Preferential Vasoconstriction to Cysteiny1 Leukotrienes in the Human Saphenous Vein Compared With the Internal Mammary Artery
Implications for Graft Performance

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**Background** Platelet aggregation with the release of their vasoactive mediators is an important factor contributing to the patency of coronary bypass grafts. However, the role of leukocyte-derived mediators on graft performance is unclear. Leukotrienes (LTs) are proinflammatory mediators released from a variety of leukocytes that possess both vasoactive and mitogenic properties. We have therefore compared the effects of the cysteiny1 LTs (C4, D4, and E4) on the human saphenous vein (SV) and human internal mammary artery (IMA).

**Methods and Results** Human SVs from 43 patients (mean age, 58 years) and IMAs from 33 patients (mean age, 57 years) were obtained from individuals undergoing coronary artery bypass surgery for coronary artery disease. The samples were set up in organ baths to record changes in vessel wall tension. In undistended SVs the cysteiny1 LTs elicited concentration-dependent contractions. The $E_{max}$ for LTE4 (4.23±1.0 mN; n=6) was significantly less than that observed with either LTC4 (25.7±4.01 mN; n=7; P<.001) or LTD4 (26.19±3.16 mN; n=7; P<.001). In addition, the LTD4 receptor antagonist ICI 198615 (30 nmol/L) significantly inhibited the LTD4 concentration-response curve but not the LTC4 responses. Furthermore, treatment of the SV with acivicin (0.05 mmol/L), a γ-glutamyl transpeptidase inhibitor, caused a significant rightward displacement of the LTC4 concentration-response curve. In contrast, LTC4 and LTD4 produced a response in IMAs from only 3 of 29 patients. LTC4 and LTD4 produced small contractions, of which the maximum responses were 3.28±1.92 mN (n=5) and 3.12±1.38 mN (n=5). LTE4 produced no responses in the IMA. Experiments in which the SV was pretreated with 1-N0-monomethyl-L-arginine (L-NMMA; 10^{-5} mol/L) or indomethacin (10^{-5} mol/L) or was denuded of endothelium had no significant effect on the $E_{max}$ values for LTE4. Also, the IMA remained unresponsive to cysteiny1 leukotrienes after treatment with L-NMMA or indomethacin or endothelium removal. In vitro autoradiography localized specific [H]-LTC4 and [H]-LTD4 binding sites (putative receptors) to the smooth muscle cells of both SV and IMA, with greater binding to the SV.

**Conclusions** Our data show that there is a preferential contraction to LTs in SV compared with IMA. This difference in smooth muscle cell reactivity to the cysteiny1 LTs suggests that endogenous LT production from circulating or infiltrating leukocytes may be an important factor contributing to graft function. *(Circulation. 1994;90:515-524.)*

**Key Words** • leukotrienes • bypass grafts • endothelium • vascular reactivity

There is increasing evidence to suggest that the function and patency of coronary bypass grafts is influenced by their biological properties.1,2 The increased patency rates of the internal mammary artery (IMA) conduits compared with the saphenous vein (SV) are thought to be partly attributed to the greater ability of IMA endothelial cells to modulate vascular tone and increase thromboresistance through the release of endothelium-derived nitric oxide3 and prostacyclin.4

The performance of coronary bypass grafts may also depend on the ability of smooth muscle cells to produce and/or respond to circulating or infiltrating inflammatory cells. This is particularly important in venous conduits that have poor antithrombogenic properties,5 which can result in an enhanced platelet and leukocyte vessel wall interaction during insertion of the graft.6,7 This cellular interaction can result in the release of a variety of mediators, including autacoids8,9 and growth factors10, which could modulate vessel tone and accelerate smooth muscle cell growth and thus influence the evolution of disease in the conduit vein.

Leukotrienes (LTs) are proinflammatory mediators released from leukocytes by a variety of stimuli that possess both vasoactive11,12 and mitogenic properties.13,14 We have previously shown that the cysteiny1 LTs C4 and D4 have a complex mode of action in vitro in the SV, including both vasodilator effects at low concentrations (picomoles) and vasoconstrictor effects at high concentrations (nanomoles), which were unaffected by endothelial removal.15 We have also found raised levels of urinary LTE4 (a stable metabolite of the cysteiny1 LTs) in patients after coronary artery bypass surgery,16 indicating an increased production of cysteiny1 LTs. LTE4 also possesses vasoactive properties; therefore, we have compared the effects of LTE4 with those of LTB4, LTC4, and LTD4 on the SV and examined the effect of the competitive LTD4 receptor antag-
onist ICI 198615 on LT responses. In addition, we studied the effects of LTB₄, LTC₄, LTD₄, and LTE₄ on the IMA to compare the reactivity of both vessels to these inflammatory mediators and used in vitro receptor autoradiography to localize specific binding sites in these two vascular conduits.

**Methods**

**In Vitro Organ Bath Experiments**

Human undistended SVs from 43 patients (mean age, 58 years; range, 44 to 69 years old), and human IMAs from 33 patients (mean age, 57 years; range, 38 to 72 years old) were obtained during coronary artery bypass surgery. The blood vessels were immediately placed in a modified Tyrode’s solution of the following composition (mmol/L concentration): NaCl 136.9, NaHCO₃ 11.9, KCl 2.7, NaH₂PO₄ 0.4, MgCl₂ 2.5, CaCl₂ 2.5, glucose 11.1, and disodium EDTA 0.04. The blood vessels were then dissected free of connective tissue, cut into 4- to 5-mm ring segments, and mounted between two L-shaped metal hooks suspended in 5-mL organ baths connected to a force displacement transducer (Grass 79D) coupled to a Grass 79D polygraph to monitor and record changes in vessel wall tension. The baths were filled with 5 mL Tyrode’s solution and aerated at 37°C with a gas mixture of 95% O₂ and 5% CO₂.

**Protocol**

SVs and IMAs were initially stretched to give a preload of 60 mN and 40 mN of force, respectively. These preloads have been shown to be optimal for maximal isometric contraction in response to 90 mmol/L KCl. The vessels were then allowed to relax and equilibrate for 60 minutes. When resting tension was stable, each segment was challenged twice with 90 mmol/L KCl to assess the contractile response of the tissue, the bathing solution being changed between doses when the KCl responses reached a plateau. After two KCl responses, the tissues were washed and allowed to equilibrate for 30 minutes. The rings were then studied in pairs, and doses of either LTB₄, LTC₄, LTD₄, or LTE₄ were added to each bath in a cumulative manner. In another group of tissues the effect of the thromboxane mimetic U46619 was compared. Only one cumulative concentration-response curve was established in each ring segment.

**Effects of ICI 198615 on LTC₄ and LTD₄ Responses**

Some tissues were incubated for 30 minutes with vehicle (30 mmol/L and 0.3 µmol/L dimethylsulfoxide) or ICI 198615 (30 mmol/L and 0.3 µmol/L) before producing concentration-response curves to each LT. In another group of tissues, segments of SV were incubated with ICI 198615 (30 mmol/L) in the presence or absence of acivicin (0.05 µmol/L), an irreversible γ-glutamyl transpeptidase inhibitor that prevents the metabolism of LTC₄ to LTD₄. In other segments, LTC₄ concentration-response curves were performed in the presence of acivicin (0.05 µmol/L) alone, whereas LTD₄ concentration-response curves were performed in the presence or absence of l-cysteine (3 mmol/L) to block the metabolism of LTD₄ to LTE₄.

**Endothelial Modulation**

In another series of experiments quiescent vessels were preincubated with L-NAME (a gift from Wellcome Laboratories; acivicin, l-cysteine, indomethacin, and Tris HCl (Sigma Chemical Company Ltd); and ICI 198615 (ICI Pharmaceuticals). Drug concentrations refer to final

We have already established that LTC₄ and LTD₄ have dilator effects in the SV; therefore, a separate series of experiments were performed to determine whether LTE₄ had any dilator effects in the SV and whether LTC₄, LTD₄, and LTE₄ have any vasodilator actions in the IMA. To induce a preconstriction in the vessel segments, norepinephrine (0.1 to 0.3 µmol/L) was added to the Tyrode’s solution bathing the tissue. Once a stable plateau had been reached, cumulative additions of LTs were made. As a positive control, relaxation to acetylcholine was performed.

**In Vitro Autoradiography**

Tritiated LT binding sites in SV and IMA were localized by in vitro autoradiography. SV and IMA were obtained from an additional nine patients undergoing coronary artery bypass surgery and immediately snap frozen before the experiments. Because of the greater supply of SV tissue, this vessel was used in the preliminary experiments (n=6) to determine the optimum incubation time (association experiments) and washout times (dissociation experiments) for the saturation experiments.

**Saturation Experiments**

Slide-mounted sections of both vessel types were initially preincubated in 50 mmol/L Tris HCl buffer, pH 7.4, for 15 minutes at 4°C to reduce levels of endogenous leukotrienes. Slides were then incubated in buffer containing 5 mmol/L CaCl₂, 0.05 mmol/L acivicin, and 20 mmol/L L-cysteine in the presence of 0.01 to 3.0 mmol/L [³H]-LTC₄ or [³H]-LTD₄ (specific activity, 154 Ci/mmol; NEN Dupont) for 60 minutes at 4°C. The acivicin prevented the metabolism of LTC₄ to LTD₄ during incubation, and l-cysteine prevented the metabolism of LTD₄ to LTE₄. The degree of nonspecific binding was established by incubating alternate sections in the presence of 1 µmol/L unlabeled LTC₄ or LTD₄ (Cascade Biochemicals Ltd). After incubation, sections were washed twice for 5 minutes (determined from the dissociation data), in buffer at 4°C, dipped in cold distilled water (4°C), and dried in a stream of cold air. Low-resolution autoradiography was performed by exposing incubated sections to Hyperfilm ³H (Amersham) for 5 weeks. The film was processed in undiluted D19 developer (Kodak) and fixed in IF23 fixer (Ilford, diluted 1 to 4 in distilled water), both for 5 minutes at 22°C. Low-resolution autoradiographs were examined and photographed on a Nikon macro system. Estimation of [³H]-LTC₄ and [³H]-LTD₄ binding was performed by wiping off tissue sections from the microscope slides with Nucwipes (National Diagnostic), which were then placed in Ultragold scintillant (4.5 mL) and counted for tritium for 2 minutes. Background counts were subtracted from all readings and specific binding, and then calculated by subtracting nonspecific binding from total binding. [³H]-LTC₄ and [³H]-LTD₄ binding sites were identified at a microscopic level (high-resolution autoradiography) by apposing sections to coverslips coated with emulsion (LM1, Amersham) and exposing for 6 weeks in lightproof boxes at 4°C. After exposure, the emulsion-covered coverslips were also processed in undiluted D19 developer (Kodak), briefly placed in stop solution (Ilford) for 15 seconds, and fixed in IF23 (Ilford, diluted 1 to 4 in distilled water), both for 5 minutes at 22°C. Tissue was then stained with Mayer’s hematoxylin and eosin for histological examination. Autoradiographs and stained tissue were examined under dark-field and bright-field illumination on an Olympus Vanox microscope.

**Drugs**

The following drugs were used: LTB₄, LTC₄, LTD₄, and LTE₄ (Cascade); [³H]-LTC₄ and [³H]-LTD₄, emulsion (LM-1), and Hyperfilm ³H (Amersham); L-NAME (a gift from Wellcome Laboratories); acivicin, l-cysteine, indomethacin, and Tris HCl (Sigma Chemical Company Ltd); and ICI 198615 (ICI Pharmaceuticals). Drug concentrations refer to final
molarity within the organ bath solution. LTs were obtained as stock solutions dissolved in solvent (MeOH:H2O:AcOH) and packed under argon. The stock solutions were divided into aliquots and stored under an atmosphere of nitrogen at −40°C. For each experiment, an aliquot was diluted with distilled water to an appropriate concentration and kept on ice. Indomethacin was dissolved in 100 mmol/L sodium carbonate and then further diluted in distilled water. L-NMMA was dissolved in distilled water, and ICI 198615 was dissolved in dimethylsulfoxide and then further diluted in distilled water.

Data Analysis

Contractions were measured as absolute tension in millinewtons or as a percentage of the maximal isometric contraction to 90 mmol/L KCl. The EC50 values for each concentration-effect curve were obtained by linear regression analysis of data points in millinewtons or percentage of KCl response versus log concentration above and below the EC50 level. These values were transformed into pD2 values (−log EC50). The pKb values (−log Kb) were calculated using the following equation: Kb=[B]/(DR−1), where [B] is the concentration of the antagonist and DR (dose ratio) is the ratio of the EC50 of agonist in the presence and absence of antagonist. The Kb value was transformed into the pKb value. All results are expressed as mean±SEM, and in all experiments, n equals the number of patients from whom the vessels were obtained. Differences between leukotrienes were compared by an unpaired Student’s t test. Comparisons between control and experimental groups were made by ANOVA. A value of P<.05 was considered to indicate a statistical difference.

Results

Effect of Cysteinyl LTs on Resting Tone

The concentration-response curves for the different LTs are shown in Fig 1. In undenstended SV preparations, LTE4 produced an Emax value of 4.23±1.0 mN (n=6), which was significantly less than that observed with either LTC4 (25.7±4.01 mN; n=7; P<.001) or LTD4 (26.19±3.16 mN; n=7; P<.001) (Fig 1, upper panel). The maximum responses of LTC4, LTD4, and LTE4 when compared as a percentage of the maximum response elicited by 90 mmol/L KCl were 80.58±14.19%, 76.40±10.04%, and 11.6±2.9%, respectively. The contractions induced by each LT were slow in onset, and duration of the maximum response was protracted. LTE4 had the longest duration of action, inducing contractions that were at least twice those caused by LTC4 and LTD4 (LTE4, 46.3±6.8 minutes versus LTC4 and LTD4, 14.8±2.0 minutes; P<.01). The chemotactic LT B2 (1 nmol/L to 0.1 μmol/L) had no effect on vascular tone in the SV (n=4).

In contrast to their effects in the SV, neither LTC4 nor LTD4 produced efficacious contractions in the IMA (Fig 1, lower panel). The IMA responded to LTC4 and LTD4 with small contractions; the Emax values were 3.28±1.92 mN to LTC4 in 3 of 5 and 3.12±1.38 mN to LTD4 in 3 of 5, which was equivalent to 20.77±10.59% and 17.54±9.78%, respectively, of the response produced by 90 mmol/L KCl. LTE4 (n=4) and LTD4 (n=6) produced no contractile responses in IMA at concentrations up to 0.1 μmol/L. When the responsiveness of the SV and IMA was examined with the thromboxane mimetic U46619 (1 nmol/L to 0.3 μmol/L), U46619 produced a similar maximum tension in both tissues: Emax, 147.5±11.5% for SV and 152±35.4% for IMA, and a lower EC50 value: pD2, 8.57±0.18 (n=4) for SV and 8.0±0.18 for IMA (n=4; P=.03).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Leukotriene (LT) contractions in human isolated saphenous vein (ring preparations [upper panel] and internal mammary artery [lower panel]). The curves show the contractions elicited by LTC4 (●; n=7 [SV] and n=5 [IMA]), LTD4 (○; n=7 [SV] and n=5 [IMA]) and LTE4 (▲; n=6 [SV] and n=6 [IMA]). The response is presented as a percentage of the response to 90 mmol/L. All values are mean±SEM. (Emax values for SV LTC4/ LTD4 vs LTE4, P<.001.)

Influence of the Endothelium on LTE4 Responses in SV

Results derived from experiments in which the SVs were pretreated with L-NMMA (100 μmol/L) or indomethacin (10 μmol/L) or denuded of their endothelium before establishing concentration-response curves to LTE4 are presented in Table 1. The data obtained from paired preparations indicated that these treatments did not significantly alter the Emax values of LTE4 in these tissues (n=6). In another series of experiments in which
the SVs were preconstricted with norepinephrine (0.1 μmol/L), LTE₄ (1 pmol/L to 10 nmol/L) did not relax the preconstricted ring segments (0% change in norepinephrine-induced tone; n=3).

**Influence of the Endothelium on Cysteiny L T Responses in IMA**

Similarly, control segments were unresponsive to LTC₄ and LTD₄ in the IMA, showing no increase in developed tension (0 mN) from the baseline. IMA segments treated with L-NMMA or indomethacin or denuded of their endothelium also remained unresponsive to LTC₄ (0 mN; n=6) or LTD₄ (0 mN; n=6) and did not unmask contractile responses to LTE₄ (0 mN; n=6). The unresponsiveness of IMA segments to LTs was not caused by vessel wall damage because the vessels responded to 90 mmol/L KCl. Furthermore, the cysteiny LTs were also unable to relax the IMA at concentrations ranging from 1 pmol/L to 10 nmol/L (0% change in norepinephrine-induced tone; n=6).

**Effect of ICI 198615 on LT Responses in SV**

Treatment of SV (n=12) with ICI 198615 (3.0 nmol/L or 0.3 μmol/L) antagonized the contractions induced by LTD₄ (Fig 2 and Table 2). Similar experiments performed with ICI 198615 (30 nmol/L) failed to inhibit LTC₄-induced contractions (n=6); however, at the higher concentration ICI 198615 (0.3 μmol/L) significantly antagonized the LTC₄ concentration-response curve (Table 2; n=6).

The results derived from experiments in which SVs were pretreated with acivicin (0.05 mmol/L) before LTC₄ concentration-response curves were established are presented in Fig 3. These data obtained from the paired preparations indicated that this treatment caused a significant displacement of the LTC₄ concentration-response curve (n=6). The LTC₄ pD₂ values were 7.67±0.05 for the control group, and 7.45±0.09 for the acivicin-treated group (P<.05). In addition, ICI 198615 (30 nmol/L) in the presence of acivicin (0.05 mmol/L) failed to inhibit the LTC₄ dose-response curve; the LTC₄ pD₂ values were not significantly different between the two groups (LTC₄+acivicin, 7.45±0.09; LTC₄+acivicin+ICI 198615, 7.55±0.09).

When SVs were pretreated with L-cysteine (3 mmol/L), the LTD₄ pD₂ values (7.89±0.10) were not significantly different from controls (7.78±0.07). The LTD₄ Eₘₐₓ was

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**Table 1. Comparison of Maximum Effective Concentration Values for LTE₄ in the Human Saphenous Vein Between Control and Experimental Group**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control L-NMMA</th>
<th>Indo</th>
<th>(-)Endo</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTE₄</td>
<td>11.6±2.9%</td>
<td>14.8±4.1%</td>
<td>15.5±3.7%</td>
</tr>
</tbody>
</table>

L-NMMA indicates L-N⁵-monomethyl-L-arginine; Indo, indomethacin; (-) Endo, endothelium-denuded; and LTE₄, leukotriene E₄.

Eₘₐₓ values are presented as a percentage of the response to 90 mmol/L KCl. Values are reported as mean±SEM.

**Table 2. Leukotriene Antagonist Effects of ICI 198615 In Human Saphenous Vein Ring Preparations**

<table>
<thead>
<tr>
<th>Drug</th>
<th>LTC₄ pKₐ Value</th>
<th>LTD₄ pKₐ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICI 198615</td>
<td>30 nmol/L</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.3 μmol/L</td>
<td>7.39±0.06</td>
</tr>
</tbody>
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The pKₐ values were calculated using the following formula: Kₐ=[antagonist]/(dose-ratio-1). These values were calculated only when there was a significant (P<.05) displacement of the agonist curves subsequent to the drug treatments. When the curves were not significantly (NS) shifted no pKₐ values were calculated.

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**Fig 2. Leukotriene (LT) D₄ concentration-response curves in human saphenous vein.** Tissues were incubated with either vehicle (●, 30 nmol/L or 0.3 μmol/L dimethylsulfoxide) or ICI 198615 (▲, 30 nmol/L; ▼, 0.3 μmol/L). Values are expressed in millinewtons. Vertical bars represent mean±SEM.

**Fig 3. Leukotriene (LT) C₄ concentration-response curves in human saphenous vein.** Tissues were incubated with either vehicle (●, 30 nmol/L dimethylsulfoxide) or ICI 198615 (30 nmol/L) in the presence (▲) or absence (▼) of acivicin (0.05 mmol/L) or LTC₄+acivicin (○). Values are expressed as a percentage of the KCl contraction. Vertical bars represent mean±SEM.
also not altered: 40.1±9.1% and 44.6±10.4% KCl response in untreated versus treated groups, respectively (n=5).

Low-Resolution Autoradiography of SV and IMA

Low-resolution autoradiography localized specific [3H]-LTC₄ and [3H]-LTD₄ concentration-dependent binding (0.03 to 3.0 nmol/L) to both the SV (Fig 4) and IMA (Fig 5). Estimation of binding data appeared to show differences in the degree of specific binding of [3H]-LTC₄ (Fig 6) and [3H]-LTD₄ (Fig 7). The IMA showed less specific [3H]-LTC₄ (Fig 8) and [3H]-LTD₄ binding (Fig 9) than the SV, although no statistical analysis was performed because of the low number of samples in each group.
High-Resolution Autoradiography of SV and IMA

High-resolution autoradiography showed [3H]-LTC\textsubscript{4} and [3H]-LTD\textsubscript{4} binding that was primarily localized to the tunica media, with less binding to the adventitia and no evidence of binding to the endothelium. Fig 10 shows a representative autoradiograph of [3H]-LTC\textsubscript{4} binding to the SV. Fig 11 shows a representative autoradiograph of [3H]-LTD\textsubscript{4} binding to the SV and IMA.

Discussion

This article describes the comparative effect of the cysteinyI LTs in human SV and IMA. Our data show for the first time that there is a preferential contraction to LTs in the SV compared with the IMA and that this disparity in responsiveness was not caused by the influence of endothelium-derived relaxing factors opposing LT-induced contractions in the IMA. In addition, there was no hyperactivity to U46619 in the SV compared with the IMA.

The cysteinyI LT responses in the IMA were unaffected by L-NMMA or indomethacin treatment, suggesting that relaxing factors released from the endothelium were not masking LT-induced contractions. Previous studies have shown preferential endothelial modulation of responses to various vasoconstrictors between the IMA and SV\textsuperscript{20}; however, it appears from our experiments that this mechanism does not account for the different responses of the IMA and SV to LTs, which is probably due to the specific LT-receptor profile of the smooth muscle cells in these vessels.

To examine further the mechanisms of the LT-induced vascular hyperactivity in the SV, we compared the expression of LT receptors in SV and IMA using a combination of in vitro receptor autoradiography and receptor pharmacology with the specific LTD\textsubscript{4} receptor antagonist ICI 198615. Analysis of binding data in the SV suggested differences between [3H]-LTC\textsubscript{4} and [3H]-LTD\textsubscript{4} binding activity. [3H]-LTC\textsubscript{4} exhibited a high concentration-dependent binding at 3 nmol/L, whereas [3H]-LTD\textsubscript{4} binding was saturable at 1 nmol/L. The higher degree of LTC\textsubscript{4} binding may be partially attributable to binding to glutathione-S-transferase.\textsuperscript{21} It is not certain whether these binding sites are indeed the receptors through which the biological effects of LTC\textsubscript{4} and LTD\textsubscript{4} are mediated in the SV. However, the contractions elicited by both LTC\textsubscript{4} and LTD\textsubscript{4} were inhibited by ICI 198615, indicating that the actions of LTs in the SV are mediated through activation of specific LT receptors. It was suggested from our previous study that these receptors are located in the medial
smooth muscle cells because endothelial removal in the vessels did not affect LT contractions.\textsuperscript{15} Our present data support this, showing that tritiated LT binding was also localized primarily to the tunica media.

The nature of the binding sites in IMA is unclear because the majority of IMAs tested did not respond to LTC\textsubscript{4} and LTD\textsubscript{4}. The reasons only three IMAs responded to LTC\textsubscript{4} and LTD\textsubscript{4} are unclear, but it has been
shown that the presence of atherosclerosis can augment the response to LTs. It is possible that these three arteries may have had some degree of early atherosclerotic changes, although this was not evident on macroscopic examination. Several possibilities exist for interpretation of the binding data. Binding sites for LTs on enzymes or transport proteins rather than functionally important receptors might account for the specific binding observed. Alternatively, the binding sites may represent LT receptors that are not linked to vasomotion. For example, LTs are known to have both mitogenic and vascular permeability effects. Lastly, the difference in vascular reactivity between the SV and IMA may be caused by other reasons such as an altered receptor number or affinity or postreceptor alterations in signal transduction in vascular smooth muscle. Further studies are needed to identify the function of these LT binding sites to clarify the mechanism of the LT-induced vascular hyperreactivity in the SV.

The actions of LTs are complex, and the pharmacological studies that attempt to characterize the cysteinyl LT receptors in several tissues are frequently compromised by secondary events such as mediator release or LT metabolism. Previous studies have shown that LTC4 in the absence of L-serine borate complex may be degraded to LTD4 by enzymatic activity of γ-glutamyl transpeptidase. In this study, the sensitivity of the LTC4 concentration-response curves in the presence of acivicin were significantly shifted to the right, and the antagonist activities of ICI 198615 were lost. These results suggest that this enzyme rapidly metabolizes LTC4 so that the major contractile effect of LTC4 in the SV is caused by its conversion to LTD4. In addition, we were unable to show that L-cysteine affects LTD4 concentration-response curves, which is similar to previous findings.

Our results suggest a heterogeneous population of LT receptors in the human SV, one which is ICI 198615-sensitive and is specific for LTD4, and the other ICI 198615-insensitive, which appears to be specific for LTC4. This heterogeneity is further supported by the reported differences in pK4 values for LT responses and the apparent differences in triitated LT binding characteristics in the SV.

These findings may have important consequences for graft function and patency. The IMA has a potent endogenous nitric oxide system that can protect against vasospasm and thrombus formation and thus prevent graft occlusion. In addition, the present findings suggest that IMA vascular smooth muscle cells have a reduced capacity to constrict to certain endogenous inflammatory mediators such as LTs, which may further aid protection against thrombus formation. In contrast, in SVs the endothelial–L-arginine pathway is much less active, and its effects are inhibited by concomitantly released endothelium-derived constriction factors. Previous studies have shown the importance of platelet-endothelial interactions in SV grafts with a decrease in the early occlusion rate with antiplatelet therapy. However, the contribution of leukocyte-endothelial interactions on graft performance is not clear. LTs are produced by activation of various white blood cells, including neutrophils, monocytes, and eosinophils. LTC4 and LTD4 are known to induce adhesion of polymorphonuclear leukocytes to human endothelial cells through the production of platelet-activating factor. There is also evidence that LTs, in particular LTβ, enhance leukocyte adhesion to the endothelium, and inhibition of endogenous LT biosynthesis prevents postischemic leukocyte adhesion and tissue injury in an animal model. The importance of leukocyte-endothelium interactions to graft function was highlighted by a

Figure 10. High-resolution autoradiographs of [3H]-leukotriene (LT) C4 binding to human saphenous vein showing dark-field/light-grain (left) photomicrographs and hematoxylin and eosin stained tissue (right). Panel A: Total [3H]-LTC4 binding. Panel B: Nonspecific binding. IEL indicates internal elastic lamina; L, lumen. (Scale bar, 100 μm.)
recent report showing an increase in platelet and leukocyte adhesion and reduced short-term patency in pig arteriovenous bypass grafts.\textsuperscript{7}

We have shown that SV vascular smooth muscle cells are reactive to LTC\textsubscript{4} and LTD\textsubscript{4} and are less reactive to LTE\textsubscript{4}. LTE\textsubscript{4}, although less potent and efficacious than either parent molecule, retains some biological activity in the SV. Owing to its stability and sustained action, LTE\textsubscript{4} may therefore persist in the circulation for a longer time than either LTC\textsubscript{4} or LTD\textsubscript{4}. This feature of LTE\textsubscript{4} may be important to graft function in light of the recent findings showing that LTE\textsubscript{4} led to a selective recruitment of eosinophils and neutrophils in vivo.\textsuperscript{35} In the event of the generation of cysteinyl LTs in patients with arterialized SV grafts, marked contractions to LTC\textsubscript{4} and LTD\textsubscript{4}, such as occur in the SV, could reduce local blood flow at sites where leukocytes are activated. In addition, LTC\textsubscript{4} may potentiate the effects of catecholamines\textsuperscript{36} and thromboxane A\textsubscript{2},\textsuperscript{37} which could further increase contraction of SV, whereas LTE\textsubscript{4} could act to recruit further leukocytes and thus favor thrombus formation. Indeed, the arterialized SV grafts remain responsive to LTC\textsubscript{4},\textsuperscript{38} indicating that LT responses are conserved after arterialization of the SV.

LTs may be produced during cardiopulmonary bypass, because extracorporeal circulation of blood is known to activate leukocytes.\textsuperscript{39} To support this, we have recently shown that LT levels are raised in patients with coronary artery disease and after coronary artery bypass surgery.\textsuperscript{16} Furthermore, leukocytes in vitro can produce concentrations of LTC\textsubscript{4} in the range of 7 to 40 ng/10\textsuperscript{6} leukocytes,\textsuperscript{40} which is in the concentration range (EC\textsubscript{50} value) for producing a biological effect in the SV. Together, these findings suggest that activated white blood cells could produce sufficiently high local concentrations of LTs to induce contraction and therefore influence blood flow through the vein graft. In addition, it is possible that LT release from white blood cells may form the basis of an amplification mechanism to further leukocyte recruitment into SV grafts.

Thus, the preferential contraction of LTs on SVs compared with IMAs, together with the recent clinical evidence of an enhanced production of LTs,\textsuperscript{16} suggests that endogenous LT production may be an important factor contributing to graft function and that LT bio-

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**Figure 11.** High-resolution autoradiographs of [\textsuperscript{3}H]-leukotriene (LT) D\textsubscript{4} binding to human internal mammary artery and saphenous vein showing dark field/light grains (left) and bright field/dark grains (middle) photomicrographs and hematoxylin and eosin stained tissue (right). (Scale bar, 100 µm.)
synthesis inhibitors and antagonists may be of therapeu-
tic value.

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