The mouse aortocaval fistula recapitulates human arteriovenous fistula maturation

Kota Yamamoto,1,2,4 Clinton D. Protack,2,3 Masayuki Tsuneki,2,5 Michael R. Hall,2,3 Daniel J. Wong,2,3 Daniel Y. Lu,2,3 Roland Assi,2,3 Willis T. Williams,2,3 Nirvana Sadaghianloo,2,6 Hualong Bai,2,3 Tetsuro Miyata,4 Joseph A. Madri,2,5 and Alan Dardik1,2,3

1Veterans Affairs Connecticut Healthcare Systems, West Haven, Connecticut; 2Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, Connecticut; 3Department of Surgery, Yale University School of Medicine, New Haven, Connecticut; 4Division of Vascular Surgery, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 5Department of Pathology, Yale University School of Medicine, New Haven, Connecticut; and 6University Hospital of Nice, Nice, France

Submitted 7 August 2013; accepted in final form 1 October 2013

Yamamoto K, Protack CD, Tsuneki M, Hall MR, Wong DJ, Lu DY, Assi R, Williams WT, Sadaghianloo N, Bai H, Miyata T, Madri JA, Dardik A. The mouse aortocaval fistula recapitulates human arteriovenous fistula maturation. Am J Physiol Heart Circ Physiol 305: H1718–H1725, 2013. First published October 4, 2013; doi:10.1152/ajpheart.00590.2013.—Several models of arteriovenous fistula (AVF) have excellent patentcy and help in understanding the mechanisms of venous adaptation to the arterial environment. However, these models fail to exhibit either maturation failure or failure to develop stenoses, both of which are critical modes of AVF failure in human patients. We used high-resolution Doppler ultrasound to serially examine mice with AVFs created by direct 25-gauge needle puncture. By day 21, 75% of AVFs dilate, thicken, and increase flow, i.e., mature, and 25% fail due to immediate thrombosis or maturation failure. Mature AVF thickened due to increased amounts of smooth muscle cells. By day 42, 67% of mature AVFs remain patent, but 33% of AVFs fail due to perianastomotic thickening. These results show that the mouse aortocaval model has an easily detectable maturation phase in the first 21 days followed by a potential failure phase in the subsequent 21 days. This model is the first animal model of AVF to show a course that recapitulates aspects of human AVF maturation.

well as the use of animals larger than mice (1, 5, 8, 12, 15, 16). A common feature of all these models is that they are good models of surgical access, with good patency; unfortunately, they fail to exhibit a percentage of animals that either fail to mature or fail to develop stenoses in long-term followup, both of which are both important aspects of understanding modes of failure of human AVF.

Recent advances in ultrasound technology have allowed increasingly accurate analysis of blood flow within small vessels, such as in a mouse, as well as allowing the ability to serially examine the same mouse over time. We used this technology to observe the time course of venous remodeling in the mouse aortocaval puncture model (23) to determine whether this model is an accurate model of human AVF maturation and, in particular, whether a certain percentage of animals fail to mature successfully.

MATERIALS AND METHODS

Antibodies. Antibodies against mouse CD31 (PECAM-1) were raised in rabbits and purified as previously described elsewhere (18). Rabbit polyclonal antibodies against mouse α-smooth muscle actin (α-actin, ab5694) and Ki-67 (ab15580) were purchased from Abcam (Cambridge, MA).

Anesthesia and surgery. All animal experiments were performed in strict compliance with federal guidelines and with approval from the Institutional Animal Care and Use Committee of Yale University. The appropriate anesthesia and analgesia were given as described in our previous study (23). Male C57BL/6 mice (age: 10 wk and body weight: 20–30 g) were used for this study.

Briefly, 4% isoflurane in 0.8 l/min O2 was delivered via an isoflurane vaporizer for induction and decreased to 2–3% after mice were anesthetized. Under general anesthesia, a midline laparotomy was made, and the aorta and inferior vena cava (IVC) were exposed. The proximal infrarenal aorta and distal aorta were dissected for clamp placement and needle puncture, respectively; the vena cava was not dissected free from the aorta. After the aorta was clamped just below the left renal artery, a 25-gauge needle was used to puncture the aorta through into the IVC. The surrounding connective tissue was used for hemostatic compression. Successful creation of the AVF (“technical success”) was characterized by hemostasis as well as visible pulsatile arterial blood flow in the IVC.

Ultrasound. We performed Doppler ultrasound both pre- and postoperatively to confirm the presence of the AVF and to measure the sizes of the aorta, IVC, and heart. The Vevo770 High Resolution Imaging System (VisualSonics) with probe RMV704 (20–60 MHz) was used under general anesthesia as described above. For confirmation of an AVF, the waveform in the IVC was recorded using...
pulse-wave mode. The presence of arterial or turbulent waveforms in the IVC that were not detected before the AVF surgery was regarded as a patent AVF.

The diameters of the aorta and IVC just above, cranial to the renal veins, were recorded, as was the diameter of the heart at maximal diastole. Ultrasound testing was performed on preoperative day 1 and on postoperative days 1, 7, 14, 21, 28, 35, and 42. Flow velocity was measured by ultrasound, and shear stress was calculated using the Hagen-Poiseuille equation (13). The following equation was used:

\[ T = 4\eta Vr, \]

where \( T \) is shear stress, \( \eta \) is blood viscosity, \( V \) is flow velocity (in cm/s), and \( r \) is the radius (in cm). Blood viscosity was assumed to be constant at 0.035 poise.

---

**Fig. 1.** Assessment of arteriovenous fistula (AVF) technical success. 
A: schematic showing the definitions of the terminology used. IVC, inferior vena cava. 
B: ultrasound findings of the waveforms in the IVC. Top: cranial to the AVF (near the diaphragm); middle, at the AVF; bottom, caudal to the AVF (near bifurcation of IVC). 
C: cross-section at the AVF. Ao, aortic lumen; IVC, IVC lumen. Overgrowth of aortic tissue into the IVC can be seen (*). 
D: partial pressures of \( \text{O}_2 \) and \( \text{CO}_2 \) of the infrarenal IVC in the sham group and infrarenal venous limb of the AVF group. *\( P = 0.0008 \) by \( t \)-test. 
E: time course of the change of osteopontin (OPN) mRNA expression in the venous limb of the successful AVF group as well as the sham group.
**Histology.** After euthanasia, the AVF was extracted en bloc after a circulatory flush with PBS followed by 10% formalin. The tissue block was then embedded in paraffin and cut into 5-μm cross-sections. Hematoxylin and eosin as well as elastin van Gieson staining were performed for all samples.

For intima-media thickness measurements, samples were cut at 50–100 μm cranial to the fistula and stained with elastin van Gieson. The thickness was measured at eight equidistant points per cross-section and then averaged.

**Immunohistochemistry.** Immunohistochemistry was performed using the Dako EnVision + Dual Link System-HRP (Dako, Carpinteria, CA). For antigen retrieval, sections were heated in citric acid buffer (pH 6.0) at 100°C for 10 min using the Lab Vision PT Module (Thermo Scientific, Kalamazoo, MI). Sections were treated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature to block endogenous peroxidase activity and incubated with 5% normal goat serum in PBS (pH 7.4) containing 0.05% Triton X-100 for 1 h at room temperature to block nonspecific protein-binding sites. Sections were then incubated at 4°C with the primary antibodies diluted at 1:100 (anti-α-actin and Ki-67) and 1:200 (anti-CD31) in PBS containing 0.05% Triton X-100. After an overnight incubation, sections were incubated with EnVision reagents for 1 h at room temperature.

Fig. 2. Systemic effects of the AVF. A: survival curve comparing AVF and sham surgery groups (Kaplan-Meier statistics). B: photograph showing collateral veins (black arrows) developing in the abdominal wall of a mouse with a successful AVF (day 21). C: photograph showing ultrasound assessment of the dilated distal IVC (*), representing venous congestion of the lower limb due to arterial blood flowing into the IVC through the AVF (arrow). D: line graph showing the change in cardiac diameter over time, as measured using ultrasound. E: body weights of mice with either sham or successful AVF surgery (day 21).
and treated with the Dako Liquid DAB+ Substrate Chromogen System (Dako) to visualize the reaction products. Finally, sections were counterstained with Dako Mayer’s Hematoxylin (Lillie’s Modification) Histological Staining Reagent (Dako).

**Blood gas experiments.** \(P_O2\) in the IVC was measured using the i-STAT system (Abbott Laboratories). Blood samples were drawn from the infrarenal IVC, e.g., the venous limb of the AVF, while animals were under anesthesia (97–98% \(O_2\)) before samples were harvested.

**RNA extraction and quantitative PCR.** Total RNA from the venous limb of the AVF was isolated using the RNeasy Mini kit with digested DNase I (Qiagen); care was taken to avoid the surrounding arterial tissue. RNA quality was confirmed by the 260-to-280-nm ratio. Reverse transcription was performed using the SuperScript III First-Strand Synthesis Supermix (Invitrogen). Real-time quantitative PCR was performed using SYBR Green Supermix (Bio-Rad Laboratories) and amplified for 35 cycles using the iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories). Correct target amplification and exclusion of nonspecific amplification was confirmed by 1.5% agarose gel electrophoresis, and primer efficiencies were determined by melt-curve analysis. All samples were normalized by GAPDH RNA amplification.

**Statistical analysis.** All data were analyzed using JMP 9.0 software (SAS Institute). Comparison between groups was performed with multivariate ANOVA followed by post hoc tests. \(P\) values of <0.05 were considered significant.

**RESULTS**

**Assessment of fistula technical success.** AVFs were created in the mouse between the aorta and IVC as previously described (23). Successful technical creation of a patent AVF was assessed by several methods. First, intraoperatively, after hemostasis was obtained, visual inspection confirmed bright arterial blood flowing turbulently into the IVC through the AVF. Postoperatively, noninvasive Doppler ultrasound was used to examine the AVF. Definitions of the anatomy of the IVC in this study are shown in Fig. 1A. Caudal to the fistula, the Doppler waveform possessed typical venous morphology; at the AVF, the waveform was arterial with spectral broadening suggesting turbulence. Cranial to the AVF, the waveform was arterial, with diminished peak velocities compared with those at the AVF (Fig. 1B). Histological assessment of the AVF showed a patent fistula between the aorta and IVC (Fig. 1C). After 21 days, the AVF showed overgrowth of aortic tissue into the IVC, suggesting the perianastomotic neointimal hyperplasia that is typically seen in human AVF (asterisk in Fig. 1C).

Physiological assessment of the AVF was performed. Mice with patent AVFs showed significantly higher \(P_O2\) in the infrarenal IVC, e.g., the venous limb of the AVF, compared with sham mice without AVF; there were no differences in \(P_CO2\) (Fig. 1D). In addition, mice with AVFs showed significantly increased osteopontin mRNA expression compared with sham mice (Fig. 1E); osteopontin typically increases significantly in AVFs (3).

The survival of mice after AVF or sham surgery is shown in Fig. 2A. Early deaths were typically due to postoperative hemorrhage. After successful AVF surgery, these mice typically showed dilated abdominal wall veins (Fig. 2B) as well as dilation of the distal IVC (Fig. 2C), consistent with lower limb venous congestion from the AVF.

Cardiac diameter was assessed using ultrasound, showing enlargement in mice with AVFs compared with those with sham surgery (Fig. 2D). However, dilated cardiac diameter was not associated with any morphological signs of cardiac failure, including increased body weight (Fig. 2E) or late death (Fig. 2A), consistent with minimal systemic toxicity from the AVF.

**AVF patency over time.** Technical success was defined as visualization of arterial blood easily flowing into the IVC at the end of surgery. Technical success was assessed after each group of 50 procedures and was noted to increase with time, suggesting a learning curve for this surgical model (Fig. 3A).

Long-term patency of the AVF was assessed only in mice with a technically successful AVF after the procedure (Fig. 3B). In ~25% of the mice with a technically successful AVF on day 0, the AVF had no detectable flow on day 1, probably due to immediate thrombosis or very low flow velocity. Between days 1 and 28, some of the AVFs showed patency despite no detectable flow in the AVF on day 1. On the other hand, some of the AVFs that were patent on day 1 were
occluded on day 21. Overall, there was a slight increase in patency between days 1 and 21. Beginning at approximately postoperative day 28, ~33% of the patent AVFs failed; this failure was noted on ultrasound, without any systemic signs of AVF failure. This behavior of the AVF suggests that this model can be easily assessed on days 0, 1, 21, and 42 (Fig. 3B), with all the mice grouped into five groups according to their technical success, immediate thrombosis, and patency at days 21 and 42 (Fig. 4). Although the results shown in Fig. 4 reflect the general behavior of the AVF course, not shown is the potential for recanalization and increased flow of an AVF that was thought to be thrombosed or have low flow on day 1.

**AVF measurements.** Doppler ultrasound was used to serially examine the aortic and suprarenal IVC diameter in mice with AVF or sham procedures. After 21 days, mice with patent AVFs (“maturation success”) had dilation of the suprarenal IVC cranial to the AVF (38% increase) as well as of the aorta proximally leading into the AVF (27% increase). After 42 days, mice with patent AVFs (“AVF success”) had dilation of both the aorta (Fig. 5A, shaded bars) as well as suprarenal IVC (Fig. 5B, shaded bars); however, AVFs that were initially patent at day 21 and then ultimately failed by day 42 (“AVF failure”) had no significant differences in either aortic or IVC diameters from sham mice at day 42 (Fig. 5, A and B, hatched bars). In this AVF failure group, both aortic and IVC diameters were increased significantly at day 21, similar to the AVF success group (data not shown), suggesting that the lack of difference between sham and AVF failure diameters at day 42 (Fig. 1, A and B, open and hatched bars) were not due

![Fig. 4. Schema of the AVF groups.](image-url)

![Fig. 5. AVF assessment with ultrasound.](image-url)

A: bar graph summarizing the changes of aortic diameter after 42 days. *P < 0.05 compared with the sham group by ANOVA. B: bar graph summarizing the changes of suprarenal IVC diameter after 42 days. *P < 0.05 compared with the sham group by ANOVA. C: blood flow velocity in the supra- and infrarenal IVC. *P < 0.01 by ANOVA. D: diameter of the suprarenal and infrarenal IVC. *P < 0.01 by ANOVA. E: shear stress in the supra-and infrarenal IVC. *P < 0.01 by ANOVA.
to lack of any dilation in the AVF failure group but rather to a
decrease in diameters after occlusion of the fistula. Blood flow
velocity, IVC diameter, and shear stress were increased at 21
days in AVF mice compared with sham mice, although both
velocity and shear stress decreased, and IVC diameter in-
creased, as we followed the blood flow cranial from the AVF
to above the renal veins (Fig. 5, C–E).

Histological assessment was performed 150–200 μm above
the AVF, in the infrarenal IVC (Fig. 6, A–C), showed a
thickened venous wall in mice with successful AVFs compared
with a thinner venous wall in both preoperative and sham mice.
Quantification of the intima-media thickness of the venous
limb of the AVF confirmed significant thickening (Fig. 6D)
that was characterized by an increased number of α-actin-
positive cells without any change in CD31-positive cells
(Fig. 6E).

Since ~33% of the AVFs patent on day 21 show ultrasound
evidence of failure by day 42 (Fig. 3B), we analyzed patent and
failed AVFs at day 42 (Fig. 7, A and B). Histology at the AVF
site showed perianastomotic AVF tissue growth; this tissue
was thin and had a lumen in patent AVFs (Fig. 7A), whereas
this tissue was thicker and had no patent lumen between the

---

Fig. 6. Histology of a successful AVF (day 21). A–C: cross-sections of the IVC just be-
low the renal veins in preoperative mice (A), sham mice at day 21 (B), and AVF mice at
day 21 (C). Scale bars = 200 μm D: bar graph showing mean venous intima-media
thickness. n = 3. *P < 0.05 by ANOVA with
post hoc test. E: photomicrographs showing
representative immunohistochemistry for CD31
(top) or smooth muscle α-actin (bottom). For each
group of pictures, the top image shows the aorta
and the bottom image shows the IVC. Scale
bars = 100 μm.
facilitate technical success of the surgical procedure (1, 5, 8, 12, 15, 16). An advantage of the mouse model is the availability of genetic strains and variants that can be used to host AVF and thus study its effects in animal models of disease. Second, although there are some studies (6, 10, 11, 17) that have performed AVF surgery on mice, these reports have generally been in the context of developing a model for right-sided heart failure, using larger-size needles (11, 17). We used a 25-gauge needle to perform the puncture and were able to detect lower limb venous congestion from the additional arterial flow into the vena cava; however, we did not detect serious adverse events in the mice, consistent with the clinical safety of most AVFs in human patients. However, Fig. 3A shows the learning curve for the mouse model; these results showed that improved practice with cessation of bleeding improves the technical success of the procedure. The residual ~20% of cases that are technical failures are likely due to either the excessive force needed to stop the puncture hole bleeding, due to compression of the fistula tract, or due to low flow that becomes detectable at later times (Fig. 3B). We continue to use compression for hemostasis as our experience with suture closure of the puncture site led to a much higher rate of vessel stenosis and fistula closure (data not shown). We believe that experience with the model is the best solution to improve the technical success rate.

In this model, there are both local and systemic effects due to the AVF. One of the interesting findings was the diminished shear stress above the renal veins. In sham mice, the increased suprarenal IVC diameter may be responsible for the relative diminution of shear stress compared with the infrarenal segment. Similarly, when the AVF is present, the tremendously increased flow (Fig. 5C) increases the infrarenal shear stress, which then diminishes in the increased diameter of the suprarenal IVC (Fig. 5E). We believe that the large amount of renal vein flow into the suprarenal IVC stabilizes the infrarenal high-velocity turbulent flow due to the AVF, resulting in more laminar flow and accordingly lower mean velocities.

In summary, this model recapitulates aspects of the AVF that is created in human patients. Due to the scarce knowledge of both the maturation and failure that occur in human AVFs, this model may become a powerful tool to study the improvement of AVF outcomes.

Conclusions. The murine aortocaval model faithfully recapitulates the maturation and patency of the human AVF, with subgroups of animals failing to mature and, of those that do mature, another subgroup that fails in long-term followup. This model does not require advanced microsurgical skills, is straightforward to analyze, and has minimal system adverse effects. Therefore, this model should be useful in the study of AVF in animal models of disease.

GRANTS
This work was supported by National Institutes of Health Grants R01-HL-095498 (to A. Dardik) and PO1-NS-062686 (to J. Madri), a Yale Department of Surgery Ohse award (to K. Yamamoto and C. Protack), the Uehara Memorial Foundation (to K. Yamamoto and M. Tsuneki), as well as through the resources and use of facilities at the Veterans Affairs Connecticut Healthcare System (West Haven, CT).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS


REFERENCES