Mouse Model of Transplant Arteriosclerosis
Role of Intercellular Adhesion Molecule-1

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Abstract—Transplant-accelerated arteriosclerosis in coronary arteries is the major limitation to long-term survival of patients with heart transplantation. The pathogenesis of this disease is not fully understood. Herein, we describe a simplified model of artery allografts in the mouse that allows us to take advantage of transgenic, knockout, or mutant animals. Common carotid arteries or aortic vessels were end-to-end allografted into carotid arteries between C57BL/6J and BALB/c mice. Neointimal lesions were observed as early as 2 weeks after surgery and had progressed at 4 and 6 weeks postoperatively. The lumen of grafted arteries was significantly narrowed due to neointima hyperplasia 4 weeks after transplantation. Using this model, we studied the role of intercellular adhesion molecule-1 (ICAM-1) in the development of transplant arteriosclerosis in ICAM-1–deficient mice. Neointimal lesions of artery grafts from ICAM-1 –/– C57BL/6J to BALB/c mice were reduced up to 60% compared with wild-type controls. MAC-1 (CD11b/18)-positive cells adhering to the surface of ICAM-1 –/– artery grafts were significantly less as identified by en face immunofluorescence, and these positive cells were more abundant in intimal lesions of artery grafts in wild-type mice. Furthermore, the major cell component of neointimal lesions 4 weeks after surgery was found to be α-actin–positive smooth muscle cells, which were significantly reduced in lesions of ICAM-1 –/– artery grafts. Thus, this model has been proven to be useful for understanding the mechanism of transplant arteriosclerosis. Our findings demonstrate that ICAM-1 is critical in the development of allograft arteriosclerosis via mediation of leukocyte adhesion to, and infiltration into, the vessel wall.

Key Words: transplant arteriosclerosis ■ mouse models ■ ICAM-1 deficiency

A llograft accelerated-transplant arteriosclerosis is the main limitation to long-term survival of patients with organ transplantation.1,2 A hallmark of lesions is mononuclear cell infiltration into the vessel wall of grafts at the early stage, followed by neointimal formation, which largely constitutes smooth muscle cells (SMCs).3 Although the pathogenesis of the disease remains to be studied, it has been observed that the earliest cellular event is leukocyte adhesion to the endothelial surface of the vessel wall within transplanted organs. Subsequently, T cells and monocytes infiltrate into the arterial wall.4 However, the molecular mechanisms by which leukocytes are continuously recruited to the vessel wall of grafted organs in vivo is not fully elucidated.

Intercellular adhesion molecule-1 (ICAM-1), a surface glycoprotein of the immunoglobulin superfamily, contains 5 immunoglobulin-like motifs in its extracellular domain, followed by a single transmembrane region and a short cytoplasmic tail.5 6 Several lines of evidence suggest that ICAM-1/MAC-1–dependent cellular interaction is involved in a number of inflammatory processes and in arteriosclerosis via mononuclear cell adhesion and migration.7–10 ICAM-1 is a counterreceptor for the β2 leukocyte integrins MAC-1 (αβ, CD11b/CD18) and LFA-1 (αβ, CD11a/CD18), and their engagement results in leukocyte adhesion and transmigration through the endothelium.11

Attracted by the well-defined genetic systems, a number of investigators have begun to use the mouse as an experimental system for transplant arteriosclerosis research.12–15 Hundreds of inbred lines have been established, the genetic map is relatively well-defined, and both congenic strains and recombinant strains are available to facilitate genetic experimentation. In just a few years, murine lipoproteins have been characterized, genetic variants of apolipoproteins identified,16–17 and genetic variation in susceptibility to atherosclerosis among inbred mouse strains demonstrated. Several mouse models manifesting lesions resembling human transplant arteriosclerosis have been developed18–22 and have helped address specific interventional issues and the mechanism of the disease. The main limitation to the mouse model is the technical complexity, which requires an extended training period for the operator to become proficient. Therefore, we
decided to establish a simplified mouse model of transplant arteriosclerosis that uses a cuff technique.

In the current study, we performed allografts of carotid arteries or aortas to carotid arteries between C57BL/6J and BALB/c mice, evaluated the role of ICAM-1 in the development of allograft lesions by using ICAM-1–deficient mice, and demonstrated that ICAM-1 plays an important role in the pathogenesis of transplant arteriosclerosis.

Methods

Mice and Artery Allograft Procedure

All animal experiments were performed according to protocols approved by the Institutional Committee for Use and Care of Laboratory Animals. Artery transplantation was performed between BALB/c (H2d) and C57BL/6J (H2b) (Charles River Laboratories, Sulzfeld, Germany) or ICAM-1–deficient mice of the C57BL/6J strain, which were purchased from The Jackson Laboratory, Bar Harbor, Me. Three genotypes of ICAM-1+/−, +/+ , and +/+ mice were identified by using The Jackson Laboratory’s polymerase chain reaction (PCR) protocol (primers: oMR017 5′-CTG AGC CAG GTC TCG-3′; oMR018 5′-GAG CGG CAG AGC AAA AGA AGC-3′; and oMR019 5′-AGG ACA GCA AGG GGG AGG ATT-3′). The mice were maintained on a light/dark (12/12-hour) cycle at 22°C and received food and water ad libitum.

Two types of arteries, the carotid artery and aorta, were used as organ donors in the current study. Mice were anesthetized with pentobarbital sodium (50 mg/kg body weight IP), and heparin (100 U in 100 μL of saline solution) was administered via the inferior vena cava. After 3 minutes, the right common carotid artery was exposed and removed (1 cm). For aorta preparation, a midline abdominal incision was made in the donor animal, the anterior thoracic cage was opened from the diaphragm and incised laterally to the internal mammary vessels, and the aorta was removed. Each vessel graft was harvested carefully to avoid mechanical injury during surgical preparation. All grafts were washed with saline solution containing 100 U/mL heparin.

Recipients were anesthetized, and atropine sulfate (1.7 mg/kg body weight IP) was administered to maintain the respiratory tract in good condition. The operation was performed under a dissecting stereomicroscope (Olympus SZH 10). The mouse was fixed in a supine position with its neck extended. A midline incision was made on the ventral side of the neck from the lower mandible to the sternum. The right cleidomastoid muscle was resected. Figure 1
shows the transplantation procedure. The right common carotid artery was mobilized free from the bifurcation in the distal end toward the proximal end as far as possible. The vessel was ligated with an 8-0 silk suture and dissected between the middle ties. The proximal and distal portions of the artery were passed through cuffs made of an autoclavable nylon tubing, 0.63 mm in outside diameter and a 0.5-mm inside diameter (catalog No. 800/200/100/200; Portex Ltd). The cuff length was 1 mm, with a 1-mm “handle” or extension. The vessel, together with the handle, was fixed by microhemostat clamps (4 mm long, Martini). The suture at the end of the artery was removed, and a segment of the artery was everted over the cuff body and fixed to the cuff with an 8-0 silk suture. Another portion of the artery was similarly prepared (Figure 1A).

The arterial segment was implanted between the 2 ends of the carotid artery by sleeving the ends of the arterial segment over the artery cuff and ligating them together with the 8-0 suture (Figures 1B to 1D). The vascular clamps were removed and evidence of pulsations was sought in both the grafted and native vessels. If there were vigorous pulsations in the transplanted vessel, the skin incision was closed with a 6-0 interrupted suture. About 30 minutes were needed to perform the whole operation, and the ischemia time of artery segments was between 5 and 10 minutes.

For histological analysis, perfusion was performed as described previously.23 In brief, mice were anesthetized, perfused with 0.9% NaCl solution via cardiac puncture to the left ventricle, and subsequently perfusion-fixed with 4% phosphate-buffered formaldehyde (pH 7.2) for 2 and 5 minutes, respectively. The grafts were harvested at 1 day or 1, 2, 4, and 6 weeks postoperatively (6 to 8 randomly chosen mice at each time point) by cutting the transplanted segments from the native vessels at the cuff end. Samples were fixed with 4% phosphate-buffered formaldehyde at 4°C for 24 hours. For frozen section preparation, mice were killed by cervical dislocation, and arterial grafts were harvested, immediately frozen in LN2, and stored at −80°C.

**Histological and Morphometric Analyses**

The grafts were processed for routine histology and embedded in paraffin. Sections (4 μm) began at the center of the graft and were stained with hematoxylin and eosin (HE) for histological evaluation. Sections were reviewed for quantification of the lesions with a BX60 microscope (Olympus Optical Co, Ltd) equipped with a Sony 3CCD camera and television monitor. The intima was defined as the region between the lumen and the internal elastic lamina. The media was defined as the region between the internal and external elastic laminae. By using a transmission scanning microscope (Zeiss LSM 510, Zeiss) equipped with a 488-nm argon-ion laser and Plan Neofluar 10×/0.3 oculars and interfaced to the program Start LSM 510, images were first scanned, saved, and then overlaid with different lines to trace the lumen, the internal elastic lamina, and the external elastic lamina. The (neo)intimal area was determined by subtracting the area of the lumen from the area enclosed by the internal elastic lamina. The medial area was determined by subtracting the area enclosed by the internal elastic lamina from the area of the external elastic lamina. Six to 8 cross sections were obtained by selecting the first of every 3 sections from each graft. Areas were measured and recorded in square micrometers. In the statistical analyses, the individual values for the area from each animal at each time point (1, 2, 4, and 6 weeks) were averaged. From these data, the neointima/media ratios were obtained.

The number of cell nuclei was counted in the intima, media, and adventitia region by using an eyepiece equipped with a measuring grid (WH10X-H with SQ10/10, Olympus). The area of the eyepiece grid measured 1×1 mm and consisted of 100 units (10×10). For counting the number of cell nuclei, a magnification of 400X was used. In each section, the number of cell nuclei was determined in 10 units measuring 0.025 mm×0.025 mm each (area of 625 μm² per unit), and the mean values were recorded for each histological section.

**Immunofluorescence Staining**

The procedure used for immunofluorescent staining was similar to that described previously.24 In brief, serial 5-μm-thick frozen sections were labeled with rat monoclonal antibodies against mouse...
MAC-1 (CD11b/18) leukocytes, CD4, and CD8 (PharMingen) or a mouse monoclonal antibody against α-actin conjugated with FITC (Sigma Chemical Co). Sections were then labeled with rabbit anti-rat antibodies conjugated with FITC. After being washed, the sections were examined with a fluorescence microscope.

En Face Immunofluorescence

The procedure used in this experiment was similar to that described previously. In short, each arterial graft segment was cut longitudinally, mounted endothelium side up on a glass slide (2.6×7.5 cm), and air-dried for 1 to 2 hours at room temperature. The segments were fixed in cold acetone (−20°C) for 10 minutes and rinsed in PBS. The segments were then incubated with appropriately diluted rat monoclonal antibody to MAC-1 for 30 minutes. After being washed in PBS, they were incubated with FITC-labeled rabbit anti-rat immunoglobulin for 30 minutes and washed again 3 times with a change of PBS every 30 minutes. All incubations were performed at room temperature. Finally, sections were mounted in gelvatol/PBS and examined with the fluorescence microscope.

Statistical Analysis

Statistical analyses were performed on a Macintosh computer (GraphPad Prism 2.0 program) using ANOVA and Student’s t test. Results are given as mean±SEM. The level for statistical significance was set at values of \( P<0.05 \) for all comparisons.

Results

Figure 1 demonstrates the procedure of an arterial segment transplanted to the carotid artery by using the cuff technique, by which 140 arterial grafts between C57BL/6J (H2b) and BALB/c (H2d) mice were studied with a surgical success rate of ≈90%. About half an hour was needed for performing the operation, and ischemia time of graft segments was between 5 and 10 minutes. The vessel grafts were harvested at various time points and histologically examined, and 5 grafts per group were analyzed by immunofluorescence.

Carotid or Aortic Allograft Arteriosclerosis

Representative histological sections of control carotid artery and arterial grafts are shown in Figure 2. In the control artery, 4 to 5 layers of cells formed the intima and media, whereas the adventitia was composed of connective tissues (Figure 2a). Obviously, cell infiltration into the media and neointimal formation were found in the allografts of C57BL/6J to BALB/c mice 2 weeks postoperatively (Figure 2b). By 4 weeks, neointimal area was enlarged, resulting in a significant stenosis of the vessel (Figure 2c).

Using the same technique, we performed experiments with aortic segments as grafts transplanted (C57BL/6J to BALB/c mice) to carotid arteries. Figure 3 shows the results, which indicate neointimal development in grafted arteries. Like-wise, stenosis of the vessels was observed by 4 weeks, and the lumen was completely occluded (2 of 6 grafts) by 6 weeks after transplantation (Figures 3e and 3f). To statistically analyze lesion development, Figure 4 summarizes data of neointima thickness measured microscopically. Neointima appeared as early as 2 weeks after surgery, although no significant difference was found compared with controls. The neointimal thickness increased in 2-week artery grafts and
significantly progressed throughout all time points. During lesion development, 4- and 10-fold increases in neointimal area were found in 4- and 6-week grafts, respectively (Figure 4). In addition, experiments with aortic isografts implanted to carotid arteries in BALB/c mice were also performed, but no neointimal lesions were observed 4 weeks after transplantation (data not shown).

**Reduced Lesions in ICAM-1 –/– Allografted Arteries**

The arterial wall of ICAM-1 –/– and wild-type mice is similarly composed of intima, media, 4 or 5 layers of smooth muscle cells and adventitia, and a small amount of connective tissue (Figures 3a and 3d). Transplanted arteries donated by wild-type C57BL/6J mice at 4 and 6 weeks (Figures 3e and 3f) showed neointimal hyperplasia, ie, thickening of the vessel wall up to 20 layers of cells. Interestingly, neointimal lesions of arterial grafts from ICAM-1 –/– to BALB/c mice showed a marked reduction at 4 as well as 6 weeks (Figures 3b and 3c). Figure 4 summarizes data of neointima thickness and the ratio of neointima and media as measured microscopically. A significant difference in lesion size of transplanted arteries derived from ICAM-1 –/– and wild-type mice was found ($P<0.01$). Neointimal lesion area of artery grafts donated by ICAM-1 –/– mice was reduced 50% to 60% compared with wild-type controls (Figure 4).

**Figure 4.** Reduced neointima thickness of artery grafts from ICAM-1 –/– mice. The procedure for animal models and the preparation of HE-stained sections are the same as described in the legend to Figure 3. Neointima and media of allografted aortas were measured microscopically. Four to 6 sections per animal were selected, and data show a graph of mean±SEM obtained from 6 to 8 animals per group. *Significant difference between ICAM-1 –/– and ICAM-1 +/+ groups, $P<0.01$.

**Reduced Lesions in ICAM-1 –/– Allografted Arteries**

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**Figure 5.** Neointimal lesions in aorta allografts from BALB/c mice to ICAM-1 +/+ or –/– mice. Areas of the lumen, neointima, and media were measured 4 weeks after surgery. Three to 5 sections per animal were selected, and data show a graph of mean±SEM obtained from 5 or 6 animals per group.

**Figure 6.** Demonstration of leukocyte adhesion to the endothelium of aortic allografts by en face immunofluorescence. Vessel graft segments (0.5 to 0.8 cm) obtained from ICAM-1 +/+ (a through c) and ICAM-1 –/– (d) mice at time 0 (b; freshly harvested aorta) or 1 day (a, c, and d) after surgery were mounted on glass slides, air-dried, fixed with cold acetone for 10 minutes at –20°C, and labeled with a rat monoclonal antibody identifying MAC-1–positive leukocytes (b through d) or with normal rat immunoglobulin (a). Positive cells were visualized by a FITC-conjugated rabbit anti-rat immunoglobulin antibody. Arrows indicate examples of positive cells. Original magnification ×250.
To study any effects of ICAM-1 expression of other cells or organs on neointimal formation, an artery transplantation from BALB/c to ICAM-1−/− knockout mice was performed. When aortic segments donated by BALB/c mice were allografted to carotid arteries of ICAM-1 −/− or ICAM-1 +/+ mice, neointimal lesions developed in both types of mice (Figure 5). The lumen stenosis and the neointimal area of grafted arteries were different between ICAM-1 +/+ and ICAM-1 −/− recipients, although no statistical significance was found.

**Decreased Leukocyte Adhesion to and Infiltration in Artery Grafts of ICAM-1 −/− Mice**

We previously adopted the vessel en face immunofluorescence method for semiquantifying cells adhering to the endothelium of vascular segments. This method was generally useful in clarifying the kinetics and phenotypes of cells adhering to the vascular endothelial surface in vivo. Nonspecific reactivity was minimal in the negative control labeled with normal rat serum (Figure 6a), and cells adhering to the endothelial surface were positively stained with a rat monoclonal antibody recognizing MAC-1–positive leukocytes (CD11b/18; Figure 6b through 6d). A large number of MAC-1–positive leukocytes were observed adhering to the endothelium of transplanted artery segments of wild-type mice 1 day after operation (Figure 6c), whereas cells adherent to the surface of artery grafts from ICAM-1 −/− mice were much less profound (Figure 6d). Occasionally, MAC-1–positive stained cells were also seen on the surface of freshly harvested artery segments (Figure 6b). These results indicate that leukocyte adhesion to the endothelium is one of the earliest cellular events in transplant-accelerated arteriosclerosis.

To assay whether cell tethering to the endothelium is followed by transmigration and localization in the grafted artery, cells infiltrated in 1-week grafts were evaluated. Figures 7a through 7d shows the histological data that indicate cell infiltration. Normal arterial walls of both ICAM-1 +/+ (Figure 7a) and ICAM-1 −/− (Figure 7c) mice exhibited a similar structure, ie, 4 to 5 layers of cells, whereas increased numbers of cells in 1-week artery grafts were evident. Interestingly, abundant cell infiltration into the vessel wall donated by ICAM-1 +/+ mice (Figure 7b) was observed compared with that from ICAM-1 −/− mice (Figure 7d). When cell nuclei in the intima and media of control and grafted vessels were counted in 10 units of the measuring grid, total cell numbers of the grafts from ICAM-1 +/+ mice were significantly higher than those from ICAM-1 −/− mice 1 week postoperatively (Figure 7e).

Using immunofluorescent techniques, we found that these infiltrated cells in 1-week artery grafts were MAC-1–positive, CD4–positive cells (Figure 8) and rare CD8–positive T cells. MAC-1–positive cells were monocytes/macrophages, natural killer cells, and granulocytes. The majority of infiltrating cells in the grafts was mononuclear cells, ie, monocytes/macrophages. Abundant infiltration of these positive cells was found in the media of 1-week artery grafts from ICAM-1 +/+ mice (Figure 8b), whereas only low numbers of MAC-1–positive cells were seen in the artery grafts of ICAM-1 −/− mice (Figure 8c). MAC-1–positive monocytes/macrophages were also detected at the luminal surface 1 week after grafting in both ICAM-1 −/− and +/+ mice (Figures 8b and 8c), but these positive cells were rarely seen in the normal vessel (Figure 8a). Figure 8d summarizes the mean data obtained from 6 to 8 animals per time point. *Significant difference from the group of ICAM-1 +/+ mice, P<0.05.
SMC Proliferation in Neointima

A previous study had demonstrated the presence of abundant SMCs in neointimal lesions of transplanted arteries. In the current study, immunofluorescence staining with monoclonal antibodies against $\alpha$-actin on frozen sections also demonstrated the presence of abundant SMCs in neointima lesions 4 and 6 weeks after surgery (Figure 9). No positive staining was seen in artery segments labeled with normal rat serum as a negative control (Figure 9a). Strong staining was observed in sections of the grafted artery from ICAM-1+/+ mice 4 weeks postoperatively (Figure 9b). Importantly, the number of positively stained SMCs was markedly reduced in artery grafts donated by ICAM-1−/− mice at 4 weeks (Figure 9c). These observations indicate that the major cells in neointimal lesions 4 weeks or thereafter postoperatively are SMCs.

Discussion

Murine genetic models in which genes are overexpressed, deleted, or mutated have been developed. These mouse models have considerable advantages over other animal systems in that they overcome the need to administer factors or their inhibitors. In the current report, we establish and characterize a new model for the study of transplant-accelerated arteriosclerosis in mice. When used with allografts from mice subjected to targeted gene deletion, the model provide a powerful tool for dissecting the relative contributions of such genes, eg, ICAM-1, in the development of neointimal hyperplasia. When used with allografts that can be treated ex vivo with drugs or gene transfer, the vessel wall allows easy penetration by small molecules and plasmids from the adventitia side. By using this model, we believe that significant progress in understanding the pathogenesis and treatment of transplant-accelerated arteriosclerosis may be seen in the near future.

Shi et al established the first mouse model of transplant arteriosclerosis by end-to-side suturing of carotid artery segments to carotid arteries, and Koula et al developed aortic transplantation to the infrarenal aorta by an end-to-end anastomosis. These mouse models have proved to be useful tools in studying the pathogenesis of transplant arteriosclerosis. In the current study, we describe a simplified mouse model of transplant arteriosclerosis that has several advantages: First, the operative procedure is simple and easy to learn. The vast majority of investigators could perform the surgery after a short period of training time. Second, the traumatic and ischemic injuries to the grafts are minimal. Half an hour is needed to perform the whole operation by our trained surgeon, and the ischemic time of artery segments is between 5 and 10 minutes. Third, the success rate of surgery is higher since the operation is performed in the neck region and takes a shorter time. Finally, neointimal lesion development in the transplanted vessel segments of both carotid and aortic arteries is comparable to that of transplanted arteries by other methods. For instance, Shi et al demonstrated that neointimal lesions were reduced by 52% in allograft arteries donated by ICAM-1−/− mice, whereas our observations indicate a 60% reduction in neointimal lesions of ICAM-1−/− arteries (Figure 4). Taken together, our mouse model has proved to be a simple and powerful tool for studying the pathogenesis of the disease and therapeutic intervention for transplant arteriosclerosis.

Figure 8. Immunofluorescent staining demonstrates MAC-1-positive mononuclear cell and CD4 T cell infiltration in artery allografts. Sections derived from aorta allografts donated by ICAM-1+/+ (a and b) and ICAM-1−/− (c) mice at 1 week were labeled with normal rat serum (a), a rat monoclonal antibody (CD11b/18) against MAC-1-positive leukocytes (b and c), or a rat monoclonal antibody against mouse CD4 (d) and visualized by a FITC-conjugated rabbit anti-rat immunoglobulin antibody. Arrows indicate examples of positive cells. Original magnification ×250. Positive cells in the intima and media were counted in a field of 400× magnification. The graph (d) shows mean±SEM obtained from 5 animals per time point. *Significant difference from the group of ICAM-1+/+ mice, P<0.05.
In addition, 2 types of donor organs, carotid arteries and aortas, have been used to transplant into carotid arteries in the current experiments. Although accelerated arteriosclerosis develops in both allografts, the following differences exist: (1) From a technical point of view, aortic segments are relatively easy to be sleeved over the cuff, which can be used for grafting the vessel donated by smaller mice. Since the body weight of some mutant mice is less than one third of the wild-type controls, the diameter of the carotid artery is not big enough to anastomose with the end of the cuff. (2) The intimal lesion area of aortic grafts is mostly larger than that of carotid arteries. Possibly, mononuclear cells adhere more easily to the uneven surface of the aortic intima owing to the existence of many intercostal arterial branches. (3) Intimal lesions of carotid artery allografts are of relatively uniform size and shape because they lack branches. Therefore, both donor organs can be used for transplantation, depending on the donor size and the skill of the investigator.

Although the importance of ICAM-1 in mediating cell adhesion to the endothelium has been established, little is known about the role of ICAM-1 expressed in allografted vessels during the development of transplant arteriosclerosis. In the current study, we demonstrated that one of the earliest cellular events is leukocyte adhesion to the surface of allografted vessels, which declines in ICAM-1 –/– allografts. Obviously, these adherent cells are destined to infiltrate into the vessel wall, because the numbers of MAC-1–positive leukocytes in ICAM-1 +/- artery grafts are significantly higher than those in ICAM-1 –/– mice (Figure 8). Therefore, ICAM-1 adhesion molecules expressed in allografted vessels are critical in mediating leukocyte adhesion and infiltration. There is evidence that infiltrated (activated) monocytes/macrophages produce a large number of cytokines and proteinases, of which matrix metalloproteinases play an important role in initiating SMC migration. We observed that a hallmark of grafted vessels from ICAM-1 +/- mice at the early stages is macrophage infiltration into the media and SMC accumulation in the intima at the late stage. Both macrophage infiltration and SMC accumulation are reduced in ICAM-1 –/– allografted vessels. We postulate that infiltrated macrophages may be critical in initiating SMC migration from the media to the intima, where they proliferate to form arteriosclerotic lesions.

Several reports provide evidence that ICAM-1 is highly expressed on endothelial cells as well as SMCs of transplanted arteries, cardiac allografts, and atherosclerotic lesions. We have also observed that neointimal SMCs express ICAM-1 (data not shown). Given the fact that SMCs of ICAM-1 –/– mice do not express ICAM-1, which is correlated with reduced neointimal lesions, we postulate a crucial role for ICAM-1 expression on SMCs in the development of intimal hyperplasia. The interaction of MAC-1 and ICAM-1 expressed on SMCs may initiate the intracellular signaling necessary for cytokine secretion by monocytes/macrophages. Support for this notion comes from the fact that macrophage inflammatory protein-1α production was induced in monocytes cultured on ICAM-1–coated plates. In addition, it has been reported that expression of ICAM-1 on SMCs may be relevant to the phenotypic change of SMCs, which is considered to be essential to their migration and proliferation in the pathogenesis of atherosclerosis. Therefore, the interaction of MAC-1 with ICAM-1 on SMCs might also play a part in the pathogenesis of transplant arteriosclerosis.

In summary, we have established a new mouse model of transplant arteriosclerosis, which has proved to be a powerful tool for studying the mechanisms of disease development. We have provided solid evidence that neointimal lesions are reduced up to 60% in artery allografts donated by ICAM-1

![Figure 9](http://atvb.ahajournals.org/)

**Figure 9.** Immunofluorescence labeling of α-actin in allograft sections. Cryostat sections from aorta allografts donated by ICAM-1 +/- (a and c) or +/- (b) mice 4 weeks after surgery were fixed with cold 5% acetone/methanol for 30 minutes, air-dried, and incubated with normal mouse serum (a) or a mouse monoclonal antibody against α-actin conjugated with FITC (b and c) for 30 minutes at room temperature. Original magnification 250×.
—/— mice. If other adhesion molecules, including vascular cell adhesion molecule and selectins, which have also been identified as being expressed in the arterial wall, could be “knocked out” or inhibited in parallel, transplant arteriosclerosis might be prevented or retarded. Thus, further study on the role of adhesion molecules could provide valuable information for the therapeutic intervention of transplant arteriosclerosis.

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