AGING AND ARTERIOSCLEROSIS

I. Development of Myointimal Hyperplasia after Endothelial Injury

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Vascular endothelial damage stimulates the proliferation of vascular smooth muscle cells and their migration into the intima of the blood vessel (1, 2). As myointimal hyperplasia is seen in arteriosclerosis, endothelial injury may be an important factor in the development of the atherosclerotic plaque (3). Endothelial damage that occurs over decades may contribute to the increased prevalence of arteriosclerosis with age. Recently, Stemerman and his coworkers (4) reported that the proliferation of vascular smooth muscle cells after balloon aortic endothelial denudation was more pronounced and lasted longer in old as compared to young rats. This suggests that the susceptibility to arteriosclerosis may increase with age. Stemerman’s important studies directed attention to an age-dependent difference in the response of vascular smooth muscle cells to injury. They did not determine whether the increased susceptibility of old animals to myointimal hyperplasia resulted from changes intrinsic to the vascular tissue or from changes in the internal milieu of the host. We set out to determine whether the increased myointimal hyperplasia following endothelial injury in old animals is an intrinsic property of vascular tissue.

In our studies, rat aortic endothelium was injured by a nondistending coiled wire catheter. We found that old rats have a greater myointimal proliferative response after endothelial injury compared to young animals. Next, we studied whether the myointimal hyperplasia observed in old rats was a function of the age of the arterial tissue or the host environment. Aortic segments from young or old Fischer 344 rats were transplanted into syngeneic recipients. We compared the myointimal hyperplasia that followed surgical trauma of transplantation of the grafted arterial segment from young and old donors. Severe myointimal hyperplastic lesions were seen 6 wk after transplantation in aortic grafts from old animals. Young aortic segments transplanted into old animals did not show this hyperplastic response. These results suggest that the age-associated change in the vascular response to endothelial injury is intrinsic to the older vascular tissue.

Materials and Methods

Experimental Animals. Male Fischer F-344 albino rats were obtained from the National Institute of Aging breeding facilities at the Charles River Laboratories, Wilmington, MA.

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Young (2–5-mo-old) rats and old rats (24–30-mo-old) were housed in steel cages, with access to chow (Purina Rodent Diet; Charleston Purina Company) and to water ad libitum. Animals were provided with light between the hours of 6 a.m. and 6 p.m. daily, in a temperature-controlled environment (76–78°F).

**Nondistending Aortic Endothelial Injury.** We modified the method of Baumgartner (5) for aortic deendothelialization without the vascular distension associated with the use of a balloon catheter. Rats were anesthetized with methohexital (Brevital) 50 mg/kg i.p. The left carotid artery was isolated from its bifurcation to the clavicle. The carotid artery was ligated distally. A Duoflex stainless steel 0.64 mm x 30.5 cm catheter guide wire (Arrow International Inc., Reading, PA) with a 30° bend 1.0 mm from tip was inserted through a small longitudinal arteriotomy in the carotid, and passed down to the level of the iliac bifurcation. The wire guide was then passed back and forth in the aorta four times, rotating the wire one-quarter turn each time. Deendothelialization of the abdominal aorta was accomplished by light external abdominal pressure on the aorta against the advancing guide wire. The carotid artery was then ligated and the neck incision closed with 4-0 nylon suture.

**Microsurgical Transplantation of Abdominal Aortic Grafts.** Animals were anesthetized and given subcutaneous injections of Brevital as necessary during the course of surgery. Hair was removed from the abdomen. The skin was then washed with water followed by Povidone iodine and 70% ethanol. The abdomen was opened from the xiphoid to ~1 cm above the pubis. The infrarenal aorta was exposed under the operating microscope from the right renal artery to the inferior mesenteric artery. Any aortic branches observed in this segment were identified, doubly ligated with 8.0 monofilament nylon suture, coagulated with a unipolar ophthalmic cautery (I-Stat; Concept Medical Industries, Clearwater, FL) and divided. The donor segment of the aorta was then isolated at its inferior and superior ends with 4-0 silk ligatures, which were placed but not tied. Two microaneurysm clips were placed, ~1.5 cm apart, at the ends of the donor segment of the abdominal aorta and cut 1.5 mm beyond the aneurysm clips. This aortic cuff was then irrigated with heparinized Ringers solution (1 u/ml) and the lumen was diluted by the insertion of the tips of microforceps and gentle dilation of the walls of the vessel. At all times, the aortic graft was kept moist with Ringers solution. Dilation of the vessel was enhanced by the topical use of a 0.5% lidocaine solution.

Exposure of the aorta was obtained similarly in recipients. Stay sutures were placed 180° apart at both the inferior and superior sites of reimplantation. The anastomosis was completed using standard microsurgical technique; 16–20 interrupted 10-0 monofilament nylon sutures completed the inferior and superior anastomosis of the vessel. Small pads of gelfoam were placed around the suture lines. The distal clamp was removed first to allow backflow of blood into the graft. At this time, any air in the graft was gently milked out through the spaces between the sutures. Any leakage of blood at the suture line usually ceased within 1 min, at which time the proximal clamp was released and blood flow through the graft was reestablished.

After the reestablishment of blood flow, the external surface of the graft was irrigated with Ringers solution and 0.5% lidocaine. The abdominal viscera were returned to their anatomic positions and the abdomen was closed in two layers with 2-0 absorbable suture in the muscle and 3-0 nylon sutures in the skin. Animals were given 10 ml Ringers solution s.c., and 25 mg of gentamycin i.m. We were careful to prevent hypothermia during surgery. After surgery, the animals were returned to their cages and normal body temperature maintained by external heating lamps until they recovered consciousness.

**Pathological Studies.** 1 h before sacrifice, animals were anesthetized with Brevital and placed on the animal operating board. The femoral vessels were identified and cannulated with a PE-50 catheter attached to a stopcock and syringe. 5 ml of Evans' Blue solution was infused, allowing the identification of undamaged endothelia, which appeared blue. 55 min after the infusion of Evans' Blue, animals received a bolus of 100 U of heparin and 10 μg papaverine through the catheter. The animals were then killed by exsanguination. The thorax and abdomen was opened from the neck to the pubis by a single longitudinal incision. The aorta was identified and dissected free with the aid of an
TABLE I
Intimal Proliferation after Deendothelialization of Aortas of Fischer 344 Rats

| Age of rat | Days after injury | Intimal thickness |
|------------|-------------------|------------------|
|            |                   | Abdominal aorta  | Thoracic aorta |
| Young      | 3                 | 9                | 5              |
| Young      | 14                | 6                | 5              |
| Old        | 3                 | 10               | 5              |
| Old        | 14                | 45               | 8              |

Endothelial cells of the abdominal aorta were removed by passing a wire catheter into the abdominal aorta with external pressure applied to the vessel. Three young and three old animals were sacrificed 3 or 14 d after surgery, and the intimal thickness of the abdominal aorta is expressed as a percentage of the medial thickness. The thickness of the intima from the uninjured thoracic aorta, expressed as a percentage of the medial thickness, served as the control.

Results

Intimal Hyperplasia after Deendothelialization of Aortas in Young and Old Rats. The aortic myointimal proliferative response after abdominal aortic deendothelialization was measured in young and old Fischer 344 rats. Endothelial injury was achieved without distension of the aorta. Evans' Blue staining confirmed complete and uniform injury to endothelial cells of the abdominal aorta with little injury to the thoracic aorta. Intimal thickness of the abdominal and thoracic aorta was measured 3 or 14 d after injury (Table I). Intimal thickness of uninjured thoracic aorta was not significantly different in old or young rats. Intimal thickness, 3 d after endothelial injury, was twice that of uninjured aorta in both old and young animals. The results were very different 14 d after injury. Intimal thickness of the injured segments of aorta in young rats had regressed and there was no significant difference in intimal thickness of injured and uninjured aorta. In contrast, in old animals, intimal thickness had increased four-fold as compared with that observed 3 d after injury. Thus, 14 d after injury, the intimal thickness was 45% of the medial thickness.

Intimal Hyperplasia in Aortic Grafts from Young and Old Rats after Transplantation into Young Syngeneic Animals. The observation that aortic intimal hyperplasia following deendothelialization was greater in old as compared with young
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TABLE II

Intimal and Medial Thickness of Grafted and Adjacent Aortic Segments

| Donor age | Recipient age | n  | Host Intima (μm) | Graft Intima (μm) | Host Media (μm) | Graft Media (μm) | Time after grafting (wk) |
|-----------|---------------|----|-----------------|------------------|----------------|-----------------|--------------------------|
| Young     | Autografts    | 6  | 3.70 ± 2.0      | 4.33 ± 1.4       | 91.8 ± 8       | 78.9 ± 11       | 6                        |
| Old       | Autografts    | 4  | 5.70 ± 1.7      | 12.53 ± 4.2      | 72.3 ± 7       | 102.7 ± 12      | 6                        |
| Young     | Young         | 5  | 2.81 ± 1.1      | 3.42 ± 2.1       | 65.9 ± 9       | 81.4 ± 7        | 6                        |
| Old       | Young         | 8  | 2.80 ± 1.9      | 23.25 ± 6.3      | 80.6 ± 5       | 75.0 ± 6        | 6                        |
| Old       | Young         | 5  | 3.75 ± 1.9      | 14.06 ± 6.2      | 83.2 ± 6       | 87.9 ± 8        | 6                        |
| Young     | Old           | 4  | 3.87 ± 1.3      | 5.88 ± 2.0       | 83.7 ± 9       | 80.9 ± 6        | 6                        |

Autografts or syngeneic grafts of aortic segments were made. Animals were killed 6 or 15 wk after surgery. The thicknesses of the intima and media of the grafted aortic segments and adjacent recipient aortas were measured.

Rats lead us to investigate whether this phenomenon was intrinsic to the vascular tissue or to the internal milieu of the recipient. Initially, autografting was performed in young and old rats (Table II). The intimal thickness of reimplanted aortic segments in young animals was comparable to adjacent, unmanipulated aorta. In old animals, the intimal thickness of the reimplanted aortic segments was twice that of the adjacent aorta. The greater intimal hyperplasia after vascular injury in old as compared to young rats was consistent with the findings obtained following catheter-induced aortic endothelial damage in intact rats.

Next, aortic segments were transplanted from young or old animals into young recipients. The intimal thickness of grafted aortic segments from young rats was comparable to that of adjacent host aorta 6 wk after surgery (Table II and Figs. 1 and 2). In contrast, the intimal thickness of aortic segments from old rats was eight times that of the adjacent host aorta 6 wk after surgery. 15 wk after surgery, the difference in intimal thickness of the aortic segments from old rats and adjacent host aorta was less than at 6 wk but was still statistically significant ($p < 0.05$). There was no significant increase in intimal thickness in grafts from young animals transplanted into old recipients. The thickness of the media was the same in host aorta and in grafts from either old or young animals.

**Cellularity of Intima and Media in Grafted and Host Aorta.** The density of cells within the intima in grafts from old and young animals was compared. Specifically, the number of nuclei per $10^4 \mu m^2$ in grafted and host aortic segments was determined (Table III). In recipients of young aortic segments, there was no difference in the number of nuclei per unit cross sectional area in the grafts, as compared with adjacent aortic tissue. In contrast, in the recipients of old aortic segments, there was a highly significant ($p < 0.005$) increase in the cellularity in the graft compared to host intima 6 wk after surgery. The cellular hyperplasia was not significantly reduced 15 wk after surgery. Thus, increased intimal thickness in aortic segments from old rats was associated with hyperplastic response not observed in aortic segments from young rats.
Discussion

Our studies indicate that the response of the aorta to injury is significantly influenced by age. Aortic segments from old rats developed greater myointimal thickness and hyperplasia than did aortic segment from young animals in two separate models of vascular injury. Stemerman and his associates (4) had shown that deendothelialization of the aorta by balloon catheter stimulated a greater proliferative response in old compared to young rats. We have extended this observation by showing that aortic endothelial injury, induced without distending the aorta, led to a greater myointimal proliferative response in old as compared with young Fischer 344 rats.

The key finding in our study is that the increased myointimal proliferative response is an intrinsic property of the arterial tissue of old animals. Thus, aortic segments from old rats, when transplanted into young rats, showed the same hyperplastic intimal lesion after endothelial injury seen in the intact old rat. The thickness and cellularity of the intima of aortic segments from old rats grafted into young syngeneic recipients were very much increased in comparison to the thickness and cellularity of the adjacent aortic tissue of the recipient. In contrast, transplanted aortic segments from young rats did not differ from the adjacent aortic tissue of the recipient. We conclude that, in these two models of vascular injury, a greater myointimal proliferative response occurs in old compared to young rats. Most important, the transplantation studies show that the altered
myointimal proliferative response depends upon the age of the aortic graft donors.

The Fischer 344 strain of rats is inbred, and skin grafts can be exchanged without rejection (6). The existence of age-associated antigens in mice has been suggested (7, 8). For this reason, we looked for evidence of histoincompatibility between young and old Fischer 344 rats. Skin grafts between old and young Fischer 344 rats survived for more than 3 mo with no macroscopic evidence of rejection, and there was no difference in the capacity of irradiated spleen cells from old or young Fischer 344 rats to stimulate the proliferation of T cells from young syngeneic animals (data not shown). Although different antigens might be expressed by the vascular tissue from young and old animals, transplantation of aortic segments from young to old Fischer 344 rats did not lead to increased intimal thickness or cellularity of the grafted young aortic tissue in comparison with the adjacent aortic tissue from the old recipient (data not shown).

The increased proliferation of cells within the intima may result from the excessive production of, or sensitivity to growth factors, or conversely to the deficient production of, or response to factors that inhibit cell growth. Whatever the precise pathogenic mechanism(s) that leads to the hyperplastic myointimal response after arterial injury, the increased prevalence of vascular disease with age probably results from both the increased susceptibility of the vasculature to injury, as well as repeated vascular injury over time that occurs during aging.

### Table III

| Donor  | Recipient | Nuclei per 10^4 μm² | Time after surgery (wk) |
|--------|-----------|---------------------|-------------------------|
|        |           | Intima | Media |                   |
|        |           | Host  | Graft | Host  | Graft |
| Young  | Young     | 33.5  | 32.1  | 59.7  | 58.3  | 6     |
| Old    | Young     | 53.4  | 146.2 | 38.0  | 68.3  | 6     |
| Old    | Young     | 29.3  | 154.3 | 48.6  | 42.4  | 15    |

The number of cell nuclei in the media and intima of the grafted and adjacent host aorta were counted in histological sections.
Summary

Old Fischer 344 rats are more susceptible to vascular lesions after arterial endothelial injury than are young animals. Thus, 20–26-mo-old Fischer 344 rats developed greater and more persistent intimal proliferative lesions than did 2–5-mo-old rats after aortic endothelial denudation. 3 d after deendothelialization, intimal thickness was increased two-fold in both old and young animals. However, 14 d after endothelial injury, intimal thickness had increased nearly five times in old animals, but had regressed to normal in young animals. Intimal thickness of young aortic grafts transplanted into young recipients did not differ significantly from adjacent host aorta or autotransplanted aortic segments 6 wk after surgery. In contrast, intimal thickness of old grafts transplanted into young recipients was eight times greater than adjacent young host aorta 6 wk after surgery. The density of cell nuclei in the intima of old grafts was also much greater than that in young grafts. Thus, in two experimental models of vascular injury, old rats have consistently had greater myointimal hyperplasia than young rats. The increased proliferative response of aortic smooth muscle cells after vascular injury of old animals may contribute to the increased prevalence of vascular disease with age.

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