Leukotriene B4 is Measurable in Serum of Smokers and Nonsmokers

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Leukotriene B4 (LTB4), an arachidonic acid metabolite released by neutrophils and macrophages, helps regulate host immune response to antigenic stimulation. LTB4 affects the chemokinesis, aggregation, and enzyme release of neutrophils and stimulates activity of cytotoxic T cells, natural killer cells, and suppressor T cells. LTB4 also has a positive affect on the margination of monocytes and macrophages in the lung in response to inflammatory stimuli. Cigarette smoking represents an inflammatory stimulus in the lung and affects (decreases) the in vitro release of LTB4 by alveolar macrophages in comparison with that by alveolar macrophages of nonsmokers. By utilizing a sensitive and specific radioimmunoassay we have detected LTB4 concentrations in the serum of smokers to be nearly 60-fold greater than those in nonsmokers, 211 (SEM 35) vs 3.6 (SEM 1.5). Because systemic quantities of LTB4 are now measurable, quantification of this immune system regulator may be useful in evaluating inflammatory disease states.

Additional Keyphrases: immune response · inflammation · radioimmunoassay

Leukotriene B4 (LTB4) is a product of arachidonic acid metabolism via the 5-lipoxgenase (EC 1.13.11.12) pathway. Produced primarily by neutrophils and macrophages (1, 2), LTB4 affects a variety of immunologic factors involved in acute inflammation and cellular and humoral response to antigens. Release of LTB4 stimulates chemokinesis and chemotaxis of neutrophils, monocytes, and macrophages (3, 4) in addition to promoting leukocyte aggregation, adhesion, and enzyme release (4, 5). Cytotoxic T cell activity is also stimulated by LTB4 (6, 7), as is the induction of suppressor T cells (7, 8) and natural killer cells (9). These diverse effects of LTB4 on the immune system allow it to play a significant role as a regulatory substance in the host immune response.

LTB4 is generally thought to act at the local level; therefore, previous assessment of systemic concentrations has been limited (10, and correlation of various disease states with circulating LTB4 quantities is poorly documented. In examining serum samples from smokers and nonsmokers for measurable LTB4, we found that LTB4 could be quantified by a sensitive radioimmunoassay method. Additionally, we found a significant increase of serum LTB4 in smokers, as compared with nonsmokers.

Materials and Methods

Study population. The study population consisted of 10 nonsmoking volunteers and 10 smoking volunteers, ages 33–92 and 53–64 y, respectively. The nonsmokers had not smoked for at least 10 y: six had never smoked, two had smoked briefly in the 1950s, one had a 39-y history of no smoking, and one had not smoked for 12 y. The smokers had smoked for a mean ± SEM of 38 ± 6 pack-years (range, 15–67 pack-years).

Samples. Whole blood was collected by antecubital venipuncture into closed, sterile containers (Vacutainer Tubes; Becton Dickinson, Rutherford, NJ). Once clotting was complete, the samples were centrifuged at 1500–2000 × g at 4 °C for 10 min. Serum was removed, aliquoted into capped storage vials, and stored at −20 to −40 °C until extraction.

LTB4 extraction. Acidify 1 mL of serum to pH 3.5–4.0 by adding 50 μL of 2 mol/L HCl, then pass the samples through a C18 bonded-phase packing cartridge (Sep-Pak C18; Waters Associates, Milford, MA). The procedure is adapted from Powell (11): in brief, pretreat the cartridges with 2 mL of methanol followed by 5 mL of de-ionized water. Apply the acidified serum samples to the cartridge, then elute sequentially with 5 mL each of de-ionized water, ethanol (100 mL), and hexane, discarding all eluates. Finally, elute the LTB4 with 5 mL of methyl formate. Divide this fraction into two aliquots of equal volume and evaporate the solvent under a stream of air. Reconstitute samples with 0.5 mL of LTB4 buffer (50 mmol/L Tris HCl, pH 8.6, containing 1 g of gelatin per liter) and store at −20 °C until assay.

LTB4 assay. To assay LTB4, we used a radioimmunoassay kit (Amersham, Arlington Heights, IL) exploiting competitive binding between radiolabeled [3H]LTB4 and unlabeled LTB4 (sample or standard) for a limited number of binding sites on the anti-LTB4 antibody. This antibody demonstrates extremely low cross reactivity with other arachidonic acid metabolites: 20-OH-LTB4 0.4%, 6-trans LTB4 0.4%, 5-hydroxyeicosatetraenoic acid <0.05%, leukotriene C4 <<0.05%, and arachidonic acid <0.05%. The quantity of radiolabeled LTB4 bound to the antibody is inversely proportional to the quantity of unlabeled LTB4 present in the sample or standard.

We reconstituted reagents according to the manufacturer's instructions, and diluted the 4 ng/mL LTB4 stock standard to prepare assay standards in concentrations of 1.6 to 200 pg per tube. These standards were used to construct the reference curve. All samples were assayed in duplicate. Initial assay of serum from smokers revealed LTB4 values exceeding the concentration of the highest standard (200 pg per tube). Consequently, we diluted all samples 10-fold before assaying. Two samples had to be diluted 100-fold (results were corrected for these dilutions).

Bound counts per minute (mean of two tubes) and normalized percent bound (%B/B0) of the standards and samples were calculated. The reference curve was established by plotting on semilog graph paper the %B/B0 vs picograms of LTB4 per tube for the standards.

Results

We assayed 20 serum samples for LTB4 after extraction with a solid-phase C18 cartridge. Initial analysis of the serum from smokers showed values in excess of the highest

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standard (200 pg per tube). A dilution of all samples gave reportable results on repeat analysis. We found the concentration of LTB4 in the serum of nonsmokers to be 3.6 ± 0.5 pg per tube vs 211 ± 35 pg per tube (mean ± SEM) for smokers [range, 1.8 to 6.6 pg per tube vs 90 to 490 pg per tube (P <0.001)]. These increased concentrations in the serum of smokers might represent metabolic products of LTB4 or other arachidonic acid products, but this is unlikely because the antiserum that we used was very specific for LTB4. Therefore, we believe that the values we obtained represent LTB4, that these concentrations are detectable in serum, and that cigarette smoking greatly increases them.

Discussion

We report measurable quantities of the arachidonic acid metabolite LTB4 in the serum of smokers and nonsmokers. Previous work with LTB4 in smokers and nonsmokers has concentrated on in vitro assessment of arachidonic acid metabolism by alveolar macrophages (12, 13). Reportedly, alveolar macrophages release LTB4 (2, 14) and, although LTB4 is the principal arachidonic acid metabolite produced, this production is inhibited by cigarette smoking (12, 15, 16). Quantities of the arachidonic acid metabolite prosta-
glandin E2 are also reported to be decreased in the alveolar mac-
rophages of smokers (17–19). Cigarette smoking apparently inhibits the 5-lipoxygenase-catalyzed metabolism of arachidonic acid (12, 16), although inhibition of phospholipase was suggested earlier (18).

Direct inhibition of LTB4 release in alveolar macrophages may be the result of cigarette smoking, but our data suggest the possibility that the inflammatory response induced by smoking could contribute to increased systemic LTB4 concentrations. Furthermore, one investigative group reported similar quantities of LTB4 released from the alveolar mac-
rophages of nonsmokers and smokers (20). More recent examination of the effects of exogenous arachidonic acid has shown that this fatty acid may act as a 5-lipoxygenase inhibitor in addition to its role as substrate (21).

The rapid metabolism of LTB4 has probably made it previously undesirable as a serum marker. However, it is not unreasonable to assume that a chronic inflammatory response such as that seen with long-term cigarette smoking would provide the constant stimulus for LTB4 generation and release. Moreover, cells other than alveolar macrophages could be responsible for release of LTB4. This would obviously include neutrophils, in the circulating pool and localized in the lung, as well as the Langerhans cells (22) or cancer cells. Previous work by Young and coworkers revealed that Lewis lung carcinoma (23) and EL4 leukemia cells (24) secrete prostaglandin E2, demonstrating that arachidonic acid is available for metabolism. It is apparent that, in the lung, alveolar macrophages are not the only cells capable of the generation and release of LTB4.

References

6. Rola-Pleszczynski M, Gagnon L, Siros P. Leukotriene B4 aug-
7. Rola-Pleszczynski M. Differential effects of leukotriene B4 on T4+ and T8+ lymphocyte phenotype and immunoregulatory func-
11. Powell WS. Rapid extraction of arachidonic acid metabolites from biological samples using octadecylsilica silica. Methods Enzy-
18. Laviolette M, Chang J, Newcombe DH. Human alveolar macro-
phages: a lesion in arachidonic acid metabolism in cigarette smoke-
20. Martin TR, Altman LC, Albert RK, Henderson WR. Leuko-
triene B4 production by the human alveolar macrophage: a poten-
21. Peters-Golden M, Shelly C. Inhibitory effect of exogenous arachidonic acid on alveolar macrophage 5-lipoxygenase metabol-