ABERRANT PRODUCTION OF LEUKOTRIENE C₄ BY MACROPHAGES FROM AUTOIMMUNE-PRONE MICE

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Eicosanoids have been implicated in the pathogenesis of autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The evidence linking eicosanoids and rheumatic diseases is primarily based upon the concept that several eicosanoids that are potent mediators of inflammation can also modify immune function and that SLE and RA are inflammatory diseases associated with altered immune responses. The specific contribution any individual lipoxygenase or cyclooxygenase product has in predisposing to lupus remains to be established. Although prostaglandins (PGs) may promote inflammation by virtue of their capacity to induce edema, erythema, and hyperalgesia (1), certain prostanooids appear to protect against the development of autoimmune disease. Parenteral administration of prostaglandin E₁ (PGE₁) prevents the expression of lupus in autoimmune-prone New Zealand (2) and MRL-lpr/lpr (3) mice. In contrast, leukotrienes (LT) may have a permissive effect on the development of autoimmunity (reviewed in reference 4). Oxygenation of arachidonic acid by a 5-lipoxygenase yields 5-hydroperoxyeicosatetraenoic acid, which can be further metabolized to leukotriene A₄ (LTA₄). In murine peritoneal macrophages (Mφ), stimulation with zymosan preferentially converts LTA₄ into the sulfidopeptide LTC₄ (5). Peptidolysis of LTC₄ sequentially produces LTD₄ and LTE₄, which collectively comprise the slow reacting substance of anaphylaxis (SRS-A). The SRS-A are bronchoconstrictors, stimulate mucous production in the trachea, increase vascular permeability, and induce the release of lysosomal enzymes (reviewed in reference 6). More recently, LTC₄, and/or its metabolites, have been reported to exert potent effects on the murine system, inducing the synthesis of IFN-γ (7) and inhibiting the proliferation of T cells (8).

Leukotriene synthesis in the setting of autoimmunity has not been previously investigated and is the focus of this study. Mice that spontaneously manifest lupus-like illnesses were chosen as the experimental model (reviewed in references 9, 10).

Materials and Methods

Mice. Mice were purchased from the Jackson Laboratory, Bar Harbor, ME. MRL-lpr/lpr, MRL-+/+, and C57BL/6-lpr/lpr mice were bred in the Animal Research Facility at the Denver Veterans Administration Medical Center, 1055 Clermont Street, Denver, CO 80220.

This work was supported by grants from the Veterans Administration, the Rocky Mountain Chapter of the Arthritis Foundation, and the National Institutes of Health (AI-26284, HL-25785, and AA-07157). T. J. Santoro is a recipient of a FIRST Award from the National Institutes of Health. Address correspondence to T. J. Santoro, Rheumatology Section (IIG), Veterans Administration Medical Center, 1055 Clermont Street, Denver, CO 80220.
Veterans Administration Medical Center. MRL-lpr/lpr mice exhibit an accelerated and severe disease that has features of both SLE and RA (10). Congenic MRL-+/+ mice, which lack the lpr gene but possess an autoimmune background (9, 10), and C57BL/6-lpr/lpr mice, which possess the lpr gene on a nonautoimmune background, both develop an indolent type of lupus without an arthritic component.

In Vitro Culture. \(2 \times 10^5\) resident peritoneal MØ were dispensed in 96-well flat-bottomed plates (Costar, Cambridge, MA) in Eagle's Basal Medium (BME) (Gibco Laboratories Chagrin Falls, OH) plus 10% FCS and incubated at 37°C in a humidified atmosphere containing 5% CO2 for 2 h. The cells were then washed and cultured for 2-24 h in BME with 0-150 \(\mu\)g/ml zymosan (Sigma Chemical Co., St. Louis, MO). The supernatants were harvested and analyzed freshly for eicosanoid activity using enzyme immunoassays (II) with tracers purchased from AIA, Inc. (Aurora, CO) or dialyzed and examined for IL-1 activity, as previously described (12). After adherence, 80-92% of cells were MØ as evaluated by nonspecific esterase staining. There were no significant differences in the percentage of MØ obtained from autoimmune vs. normal mice. In experiments using HPLC, \(10^6\) MØ were dispensed in 35-mm petri dishes in 2 ml BME and cultured for 2 h with 150 \(\mu\)g/ml of zymosan at 37°C in 5% CO2.

HPLC Analyses. Supernatants from freshly prepared samples were spiked with \([3H]LTC_4\) (New England Nuclear, Boston, MA) (16,000 dpm, 120 pg) and \([3H]LTD_4\) (15,000 dpm, 90 pg) to determine recovery and with PGB2 (Cayman Chemicals, Ann Arbor, MI) (500 ng) to provide a reference point for chromatographic analysis. HPLC analysis was performed using a liquid chromatograph (model 1090; Hewlett-Packard Co., Palo Alto, CA). The gradient was run at a flow rate of 1 ml/min with the initial mobile phase being methanol/water/phosphoric acid (30:70:0.02, vol/vol, pH 5.7, with ammonium hydroxide) for 6 min, followed by a linear gradient to 100% methanol over 44 min. The overall recovery of radioactivity was 81 ± 3% for \([3H]LTC_4\) and 74 ± 3% for \([3H]LTD_4\) in these samples.

Results

The profile of lipoxygenase metabolites produced by zymosan-stimulated peritoneal MØ from 16-wk-old autoimmune-prone and immunologically normal mice was initially investigated using reverse-phase HPLC and a gradient-mobile phase. The chromatogram obtained in zymosan-induced MØ from normal C57BL/6 +/+ mice (Fig. 1 A) shows a peak that elutes with a retention time identical to that of authentic LTC4 (Fig. 1, peak 1) and with a UV spectrum (Fig. 1, insert) characteristic of a leukotriene. Further analysis of this peak by enzyme immunoassay (EIA) (Fig. 1 A histogram) confirmed the presence of LTC4. The second peak represents the 11-trans isomer of LTC4 (Fig. 1 A). PGB2, the internal standard (peak 3) elutes at 30.3 min. LTB4 was not detectable in the MØ supernatants. Similar profiles were observed in zymosan-activated MØ from autoimmune C57BL/6-lpr/lpr, MRL-+/+, and MRL-lpr/lpr mice (Fig. 1, B-D, respectively) and from immunologically normal C3H/HeN mice (data not shown). Thus, MØ obtained from autoimmune and normal mice demonstrate qualitatively comparable lipoxygenase products on stimulation with zymosan, and in all cases the predominant leukotriene synthesized is LTC4.

The capacity of MØ from autoimmune-prone MRL mice and immunologically normal C3H/HeN mice of various ages to produce LTC4 was next investigated by directly measuring eicosanoid activity in the supernatants of zymosan-induced cultures using EIA. An age-associated increase in the ability of MRL-lpr/lpr MØ to produce LTC4 in response to zymosan was observed (Fig. 2). In MØ from MRL-+-/+ mice, no such enhancement was seen (Fig. 2), and, in response to zymosan, MØ from the latter strain produced levels of LTC4 that were comparable with those from C3H/HeN mice (not shown). Zymosan stimulation of MØ from 6- and 20-wk-old...
autoimmune C57BL/6-lpr/lpr mice produced levels of LTC₄ that were equivalent to those generated by Mφ from age-matched control C57BL/6-+/+ and BALB/c mice (data not shown).

It remained possible that increased LTC₄ production by Mφ from MRL-lpr/lpr mice was the consequence of enhanced responsiveness to zymosan. This was investigated by optimally stimulating Mφ (10⁶/ml) from young (3-5 wk) and old (12-20 wk) MRL mice with zymosan (150 µg/ml) for 24 h, then testing the dialyzed
supernatants for IL-1 activity. Comparable levels of IL-1 were produced by Mφ from MRL-+/+ mice (74 ± 5 U/ml) and MRL-lpr/lpr mice (80 ± 6 U/ml) at all ages tested. Similar results were obtained when both the total time of culture and the dose of zymosan were varied (data not shown).

In contrast to those from normal mice, a significant increase in spontaneous LTC4 production was observed in Mφ from MRL-lpr/lpr mice. A systematic survey of various autoimmune-prone strains revealed high spontaneous production of LTC4 to be a common feature of Mφ from mice that manifested lupus-like illnesses (Fig. 3). The augmented spontaneous LTC4 activity was found to increase further with age and was unrelated to either gender (not shown) or to MHC haplotype. Spontaneous LTC4 release was most marked in Mφ from MRL-lpr/lpr mice (Fig. 3). Measurements of PGE2 in Mφ cultures from 4- (not shown) and 16- (Fig. 3) wk-old autoimmune mice for up to 24 h revealed no differences in the spontaneous release of prostanoids relative to normal mice.

Discussion

The results presented herein demonstrate that Mφ from autoimmune-prone mice exhibit a novel aberration in arachidonic acid metabolism, producing levels of LTC4 that were up to 10 times greater than those from age-, sex-, and MHC-matched immunologically normal mice in the absence of deliberate addition of exogenous stimulants. Levels of LTC4 comparable with those spontaneously released by Mφ from MRL-lpr/lpr mice 8 wk of age or older (~10^{-8} M) have been shown in vitro to (a) stimulate DNA synthesis in human epidermal keratinocytes (13); (b) augment the proliferation of human cultured fibroblasts (14); (c) induce the mitogenesis of human glomerular epithelial cells (15); and (d) replace the helper cell requirement for immune IFN production by murine T cells (7). The age-associated increase in spontaneous LTC4 production was independent of sex and was shared by Mφ from MHC disparate autoimmune mice with distinct patterns of disease. The data suggest that augmented spontaneous LTC4 release may be a common feature of mu-
rine lupus. That Mo from certain strains (e.g., MRL-+/+) display enhanced spontaneous production of LTC₄ at a time when no overt manifestations of autoimmunity are present indicates that this aberration may be of etiopathogenetic significance. Mo from MRL-lpr/lpr mice, which manifest the most aggressive lupus-like illness of all strains tested, possessed the greatest capacity to produce LTC₄ both spontaneously and in response to zymosan stimulation. The relationship between disease severity and augmented LTC₄ production further indicates that the two phenomena may be pathogenetically linked. The mechanism by which spontaneous production of LTC₄ may predispose to the development of autoimmune disease is a matter of speculation. However, by increasing vascular permeability and inducing the release of lysosomal enzymes (4, 5), LTC₄ could contribute to the inflammation and tissue destruction characteristically seen in lupus.

Summary

Eicosanoids have been implicated in the pathogenesis of autoimmune diseases. In this study, peritoneal macrophages from autoimmune-prone mice were examined for their capacity to produce proinflammatory 5-lipoxygenase metabolites. The results indicate that enhanced production of leukotriene C₄ is a common feature of murine autoimmunity and suggest further that aberrations in 5-lipoxygenase activity may play a role in the development of lupus.

Received for publication 1 March 1988 and in revised form 16 May 1988.

References


