Myosin Light Chain Kinase Regulates Capacitative Ca\(^{2+}\) Entry in Human Monocytes/Macrophages

Quang-Kim Tran, Hiroshi Watanabe, Hong-Yen Le, Ling Pan, Minoru Seto, Kazuhiko Takeuchi, Kyoichi Ohashi

Abstract—Monocytes/macrophages are present in all stages of atherosclerosis. Although many of their functions depend on changes in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), mechanisms regulating [Ca\(^{2+}\)]\(_i\) in these cells remain unclear. We aimed to explore the role of myosin light chain kinase (MLCK) in Ca\(^{2+}\) signaling in freshly isolated human monocytes/macrophages. Large capacitative Ca\(^{2+}\) entry (CCE) was observed under fura 2 fluoroscopy in human monocytes/macrophages treated with thapsigargin and cyclopiazonic acid. ML-9 and wortmannin, 2 structurally different inhibitors of MLCK, dose-dependently (1 to 100 \(\mu\)mol/L) prevented CCE and completely did so at 100 \(\mu\)mol/L, whereas inhibitors of tyrosine kinase and protein kinase C had only partial effects. Western blotting showed that thapsigargin significantly caused myosin light chain phosphorylation, which was almost completely blocked by ML-9 (100 \(\mu\)mol/L) and wortmannin (100 \(\mu\)mol/L). ML-9 also dose-dependently (1 to 100 \(\mu\)mol/L) inhibited this phosphorylation, which was well correlated with its inhibition of CCE. Transfection with MLCK antisense completely prevented CCE in response to thapsigargin and cyclopiazonic acid, whereas MLCK sense had no effect. These data strongly indicate that MLCK regulates CCE in human monocytes/macrophages. The study suggests a possible involvement of MLCK in many Ca\(^{2+}\)-dependent activities of monocytes/macrophages. (Arterioscler Thromb Vasc Biol. 2001;21:509-515.)

Key Words: monocytes/macrophages ■ capacitative Ca\(^{2+}\) entry ■ myosin light chain kinase

Monocytes/macrophages are present in all stages of atherosclerosis. 1–3 Many of their functions depend on changes in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)). For example, leukocytes adhering to endothelial cells demonstrate multiple Ca\(^{2+}\) transients; the binding of oxidatively damaged red blood cells by macrophages is almost totally Ca\(^{2+}\) dependent; and oxidized LDL–induced activation of protein kinase C (PKC) leading to macrophage growth also involves a rise in [Ca\(^{2+}\)]\(_i\). In addition, optimal oxidative modification of LDL by monocytes/macrophages requires Ca\(^{2+}\) release from intracellular stores and Ca\(^{2+}\) entry, and LDL receptor–mediated lipoprotein degradation is also Ca\(^{2+}\) dependent.

In many cells, intracellular Ca\(^{2+}\) stores are mobilized after either the production of inositol trisphosphate or blockade of the endoplasmic reticulum (ER) Ca\(^{2+}\) pump, and it has been postulated that Ca\(^{2+}\) store depletion can trigger transmembranous Ca\(^{2+}\) entry, the capacitative Ca\(^{2+}\) entry (CCE) model. Such a mechanism has not been demonstrated in human monocytes/macrophages. Furthermore, although more human homologues of the Drosophila gene products transient receptor potential and transient receptor potential-like are being cloned and have proven to be candidate genes for CCE channels, 10 further information is required to determine how intracellular Ca\(^{2+}\) store depletion can activate CCE. Numerous second messengers have been implicated in CCE activation, including inositol trisphosphate, \(^{11}\) cGMP, \(^{12}\) a Ca\(^{2+}\) influx factor, \(^{13}\) a product of Cytochrome P 450 activity, \(^{14}\) and tyrosine phosphorylation. \(^{15,16}\) We have recently reported that myosin light chain (MLC) kinase (MLCK) inhibitors prevent agonist-stimulated, fluid flow–stimulated, and chloride-sensitive Ca\(^{2+}\) entry in endothelial cells. \(^{17,18}\) In monocytes/macrophages, MLCK is implicated in several activities important in atherosclerosis. Thus, MLCK is important for macrophage motility \(^{19}\) and regulates neutrophil migration across the endothelium.\(^{20}\) MLCK activation was recently proven to be critical in cytoskeletal changes resulting in pseudopod formation during phagocytosis.\(^{21}\) Migration and recruitment of macrophages and macrophage-derived foam cells are, in turn, stimulated by increased Ca\(^{2+}\) concentration.\(^{22}\) Although these observations, taken together, indicate a likelihood of an involvement of MLCK in Ca\(^{2+}\) signaling and thus in many Ca\(^{2+}\)-dependent activities of these cells, the role of MLCK in monocyte/macrophage Ca\(^{2+}\) signaling has never been investigated.

Received August 21, 2000; revision accepted December 12, 2000.
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In the present study, we have investigated the effects of various inhibitors of MLCK and other protein kinases on CCE and MLC phosphorylation caused by thapsigargin (TG) and cyclopiazonic acid (CPA) in human monocytes/macrophages, and we have tested the effects of MLCK sense and antisense oligonucleotides on the CCE. Our results indicate that MLCK is an important regulator of CCE in human monocytes/macrophages and suggest the possible involvement of the kinase in many Ca\(^{2+}\)-dependent activities of these cells.

**Methods**

**Cell Isolation**

The investigation was approved by the institutional ethics committee. Informed consent was obtained from healthy volunteers, and heparinized blood was collected, diluted in PBS containing 2% FCS, and subjected to gravity centrifugation (400g) in Ficoll-Paque isolating solution (Pharma Biotech) for 30 minutes at 18°C. The upper plasma layer was removed, leaving the leukocyte layer undisturbed; this layer was then gently collected and washed several times with PBS containing 2% FCS at 4°C. The final pellets were suspended in RPMI 1640 medium containing 2% FCS, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 &micro;mol/L streptomycin at a density of \(2 \times 10^6\) cells/mL, aliquoted onto polybiphenyl dishes fixed on 10-10-mm glass coverslips, and incubated at 37°C under 5% CO\(_2\) for several hours. Before the experiments, samples were washed several times with the same culture medium to remove nonadhering leukocytes, leaving only adherent monocytes/macrophages on glass coverslips. Differential cell counts under Wright’s Giemsa staining showed that >90% of the isolated cells were mononucleocytes. Cell viability was >95%, as determined by trypan blue dye exclusion test.

**[Ca\(^{2+}\)] Measurement**

\([\text{Ca}^{2+}]\), was measured as previously described.\(^\text{18}\) TG, CPA, ML-9, genistein, herbinymycin A, staurosporine, bisindolylmaleimide I, MLCK sense, MLCK antisense, and Superfect reagent (QIAGEN Inc) at the concentrations used in the present study did not affect fura 2 fluorescence or autofluorescence of unloaded cells.

**Western Blotting**

MLC phosphorylation in monocytes/macrophages was measured by separation of nonphosphorylated and phosphorylated forms by glycerol-PAGE according to their respective charges of phosphate molecules, followed by electrophoretic transfer of the proteins to a nitrocellulose membrane. The relative amounts of each form were quantified by immunoblotting with an anti-MLC antibody, as described in a previous study with \(\gamma\)-\text{ATP} incorporation into separated MLC bands and phosphomyosin-specific phosphatase.\(^\text{23}\) It has been demonstrated that electrophoresed protein bands represent nonphosphorylated and phosphorylated MLC. Briefly, after stimulation with TG in the presence or absence of wortmannin or ML-9, cells were exposed to 5% trichloroacetic acid containing 2 mmol/L dithiothreitol. After centrifugation at 2500g for 3 minutes, the pellet was washed with 10 mmol/L dithiothreitol/acetone, resuspended in urea sample buffer, and processed for urea/glycerol/PAGE and immunoblotting by a modification of the method by Persechini et al.\(^\text{24}\) The urea extracts before and after stimulation with TG contained nonphosphorylated and phosphorylated MLC. Relative quantification of nonphosphorylated and phosphorylated MLC was made by densitometry.

**MLCK Sense and Antisense Transfection**

MLCK antisense oligonucleotides (5’ GGCGCTTTCATCTCCG 3’) were produced according to the mRNA of porcine smooth muscle MLCK (DDBJ access identification No. D89497). The target sequence started at the 170-base position. Respective sense oligonucleotides were produced for use as controls. The oligonucleotides were dissolved in Tris-HCl plus EDTA (pH 7.4) to 0.1 &micro;g/mL. Then 10 &micro;L of this mixture was diluted in RPMI medium containing no serum, proteins, or antibiotics to 300 &micro;L. The solution was mixed for a few seconds before Superfect transfection reagent (30 &micro;L, QIAGEN Inc) was added and mixed, and the mixture was left at room temperature for 10 minutes to allow complex formation. Meanwhile, the cells were washed with PBS several times, leaving the adherent ones on glass coverslips. Then, 2 mL of RPMI solution containing 10% FCS and antibiotics was added to the reaction tube containing the transfection complexes. The solution was mixed, immediately transferred to the cell wells at 500 &micro;L each, and incubated for 3 hours at 37°C and 5% CO\(_2\). The medium was then removed, the cells were washed once with PBS, and the culture medium containing serum and antibiotics was added. The samples were incubated for 20 hours. Over 95% of the cells remained viable under the trypan blue exclusion test before the experiments. To assess whether the oligonucleotides were successfully transfected, the S’ ends were conjugated with fluorescein isothiocyanate (FITC) and transfected, and then the cells were observed under a fluorescence microscope (Axioskop, Zeiss).

**Statistical Analysis**

Data are expressed as mean±SD from at least 3 separate experiments. Statistical analysis was performed with the Student t test for unpaired data. Correlation analysis was performed by using GraphPad Prism (GraphPad Software, Inc). A value of \(P<0.05\) was considered significant.

**Results**

**CCE Exists in Human Monocytes/Macrophages**

To investigate Ca\(^{2+}\) signaling in human monocytes/macrophages, we used TG, an irreversible inhibitor of the ER Ca\(^{2+}\)-ATPase,\(^\text{25}\) and CPA, a reversible inhibitor of the enzyme.\(^\text{26}\) Under Ca\(^{2+}\)-free conditions, TG (1 &micro;mol/L) and CPA (100 &micro;mol/L) increased the fluorescence ratios at 340 and 380 nm (F340/380 ratios) very slightly, from 0.88±0.10 and 0.92±0.02 to 1.07±0.06 and 1.10±0.11 after 90 seconds of TG and CPA treatment, respectively (open circles, Figures 1A and 1B). In Ca\(^{2+}\)-containing medium, TG (1 &micro;mol/L) significantly increased the F340/380 ratio from 0.87±0.17 to 4.28±0.39 and 4.00±0.30 after 2 and 8 minutes, respectively (closed circles, Figure 1A). Likewise, CPA (100 &micro;mol/L) largely increased the F340/380 ratio from 0.92±0.12 to 4.10±0.41 and 3.15±0.32 after 3.5 and 8 minutes, respectively (closed circles, Figure 1B). To determine whether CCE was at work, the reversible ER Ca\(^{2+}\)-ATPase inhibitor CPA (100 &micro;mol/L) was first applied in Ca\(^{2+}\)-free medium, which slightly increased the F340/380 ratio. CPA was then washed out, and the cells were kept in Ca\(^{2+}\)-free medium for 5 minutes. When 1 mmol/L Ca\(^{2+}\) was introduced without CPA, the F340/380 ratio was largely increased (Figure 1C). These results clearly implicate CCE in human monocytes/macrophages.

**MLCK Inhibitors Prevented Human Monocyte/Macrophage CCE**

To investigate the role of MLCK in Ca\(^{2+}\) signaling in human monocytes/macrophages, we first examined the effects of ML-9, a strong MLCK inhibitor,\(^\text{27}\) on TG-induced Ca\(^{2+}\) response. Five-minute ML-9 pretreatment did not increase the basal F340/380 ratio but dose-dependently (1 to 100 &micro;mol/L) prevented the TG-induced Ca\(^{2+}\) response, with almost complete inhibition at 100 &micro;mol/L (Figure 2A). In the same manner as used with ML-9, a 30-minute pretreatment with...
wortmannin, a different MLCK inhibitor, did not increase the basal F340/380 ratio but dose-dependently (1 to 100 μmol/L) inhibited the TG-induced Ca\(^{2+}\) response, with complete inhibition at 100 μmol/L (Figure 2B).

To confirm that MLCK inhibition could block CCE, CPA was applied for 5 minutes under Ca\(^{2+}\)-free conditions, which slightly raised the F340/380 ratio. CPA was then removed, and ML-9 (100 μmol/L) was applied 5 minutes before 1 mmol/L Ca\(^{2+}\) was added without CPA.

TG-induced MLC phosphorylation in monocytes/macrophages (Figure 4). In control conditions, MLC was not phosphorylated (the total extracted MLC being in the non-phosphorylated form); TG (1 μmol/L) increased phosphorylated MLC to as much as 77% of the total extracted MLC. Wortmannin (100 μmol/L) pretreatment for 30 minutes or ML-9 (100 μmol/L) pretreatment for 5 minutes completely inhibited the formation of phosphorylated MLC bodies, which were now only 6% and 0%, respectively, of the total extracted MLC. Wortmannin (100 μmol/L) pretreatment was administered for 30 minutes before TG treatment (1 μmol/L). Values represent peak F340/380 ratios. B, Medium contained 1 mmol/L Ca\(^{2+}\). Wortmannin (WT, 1 to 100 μmol/L) pretreatment was administered for 30 minutes before TG treatment (1 μmol/L). C, Ca\(^{2+}\)o was removed 2.5 minutes before CPA (100 μmol/L) was applied for 5 minutes. CPA was then removed, and ML-9 (100 μmol/L) was introduced. After 5 minutes, 1 mmol/L Ca\(^{2+}\)o was added with ML-9 maintained at 100 μmol/L for 7.5 minutes before it was removed.

MLCK Inhibitors Blocked TG-Stimulated MLC Phosphorylation

Because a key function of MLCK is to phosphorylate MLC, we investigated the effects of the MLCK inhibitors onTG-induced MLC phosphorylation in monocytes/macrophages (Figure 4). In control conditions, MLC was not phosphorylated (the total extracted MLC being in the non-phosphorylated form); TG (1 μmol/L) increased phosphorylated MLC to as much as 77% of the total extracted MLC. Wortmannin (100 μmol/L) pretreatment for 30 minutes or ML-9 (100 μmol/L) pretreatment for 5 minutes completely inhibited the formation of phosphorylated MLC bodies, which were now only 6% and 0%, respectively, of the total extracted MLC (Figure 4A). ML-9 dose-dependently (1 to 100 μmol/L) inhibited this MLC phosphorylation (Figure 4B), and the effects of ML-9 to inhibit CCE and MLC phosphorylation were closely correlated (P<0.05, r=0.95197; Figure 4C). These findings substantiate MLCK involvement in the effects shown by both inhibitors on CCE.
Effects of Other Kinase Inhibitors on CCE

Various protein kinases have been implicated in CCE. Therefore, we compared the effects of inhibitors of MLCK, protein tyrosine kinase (PTK), and PKC on TG- and CPA-induced Ca\(^{2+}\) responses. Figure 5 compares the effects on the peak and sustained phases of the Ca\(^{2+}\) responses to 1 \(\mu\)mol/L TG (Figure 5A) and 100 \(\mu\)mol/L CPA (Figure 5B) by the MLCK inhibitors ML-9 (100 \(\mu\)mol/L) and wortmannin (100 \(\mu\)mol/L), the PTK inhibitors genistein (100 \(\mu\)mol/L) and herbimycin A (100 \(\mu\)mol/L), and the PKC inhibitors bisindolylmaleimide I (10 \(\mu\)mol/L) and staurosporine (0.3 \(\mu\)mol/L).

Pretreatment for 5 minutes with either the PTK or PKC inhibitors significantly, but only partially, inhibited TG- and CPA-induced Ca\(^{2+}\) influxes, whereas the MLCK inhibitors almost abolished these influxes. Five minutes was sufficient for the inhibitors to exert their maximal effects, except for wortmannin, which required 30 minutes.

Effects of MLCK Sense and Antisense on TG- and CPA-Induced Ca\(^{2+}\) Responses

To provide further independent evidence that MLCK regulates CCE, we transfected human monocytes/macrophages with MLCK sense or antisense oligonucleotides and tested Ca\(^{2+}\) responses to TG and CPA in these cells. To confirm that the oligonucleotides were transfected into cells, the 5'-ends were conjugated with FITC, the complexes were transfected, and the cells were observed under a fluorescence microscope. All the cells displayed bright FITC fluorescence, indicating that they all contained MLCK sense and antisense oligonucleotides (please see http://atvb.ahajournals.org). Figure 6 shows the Ca\(^{2+}\) responses of control, sense, and antisense cells to 1 \(\mu\)mol/L TG (Figure 6A) and 100 \(\mu\)mol/L CPA (Figure 6C). In antisense cells, Ca\(^{2+}\) responses to TG and CPA were completely prevented; there were only small transient rises similar to those observed under Ca\(^{2+}\)-free conditions. However, MLCK sense oligonucleotides had no effects on TG- and CPA-induced Ca\(^{2+}\) responses. In Ca\(^{2+}\)-free medium, small and transient rises in \([Ca^{2+}]_i\) were still observed after treating MLCK antisense cells with 1 \(\mu\)mol/L TG (Figures 6B) or 100 \(\mu\)mol/L CPA (Figure 6D). A similar transfection protocol with only Superfect Reagent showed no effect on TG- and CPA-induced CCE (data not shown).

Discussion

The ER Ca\(^{2+}\)-ATPase inhibitors TG and CPA largely increased [Ca\(^{2+}\)], in human monocytes/macrophages in Ca\(^{2+}\)-containing medium but did so only very slightly under Ca\(^{2+}\)-free conditions. This indicates that the bulk of [Ca\(^{2+}\)]\(_i\) rise was due to Ca\(^{2+}\) entry. In Figure 1C, Ca\(^{2+}\) entry was observed on the restoration of extracellular Ca\(^{2+}\) after a short incubation in Ca\(^{2+}\)-free conditions after CPA removal. Because the store depletion stimulated by CPA was apparently the only mechanism to trigger the Ca\(^{2+}\) entry under this condition, this observation clearly indicates CCE in human monocytes/macrophages. This is similar to Ca\(^{2+}\) entry mechanisms in many other cell types.\(^{28}\)
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Figure 5. Comparative effects of various kinase inhibitors on human monocyte/macrophage CCE. Cells (n = 14) were incubated with 2 μmol/L fura 2-AM for 45 minutes at room temperature. Medium contained 1 mmol/L Ca2+. The cells were pretreated for 5 minutes with various kinase inhibitors; wortmannin pretreatment lasted for 30 minutes. Solid and shaded bars indicate peak and plateau Ca2+ responses, respectively, after treatment with TG (A) and CPA (B). Concentrations are as follows: TG, 1 μmol/L; CPA, 100 μmol/L; ML-9, 100 μmol/L; wortmannin (WT), 100 μmol/L; genistein (GEN), 100 μmol/L; herbimycin A (HA), 100 μmol/L; bisindolylmaleimide I (BIM), 10 μmol/L; and staurosporine (STR), 0.3 μmol/L. *p < 0.05 vs respective values by TG and CPA; †p < 0.05 vs respective values by ML-9.

The first clue to the involvement of MLCK in the regulation of CCE in human monocytes/macrophages was that ML-9 and wortmannin, structurally different MLCK inhibitors, completely prevented this entry at 100 μmol/L. ML-9 is a potent MLCK inhibitor (Kᵢ = 3.8 μmol/L) that competes with ATP for binding to the kinase.29 Because ML-9 at high doses can also inhibit PKC (Kᵢ = 54 μmol/L) and PKA (Kᵢ = 32 μmol/L), its dose-dependent inhibition of the Ca2+ response was not enough to implicate MLCK in CCE. However, similar effects were observed with structurally unrelated wortmannin, an inhibitor of phosphatidylinositol-3 kinase and MLCK.30 It was used in the present study as an MLCK inhibitor, because TG and CPA mobilize ER Ca2+ not through the activation of phosphatidylinositol-3 kinase. This similarity suggests specificity to MLCK of the effects by both agents to inhibit CCE. The peaks of the Ca2+ responses in Figure 2B seemed delayed, but this cannot be interpreted as an inhibitory effect of wortmannin on store depletion. Ca2+ rises due to store release constitute a very small portion of the rising phases of the Ca2+ response curves. Furthermore, as shown in Figure 3, wortmannin did not affect store mobilization. This delay was not seen with ML-9. In Figure 2C, Ca2+ entry was observed only after the removal of ML-9. This observation and a comparison with Figure 1C clearly show that ML-9 was able to inhibit CCE in monocytes/macrophages.

Further clues to the involvement of MLCK in CCE came from the findings that wortmannin and ML-9 were able to completely inhibit TG-induced MLC phosphorylation at concentrations that completely inhibited TG-induced CCE. IC₅₀ values for ML-9 to block MLC phosphorylation and CCE were also similar, according to Figure 4C. The correlation between the effects of ML-9 to inhibit CCE and MLC phosphorylation further indicates that MLCK was involved in the effect of the compound to inhibit CCE. MLCK phosphorylation by MLCK is linked to cellular contractile activity; therefore, it could be that MLCK activation after Ca2+ store release might rearrange the cytoskeleton, which would open the gate for Ca2+ entry. Nevertheless, MLCK-regulated CCE and MLC phosphorylation could just as likely be independent events; thus, the extent of MLCK phosphorylation would be considered in this regard only as a measure of MLCK activity. In fact, in another series of experiments, calyculin A, a phosphatase inhibitor that should increase MLC-phosphorylated forms by preventing them from being degraded, neither caused any Ca2+ entry nor enhanced the TG-induced Ca2+ responses (authors’ unpublished data, 2000).

Various kinases have been implicated in CCE. Particularly, the PKT inhibitor genistein was found to inhibit CCE in various cells, including fibroblasts,15 lymphocytes,31 platelets,32 and endothelial cells.3316 PKC involvement in CCE has been controversial. In Xenopus oocytes, activation of PKC was found to inhibit CCE,34 whereas in endothelial cells, we showed that inhibitors of PKC and PKA did not affect agonist-induced Ca2+ entry.35 In the present study, ML-9 and wortmannin abolished TG- and CPA-induced CCE, whereas inhibitors of PKT and PKC had only partial effects. The observations that bisindolylmaleimide I, a strong PKC inhibitor (Kᵢ = 0.01 μmol/L), PKA (Kᵢ = 2.0 μmol/L),36 and staurosporine only partially inhibited TG- and CPA-induced CCE make it more likely that the effects seen with ML-9 shown in
Figure 2A were due to MLCK inhibition. These data suggest that MLCK may play a more important role than do PTK and PKC in CCE in human monocytes/macrophages. MLCK has several potential phosphorylatable sites that can be phosphorylated by other protein kinases, including PKC and PTK. In fact, the PTK inhibitor genistein was shown to reduce MLCK phosphorylation. It is possible, then, that MLCK, partially influenced by PKC and PTK, lies downstream from these 2 kinases in the CCE signaling cascade and plays a more decisive role in the regulation of this cascade in human monocytes/macrophages. This hypothesis seems to explain the proportional contribution of PTK, PKC, and MLCK to the regulation of CCE in human monocytes/macrophages. The experiments above suggest that MLCK may be important in CCE activation in human monocytes/macrophages and that MLCK may be more important than PTK and PKC in this regard. Nevertheless, because all the inhibitors used significantly inhibited CCE, they could be accomplishing this by some nonspecific effects on the cells. The observations that transfection with MLCK antisense completely prevented CCE but that MLCK sense had no effect provide solid independent evidence that MLCK plays a crucial role in the regulation of CCE in human monocytes/macrophages. The coupling role is clear because neither the MLCK inhibitors nor MLCK antisense affected the emptying of the Ca2+ store by TG and CPA.

In conclusion, we have provided solid evidence that MLCK has a crucial role in the regulation of CCE in human monocytes/macrophages. Together with our previous findings in endothelial cells, the present study suggests a possible involvement of MLCK in the pathogenesis of atherosclerosis, the initiation of which involves both of these cell types. The present study suggests the involvement of MLCK in many Ca2+-dependent activities of these cells. Further investigations are under way to test these hypotheses.

Acknowledgment

This study was supported by a grant-in-aid for scientific research (No.10670642) from the Ministry of Education, Science, Sports, and Culture of Japan.

References

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doi: 10.1161/01.ATV.21.4.509

_Arteriosclerosis, Thrombosis, and Vascular Biology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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