages from STAT6^{-/-} mice that have attenuated responses to both cytokines. STAT6 deficiency did not influence the abundance of the protein in macrophages. Therefore, neither IL-4 nor IL-13 secretion is a requirement for macrophage 15-LO expression in vivo. *The Journal of Immunology*, 1998, 161: 1477–1482.

The enzyme 15-lipoxygenase (LO)³ has been implicated in a wide variety of disease states including atherosclerosis, asthma, and psoriasis (1), and has been shown to generate an extensive variety of products that may be implicated in these disease processes (2). The 15-LO within macrophages is thought to contribute most to pathogenesis in a number of disease states, but reticulocytes and airway epithelia are also major sources of this enzyme. A homologous enzyme has been identified in the mouse that inserts molecular oxygen predominantly into carbon 12 of arachidonic acid; this enzyme is termed 12/15-LO (3, 4).

12/15-LO expression in cultured monocyte-derived macrophages (MDMs) is regulated by the presence of exogenously applied cytokines. Cultured human MDMs do not express 15-LO unless they are incubated with IL-4 or IL-13 (5). Both of these cytokines are released from the Th2 subtype of CD4⁺ helper cells (6). IL-4- and IL-13-stimulated 15-LO expression in cultured

MDMs is inhibited by IFN- γ that is secreted from Th1 cells (7, 8). Unlike human MDMs, mouse peritoneal macrophages express considerable 12/15-LO activity at the time of their isolation. The expression of the enzyme in mouse peritoneal macrophages is not altered in mice lacking IL-4; this lack of alteration is possibly due to redundancy in the cytokine system, since the same effects are observed with IL-13 (5). IFN- γ does not have a profound effect on the 12/15-LO activity that is present upon isolation in mouse peritoneal macrophages, indicating that it probably exerts its inhibitory effects through a transcriptional effect (5, 9). While 12/15-LO activity has been detected in mouse peritoneal macrophages at the time of isolation, the enzyme is not expressed uniformly in every cell (10).

Since it has been hypothesized that IL-4 and IL-13 released from activated lymphocytes regulate the expression of 12/15-LO, we hypothesized that the total absence of mature lymphocytes would influence the activity of this enzyme. To address this hypothesis, we quantified 12/15-LO expression in mice with specific immune deficiencies. One strain used was recombinase activator gene (RAG)-2^{-/-} mice that are incapable of V(D)J recombination, leading to a total deficiency of mature T and B lymphocytes (11). This immune deficiency also leads to an absence of the TCR and ablates lymphocyte activation and its associated cytokine secretion. To determine whether 12/15-LO originated from nonlymphoid sources of IL-4 or IL-13, STAT6^{-/-} mice that do not respond to either cytokine were used. These data demonstrate that IL-4 and IL-13 secretion is not a requirement for macrophage 12/15-LO synthesis in vivo.

Materials and Methods

Animals

Strain-matched, 5- to 7-wk-old RAG-2^{+/+} and RAG-2^{-/-} mice were purchased from Taconic (Germantown, NY); STAT6^{-/-} mice and strain-matched controls have been described previously (12). All mice were fed a standard laboratory diet and given water ad libitum.

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³ Abbreviations used in this paper: LO, lipoxygenase; MDM, monocyte-derived macrophage; RAG, recombinase activator gene; wt, wild-type; 13-HODE, 13-hydroxyoctadecadienoate.

Isolation of resident peritoneal macrophages

Mice were anesthetized with metaphane and killed via cervical dislocation. Macrophages were harvested via peritoneal lavage using saline (5 ml). Cells were resuspended in DMEM (catalog no. 10-013-LV; Mediatech, Washington, DC) containing heat-inactivated FBS (10% v/v), penicillin (10 U/ml), streptomycin (10 μ g/ml), and fungizone (25 ng/ml), and plated into 6-well plates (Corning Costar, Cambridge, MA). Cells were incubated at 37°C with 5% CO₂, 95% air for the times indicated. After incubation, media was removed, and cells were prepared for protein characterization or immunocytochemistry. For protein characterization, cells were washed twice with cold PBS and scraped into PBS (1 ml); aliquots were recovered for cell counting and for the determination of protein concentration (13). The remaining cells used for the assessment of enzyme activity were centrifuged at 13,000 \times g for 30 s at room temperature; supernatants were subsequently removed, and cell pellets were frozen in liquid nitrogen and stored at -80°C.

The macrophages used for immunocytochemistry were isolated as described above and plated at an equivalent density onto Lab-Tek 8-chamber glass slides (Nunc, Naperville, IL). After incubation and washing, cells were fixed with paraformaldehyde (4% w/v) and permeabilized with ethanol at 4°C.

Western blot analyses

Total cell proteins from mouse peritoneal macrophages were resolved under reducing conditions on 10% SDS polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The immunoreactivity of the proteins on the membranes was assessed using sheep antiserum to rabbit reticulocyte 15-LO, which recognizes the murine homologue 12/15-LO, as described previously (5). Immunoblots were simultaneously incubated with a mAb to β -actin (Sigma, St. Louis, MO). Horseradish peroxidase-conjugated Abs to sheep and mouse IgG were used for detection in combination with chemiluminescent reagents according to the manufacturer's instructions (enhanced chemiluminescence, Amersham, Arlington Heights, IL). The intensity of the immunoreactive band for 12/15-LO was compared with that for β -actin within each sample by image analysis (SigmaGel, Jandel Scientific, San Rafael, CA).

Measurement of 12/15-LO products

The activity of 12/15-LO in cells was assessed as described previously (5). Briefly, cell pellets were sonicated in PBS containing 0.2% sodium cholate. Aliquots (100 μ l) of the sonicated cell suspension were incubated with 360 μ M of linoleic acid that was dissolved in a small volume of ethanol. The reaction was allowed to proceed on ice for 10 min and was stopped by the addition of an equal volume of mobile phase (acetonitrile/water/methanol/acetic acid; 350:250:150:1). The hydroperoxides produced were reduced to their corresponding hydroxy acids by the addition of trimethyl-phosphite. Products were identified and quantified by reverse phase HPLC against known standards. Additional studies have demonstrated that 13-hydroxyoctadecadienoate (13-HODE) measured by this method is of the (S) configuration and therefore accurately measures enzymatic activity.

Elicitation of peritoneal macrophages and incubation with IL-4

Macrophages were elicited by i.p. injection of a suspension of Sephacryl beads (1 ml, 2% w/v; Pharmacia, Piscataway, NJ) or thioglycolate (1 ml, 3% w/v; Sigma). At 4 days after the administration of Sephacryl or thioglycolate, macrophages were isolated by peritoneal lavage and cultured onto glass slides for 3 h as described above. Some cells from Sephacryl-treated and nontreated mice were washed and cultured for 2 days with fresh media containing murine rIL-4 (10 ng/ml; R&D Systems, Minneapolis, MN).

Immunocytochemistry and cell size analyses

Immunocytochemistry was performed using Vector avidin-biotin complex Elite kits (Vector Laboratories, Burlingame, CA) as described previously (14). Immunoreactivity was visualized using the red chromagen, aminoethyl carbazole (Biomed, Foster City, CA). Macrophages were detected using rabbit antiserum to murine macrophages (Accurate, Westbury, NY) and 12/15-LO with sheep antiserum to rabbit reticulocyte 15-LO. The immune serum was compared with the appropriate nonimmune serum in each case. Cell size was calculated with ImagePro (Media Cybernetics, Silver Spring, MD) software using images of cells that had been immunostained with the macrophage antiserum.

Statistical analyses

Analyses of data for the determination of statistical significance were performed with SigmaStat (Jandel Scientific). Data that met appropriate cri-

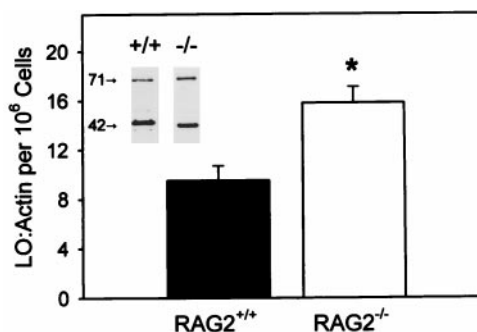


FIGURE 1. 12/15-LO protein mass. Western blotting was performed on peritoneal macrophage protein from RAG-2^{+/+} and RAG-2^{-/-} mice. Abundance of 12/15-LO was assessed by immunoreactivity with sheep antiserum to 15-LO and a mAb to β -actin simultaneously. Immunoreactive bands for 12/15-LO and β -actin were observed in each sample at 71 and 42 kDa, respectively, corresponding to the molecular mass of these proteins (inset). The relative intensity of 12/15-LO bands was compared with that for β -actin within each sample by image analysis. Macrophages from RAG-2^{-/-} mice (\square) had a significantly higher cellular ratio of 12/15-LO than cells from RAG-2^{+/+} mice (\blacksquare). Histograms represent the mean of five observations per group, and bars represent the SEM (* p < 0.006).

teria of equivalent variances and normal distribution were analyzed with the Student *t* test (two-tailed), while those that did not were analyzed by the Mann-Whitney rank-sum test. Data are expressed as mean \pm SEM.

Results

12/15-LO protein mass and activity is increased in macrophages from RAG-2^{-/-} mice

The mass of 12/15-LO and β -actin protein in individual peritoneal macrophage preparations from RAG-2^{-/-} and RAG-2^{+/+} mice was determined by Western blotting. 12/15-LO and β -actin were detected in each sample by the simultaneous use of sheep antiserum to rabbit reticulocyte 15-LO and a mAb to β -actin. In each of five mice per group, 12/15-LO and β -actin were detected at ~71 and ~42 kDa, respectively, which was consistent with the expected molecular mass of these proteins (Fig. 1, inset). The intensity of the 12/15-LO band was compared with that of β -actin within each sample using image analysis. Peritoneal macrophage populations from RAG-2^{-/-} mice had a significantly higher 12/15-LO/ β -actin ratio per cell than those from RAG-2^{+/+} mice (p < 0.006; Fig. 1).

The enzymatic activity of 12/15-LO was assessed by the formation of 13-HODE when cell homogenates were incubated with linoleic acid. Consistent with the increased protein mass, macrophage populations from RAG-2^{-/-} mice showed significantly more activity per cell than those from RAG-2^{+/+} mice (p < 0.02; Fig. 2). A previous analysis of product chirality from this assay revealed a predominance of the S isomer, which is consistent with enzymatic activity.

12/15-LO protein distribution in peritoneal macrophage populations differs in RAG-2^{+/+} and RAG-2^{-/-} mice

To visualize the distribution of 12/15-LO protein in peritoneal macrophage populations, immunocytochemical tests were performed on cultured cells from RAG-2^{+/+} and RAG-2^{-/-} mice. Resident cells were harvested from the peritoneal cavity and cultured for 3 h. Although all adherent cells from RAG-2^{+/+} mice immunostained as macrophages (Fig. 3A), only the larger population exhibited positive 12/15-LO immunostaining (Fig. 3B). The smaller population was defined as macrophages based on immunostaining with antisera that did not react with the

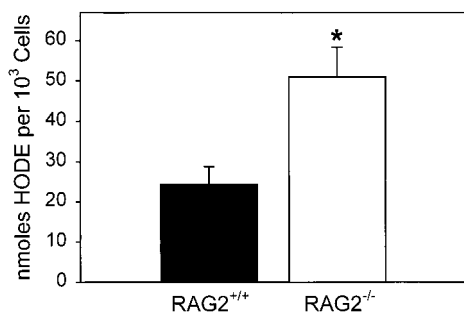


FIGURE 2. 12/15-LO enzyme activity. Peritoneal macrophage preparations were incubated with linoleic acid, and the formation of 13-HODE was quantified by reverse phase HPLC. Macrophages from RAG-2^{-/-} mice (□, *n* = 14) produced significantly more 13-HODE per cell than those from RAG-2^{+/+} mice (■, *n* = 10). Histobars represent the means of all observations, and bars denote the SEM (**p* < 0.02).

other cell types present in the peritoneal exudate. Furthermore, with increased duration of culture, these cells exhibited a spreading pattern that is consistently seen with macrophages. In contrast to the results obtained from RAG-2^{+/+} mice, all macrophages from RAG-2^{-/-} mice (Fig. 3*D*) immunostained for 12/15-LO (Fig. 3*E*). Equivalent dilutions of nonimmune serum were not immunoreactive (Fig. 3, *C* and *F*). Furthermore, upon visualization of immunostained cells, it became obvious that the pattern of 12/15-LO immunostaining in resident peritoneal macrophages corresponded to cell size. Macrophages from RAG-2^{+/+} mice that did not immunostain for 12/15-LO were smaller than those that were immunoreactive for this protein.

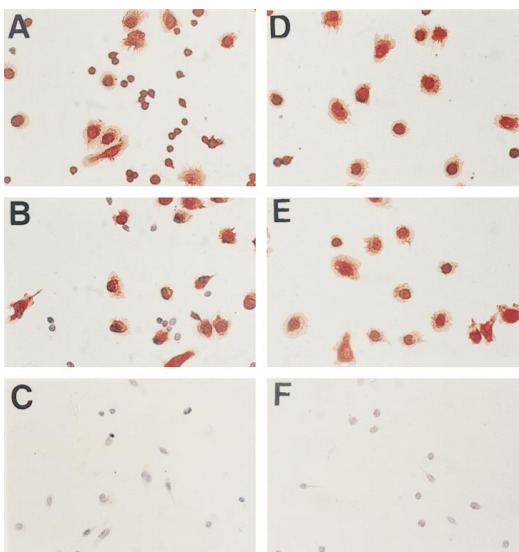


FIGURE 3. Distribution of 12/15-LO protein in peritoneal macrophage populations. Nonelicited cells were cultured for 3 h, fixed with paraformaldehyde, permeabilized, and immunostained. Immunoreactive cells were visualized with a red chromagen, and nuclei were counterstained with hematoxylin. Peritoneal macrophages from RAG-2^{+/+} mice are represented in *A–C*; cells from RAG-2^{-/-} mice are shown in *D–F*. Immunostaining using a rabbit antiserum indicated that all adherent cells from both strains were macrophages (*A* and *D*). The detection of 12/15-LO using sheep antiserum (*B* and *E*) was compared with the results of staining with nonimmune serum (*C* and *F*). Cells were photographed under $\times 400$ magnification.

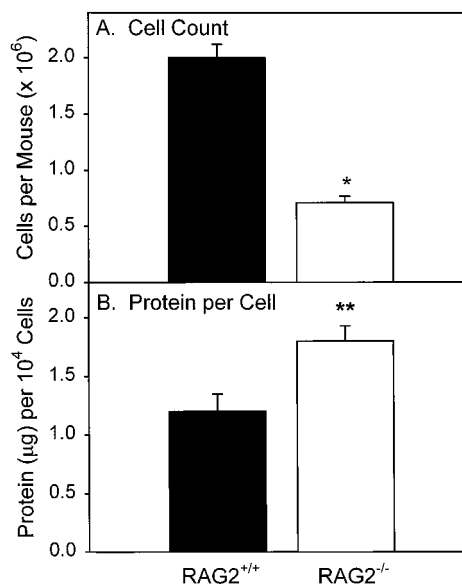


FIGURE 4. Numbers of peritoneal macrophages harvested and cellular protein content. The total number of isolated macrophages and the cellular protein content from individual mice of each strain were examined. *A* shows that RAG-2^{-/-} mice had significantly fewer resident peritoneal macrophages than did RAG-2^{+/+} mice (**p* < 0.001). Cellular protein mass was significantly higher in RAG-2^{-/-} mice (*B*) (***p* < 0.02). Closed histobars represent the mean of 10 observations for RAG-2^{+/+} mice, open histobars represent the mean of 9 observations for RAG-2^{-/-} mice, and bars denote the SEM.

Phenotypes of resident peritoneal macrophages differ in RAG-2^{+/+} and RAG-2^{-/-} mice

Morphologic differences that corresponded to the pattern of 12/15-LO immunostaining were observed between peritoneal macrophages from the two strains of mice. Macrophages from each strain were characterized to determine whether heterogeneity of 12/15-LO expression in RAG-2^{+/+} mice was related to the maturation state of resident cells.

RAG-2^{-/-} mice had significantly fewer resident peritoneal macrophages than RAG-2^{+/+} mice (*p* < 0.001; Fig. 4*A*). Furthermore, macrophages harvested from RAG-2^{-/-} mice had more total protein per cell than those from RAG-2^{+/+} mice (*p* < 0.02; Fig. 4*B*).

As noted above, a microscopic visual inspection of cells after 3 h in culture revealed a difference in the extent of cell spreading. Macrophages from RAG-2^{-/-} mice were distributed within a single size distribution with an area range of 140 to 260 μm^2 , whereas macrophages from RAG-2^{+/+} mice were distributed within two area ranges corresponding to <100 μm^2 and 140 to 260 μm^2 (Fig. 5). The larger cells in both RAG-2^{-/-} and RAG-2^{+/+} expressed 12/15-LO extensively; immunostaining was absent in the smaller cells.

12/15-LO is not expressed in newly recruited peritoneal macrophages from RAG-2^{-/-} mice

To determine whether 12/15-LO synthesis occurred immediately upon entry into the peritoneum, an i.p. injection of Sephacryl beads or thioglycolate was used to elicit macrophages in RAG-2^{-/-} mice. These agents recruit macrophages that exhibit mild activation compared with that exhibited by resident cells (15). Sephacryl increased the number of resident peritoneal macrophages by $289 \pm 44\%$, while thioglycolate increased this number by $113 \pm 4\%$ in RAG-2^{-/-} mice. In RAG-2^{-/-} mice, an injection of Sephacryl

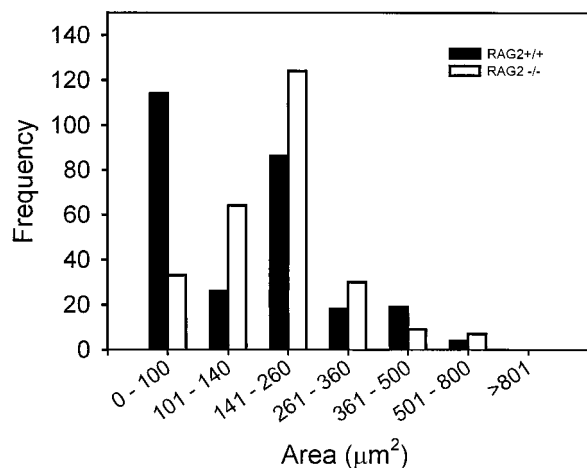


FIGURE 5. Quantification of macrophage cell size after 3 h in culture. Macrophages from each strain were cultured for 3 h and immunostained using rabbit antiserum; cell size was determined by image analysis. After 3 h in culture, RAG-2^{-/-} macrophages were all distributed within a size range of 140 to 260 μm², whereas RAG-2^{+/+} cells were distributed within two distinct ranges of <100 μm² and 140 to 260 μm². RAG-2^{+/+} data are represented by closed histograms, and RAG-2^{-/-} data are represented by open histograms (>300 cells per mouse; four mice in each group).

resulted in the recruitment of a considerable number of macrophages to the peritoneum that did not express 12/15-LO, in contrast with the uniform expression observed in resident cells (Fig. 6). Size analysis showed a uniform distribution of cells within an area range of 140 to 360 μm² for both nonelicited and Sepsacryl-elicited cells from RAG-2^{-/-} mice (Fig. 7). Similar results for immunostaining and size distribution were obtained after an injection of thioglycolate (data not shown), indicating that 12/15-LO

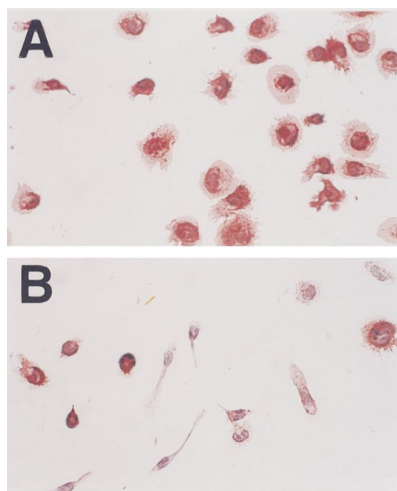


FIGURE 6. Expression of 12/15-LO in Sepsacryl-elicited macrophages. RAG-2^{-/-} mice were injected i.p. with Sepsacryl beads; peritoneal macrophages were harvested after 4 days, and cells were immunostained after 3 h in culture. Immunoreactive cells were visualized with a red chromagen, and nuclei were counterstained with hematoxylin. The administration of Sepsacryl to RAG-2^{-/-} mice increased the number of peritoneal macrophages isolated by 289 ± 44% over those isolated from nontreated mice. All macrophages from nontreated RAG-2^{-/-} mice were immunoreactive for 12/15-LO (A). However, the administration of Sepsacryl also recruited a population of macrophages to the peritoneum in RAG-2^{-/-} mice that did not express 12/15-LO (B).

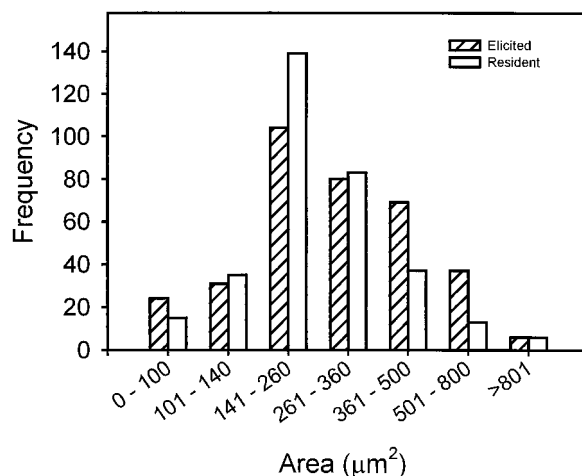


FIGURE 7. Quantification of cell size after Sepsacryl administration to RAG-2^{-/-} mice. Cell size analysis showed a uniform distribution of cells within the size range of 140 to 360 μm² for both untreated (cross-hatched histograms) and Sepsacryl-elicited macrophages from RAG-2^{-/-} mice (□). (>300 cells per mouse; four mice in each group).

synthesis is stimulated only after prolonged residence in the peritoneal cavity.

To determine whether the newly recruited cells were responsive to the lymphocyte-derived cytokines that increased 12/15-LO expression, IL-4 was incubated with macrophages from Sepsacryl-treated RAG-2^{-/-} mice. IL-4 greatly increased the number of newly recruited macrophages that immunostained for 12/15-LO (data not shown) compared with immunostaining in equivalent cell populations that were not incubated with the cytokine.

12/15-LO protein mass is unaltered in mice that are unable to respond to IL-4 or IL-13

There was no statistically significant difference in the abundance of 12/15-LO in peritoneal macrophages from STAT6^{-/-} and STAT6^{+/+} mice according to the relative content of 12/15-LO and β-actin as determined by Western blotting (Fig. 8). The morphology of peritoneal macrophages from STAT6^{-/-} was the same as that of the wild-type (wt) strain match control with two discernible

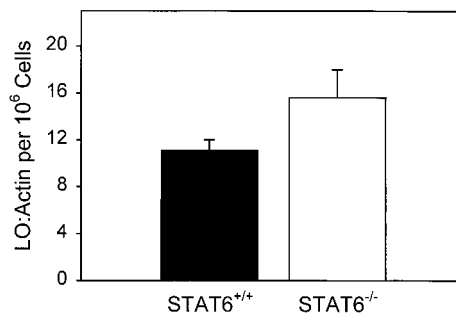


FIGURE 8. 12/15-LO: β-actin protein mass in mice that are unable to respond to either IL-4 or IL-13. Western blotting was performed on peritoneal macrophage protein from STAT6^{+/+} and STAT6^{-/-} mice. An abundance of 12/15-LO was assessed by immunoreactivity with sheep antiserum to 15-LO and a mAb to β-actin. The relative intensities of the 12/15-LO bands were compared with those for β-actin within each sample by image analysis. Macrophages from STAT6^{-/-} mice (□) had a higher cellular ratio of 12/15-LO than did STAT6^{+/+} cells (■), although this difference was not statistically significant. Histograms represent the mean of five observations per group, and bars represent the SEM.

populations. As described above, only the large population of cells immunostained for the presence of 12/15-LO.

Discussion

The presence of the lymphocyte-derived cytokines IFN- γ , IL-4, and IL-13 has a profound effect on the expression of 12/15-LO in cultured MDMs (5, 7, 8). Therefore, we hypothesized that the total absence of lymphocytes in vivo would abolish both stimulatory and inhibitory effects, with a net result of markedly decreased 12/15-LO expression in mouse peritoneal macrophages. However, contrary to our expectations, there was a significant increase in both the mass and activity of 12/15-LO in peritoneal macrophages derived from RAG-2^{-/-} mice compared with the mass and activity seen in cells from strain-matched, wt controls. This increase was due to the uniform expression of 12/15-LO in all macrophages from RAG-2^{-/-} mice. In contrast, there was a subpopulation of macrophages recruited from strain-matched RAG-2^{+/+} mice that did not express the enzyme. Furthermore, there was no attenuation of 12/15-LO abundance in STAT6^{-/-} mice that were unable to respond to IL-4 and IL-13.

Although IL-4 was the first cytokine shown to increase 15-LO activity in cultured macrophages, we have demonstrated previously that the expression of the enzyme in vivo is not influenced by a deficiency of IL-4 (5). The subsequent finding that IL-13 mimics the effect of IL-4 on 15-LO expression in cultured macrophages may account for the lack of effect of IL-4 deficiency in vivo. Lymphocytes from RAG-2^{-/-} mice cannot be activated to secrete IL-4 or IL-13. Therefore, the finding that 12/15-LO was present in peritoneal macrophages from RAG-2^{-/-} mice may be indicative of other as yet undefined activators of synthesis. Alternatively, the stimulation of 12/15-LO synthesis may be restricted to IL-4 and IL-13, but these cytokines may originate from nonlymphoid sources. The most likely nonlymphocyte source of IL-4 and IL-13 is mast cells, which are present in the peritoneum. However, the enzyme is still abundant in peritoneal macrophages from STAT6^{-/-} mice, which are unresponsive to both IL-4 and IL-13 due to an ablation of the common signaling pathway (12).

A possible cause of the increase in 12/15-LO expression in peritoneal macrophages from RAG-2^{-/-} mice could be the lack of the inhibitory effect of IFN- γ (7). Indeed, even in T and B lymphocyte-deficient animals, there can be abundant IFN- γ secreted from NK cells (16). However, an effect of IFN- γ is unlikely, since STAT6^{-/-} mice have no known defect with regard to the secretion of this cytokine; however, the strain-matched wt control had an equivalent abundance of 12/15-LO.

A surprising result was the marked difference in the macrophage phenotypes observed in RAG-2^{-/-} and RAG-2^{+/+} mice. Incubating cultured macrophages with exogenously applied lymphocyte-derived cytokines has profound effects on the properties of these cells. Since there are no known effects of RAG-2 deficiency that would directly result in changes in macrophage behavior, it is assumed that the changes in morphology were the result of an influence of lymphocytes. There is as yet no obvious mechanism by which lymphocytes affect macrophage morphology in the peritoneum of noninfected mice.

Although fewer macrophages were harvested from RAG-2^{-/-} than from RAG-2^{+/+} mice, these cells adhered and spread rapidly and relatively uniformly. In contrast, macrophages from wt mice were heterogeneous in their spreading characteristics at 3 h after plating. Changes in macrophage phenotype could be due to changes in recruitment, activation, or residence time. Fewer resident macrophages in the peritoneum from RAG-2^{-/-}

mice, the uniform spreading, and the expression of 12/15-LO in every cell are consistent with a decreased recruitment of cells to the peritoneum of this strain. Unfortunately, all of the common modes of stimulating macrophage recruitment, including the administration of thioglycolate and Saphacryl, result in some degree of activation. Both of these agents greatly increased the number of peritoneal macrophages harvested. Unlike resident cells from RAG-2^{+/+} mice, macrophage populations from Saphacryl-injected RAG-2^{-/-} mice spread on culture dishes to an extent that was similar to that seen with cells from untreated mice. However, Saphacryl converted the expression of 12/15-LO in RAG-2^{-/-} mice to a heterogeneous pattern, as seen in the wt mice. These data indicate that a deficiency of RAG-2 influences recruitment to the peritoneum and consequently the pattern of 12/15-LO expression.

A more precise definition of 12/15-LO regulation in macrophages may provide information regarding the mechanisms of several disease processes. At present, the local release of lymphocyte-derived cytokines is the only proposed mode of modulation of 12/15-LO expression in macrophages. For example, atherosclerosis is associated with a marked heterogeneity in the expression of 15-LO within lesion macrophages from both experimental animals and humans (17, 18). Atherosclerotic lesions contain many activated T lymphocytes, which may synthesize cytokines intramurally to modulate 15-LO expression (19). Although the enzyme is not expressed in all lesion macrophages, the recent demonstration of the pronounced decrease in the extent of atherosclerosis after the administration of a novel specific inhibitor of 15-LO is consistent with the importance of the enzyme to the disease process (20).

In summary, we have demonstrated that in two mouse strains, one that does not secrete IL-4 or IL-13 from lymphocytes and one that fails to respond to either of these cytokines, there is no decrease in the abundance of macrophage 12/15-LO as predicted from cell culture studies. However, the absence of 12/15-LO expression in newly recruited peritoneal macrophages and its presence in resident cells demonstrates that an unidentified factor that is unrelated to T lymphocytes has the ability to stimulate the synthesis of this enzyme.

Acknowledgments

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