CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

Association of intraluminal thrombus in abdominal aortic aneurysm with local hypoxia and wall weakening

David A. Vorp, PhD,[a,b,c] Paul C. Lee, MD,[a] David H. J. Wang, MS,[a,b] Michel S. Makaroun, MD,[a] Edwin M. Nemoto, PhD,[d] Satoshi Ogawa, MD,[e] and **Marshall W. Webster, MD,[a]** *Pittsburgh, Pa, and Kanazawa, Japan*

Purpose: **Our previous computer models suggested that intraluminal thrombus (ILT) within an abdominal aortic aneurysm (AAA) attenuates oxygen diffusion to the AAA wall, possibly causing localized hypoxia and contributing to wall weakening. The purpose of this work was to investigate this possibility.**

Methods: **In one arm of this study, patients with AAA were placed in one of two groups: (1) those with an ILT of 4-mm or greater thickness on the anterior surface or (2) those with little (< 4 mm) or no ILT at this site. During surgical resection but before aortic cross-clamping, a needle-type polarographic partial pressure of oxygen (Po_2) electrode was inserted into the wall of the exposed AAA, and the Po_2 was measured. The probe was advanced, and measurements were made midway through the thrombus and in the lumen. Mural and mid-ILT Po_2 measurements were normalized by the intraluminal Po_2 measurement to account for patient variability. In the second arm of this study, two AAA wall specimens were obtained from two different sites of the same aneurysm at the time of surgical resection: group I specimens had thick adherent ILT, and group II specimens had thinner or no adherent ILT. Nonaneurysmal tissue was also obtained from the infrarenal aorta of organ donors. Specimens were subjected to histologic, immunohistochemical, and tensile strength analyses to provide data on degree of inflammation (% area inflammatory cells), neovascularization (number of capillaries per high-power field), and tensile strength (peak attainable load). Additional specimens were subjected to Western blotting and immunohistochemistry for qualitative evaluation of expression of the cellular hypoxia marker oxygen-regulated protein.**

Results: **The Po_2 measured within the AAA wall in group I (n = 4) and group II (n = 7) patients was 18% ± 9% luminal value versus 60% ± 6% (mean ± SEM; $P < .01$). The normalized Po_2 within the ILT of group I patients was 39% ± 10% ($P = .08$ with respect to the group I wall value). Group I tissue specimens showed greater inflammation ($P < .05$) compared with both group II specimens and nonaneurysmal tissue: 2.9% ± 0.6% area (n = 7) versus 1.7% ± 0.3% area (n = 7) versus 0.2% ± 0.1% area (n = 3), respectively. We found similar differences for neovascularization (number of vessels/high-power field), but only group I versus control was significantly different ($P < .05$): 16.9 ± 1.6 (n = 7) vs 13.0 ± 2.3 (n = 7) vs 8.7 ± 2.0 (n = 3), respectively. Both Western blotting and immunohistochemistry results suggest that oxygen-regulated protein is more abundantly expressed in group I versus group II specimens. Tensile strength of group I specimens was significantly less ($P < .05$) than that for group II specimens: 138 ± 19 N/cm^2 (n = 7) versus 216 ± 34 N/cm^2 (n = 7), respectively.**

Conclusion: **Our results suggest that localized hypoxia occurs in regions of thicker ILT in AAA. This may lead to increased, localized mural neovascularization and inflammation, as well as regional wall weakening. We conclude that ILT may play an important role in the pathology and natural history of AAA. (J Vasc Surg 2001;34:291-9.)**

From the Departments of Surgery,[a] Bioengineering,[b] Mechanical Engineering,[c] and Neurological Surgery,[d] University of Pittsburgh, and the Department of Neuroanatomy,[e] Kanazawa University Medical School.

Competition of interest: nil.

Supported in part by a biomedical engineering research grant by the Whitaker Foundation.

Reprint requests: David A. Vorp, PhD, University of Pittsburgh, Department of Surgery, A-1011 P.U.H., 200 Lothrop Street, Pittsburgh, PA 15213 (e-mail: VorpDA@msx.upmc.edu).

Published online Jun 8, 2001.

Copyright © 2001 by The Society for Vascular Surgery and The American Association for Vascular Surgery.

0741-5214/2001/$35.00 + 0 **24/1/114813**
doi:10.1067/mva.2001.114813

Rupture of abdominal aortic aneurysm (AAA) is the end-stage, catastrophic failure of the diseased aortic wall and results in approximately 15,000 deaths per year in the United States.[1] AAA rupture occurs when the diseased aortic wall degenerates to the point where its mechanical strength is insufficient to sustain the pressure-induced forces acting on it. Our laboratory has previously shown that the mechanical strength of the aneurysm wall is reduced to 50% of that of nonaneurysmal aorta.[2] The precise mechanisms of this weakening are not yet known. However, understanding aneurysm wall weakening is important in the study of the natural history of AAA and

may lead to new treatment modalities that could inhibit or reverse the weakening process.

Studies have demonstrated microstructural derangements in the aneurysm wall, providing a structural basis behind the observed wall weakening associated with AAA.[3-6] These are thought to be caused by defective extracellular matrix production associated with an unknown genetic disorder[7] or by the exuberant proteolytic enzyme expression known to occur in AAA as compared with nonaneurysmal aorta.[4,8-11] Possible sources of the proteolytic enzymes are inflammatory cells found in the AAA wall[10-14] or intraluminal thrombus (ILT)[15] or mural smooth muscle cells and fibroblasts activated in response to some stimuli associated with the disease process.

Because ILT is found in about 75% of all AAAs,[16] we have recently turned our attention to this structure and its possible role in the pathogenesis of AAA.[17,18] This work has suggested that ILT alters the biophysical environment of the AAA wall. In one study, computer modeling suggested that ILT may result in attenuated oxygen flow from the lumen to the underlying AAA wall.[18] The purpose of this work was to address the hypothesis that the presence of ILT in AAA leads to various degrees of cellular hypoxia and contributes to diminished wall strength. Indeed, clinical studies have supported the direct relationship of ILT to AAA rupture risk.[19-22] We demonstrate here that the AAA wall adjacent to a thick layer of ILT is weaker and exhibits greater degrees of cellular hypoxia and inflammation with respect to wall specimens from the same AAA but adjacent to a thinner layer of ILT. This suggests that ILT may indeed play an active role in the pathogenesis of AAA.

METHODS

Intraoperative measurement of partial pressure of oxygen. In one arm of this study, patients with AAA were placed in one of two groups on the basis of the thickness of ILT at the apical anterior position: (1) those with an ILT of 4-mm or greater thickness or (2) those with little (< 4 mm) or no ILT at this site. Before aortic cross-clamping was performed, a custom-made, calibrated, needle-type, platinum-iridium, polarographic partial pressure of oxygen (Po_2) microelectrode (tip diameter 25 μm) was inserted into the wall of the exposed AAA, and the Po_2 was measured. Guided by a 3.5-MHz ultrasound probe covered with a sterile sleeve, the Po_2 microelectrode tip was advanced to points centrally within the thrombus (in patients with ILT) and within the lumen, and the Po_2 at these positions was measured. All measurements were normalized by the intraluminal Po_2 to correct for patient variability.

Human aortic tissue. In a second arm of this study, paired tissue samples were obtained during operation from the anterior aspect of a separate set of AAA during surgical resection. Group I samples were obtained from the region where preoperative computed tomography revealed that ILT was thickest (usually at or near the apex), and group II samples were obtained from a location where ILT was thinnest or absent (usually distal to the apex). Care was taken to leave any adherent ILT attached to the AAA wall during harvest. ILT thickness was measured at three different positions for each sample, averaged, and recorded. The ILT was then gently removed from the AAA wall and discarded. Nonaneurysmal infrarenal aortic wall tissue specimens were also obtained from organ donors to serve as nonaneurysmal controls. Most aortic wall samples were divided into a larger portion and a smaller portion. The larger segment was placed in saline solution for subsequent tensile testing, whereas the smaller portion was fixed with 4% paraformaldehyde for 24 hours and cryoprotected with 2.3 mol/L sucrose overnight. The fixed samples were then rapidly frozen with liquid nitrogen in Tissue Freezing Medium (Triangle Biomedical Sciences, Durham, NC) for subsequent histologic and immunohistochemical analysis.

Immunohistochemistry. Six separate sections (10 μm) were cut from different layers through the aortic samples with a cryostat microtome and mounted on 0.5% gelatin-coated slides. Immunohistochemistry was performed with an immunofluorescence technique. Sections were washed three times with a blocking solution of phosphate-buffered saline solution containing 0.15% glycine and 0.5% bovine serum albumin, pH 7.4 (buffer A) to minimize nonspecific binding of the primary and secondary antibodies. Sections were then incubated with purified goat immunoglobulin G (50 mg/mL) for 40 minutes at 25°C followed by three washes with buffer A, then incubated for 60 minutes with the primary antibody to von Willebrand factor (1:800 dilution; Sigma Chemical Co, St Louis, Mo). This was followed by three washes in buffer A and 60 minutes incubation with the secondary antibody that was fluorescently tagged (Fluorophore Cy3, 1:3000 dilution; Sigma Chemical Co). Negative controls were created from additional sections following the same procedure but with the primary antibody omitted. All sections were then washed with buffer A three times, mounted in Gelvatol (Monsanto Corporation, St Louis, Mo), secured with a coverslip, and examined under a Nikon Microphot-FXL immunofluorescence microscope (Nikon Corp, Tokyo, Japan).

From one additional set of group I and group II specimens, serial sections were fluorescently labeled with antibodies to oxygen-regulated protein (ORP)[23,24] and CD45. ORP is a 150-kD polypeptide recently found to be expressed in human vascular tissue specifically in response to hypoxia.[23] CD45 was used to detect inflammatory cells. Negative controls were included for both as above. Counterstaining adjacent sections with Hoescht allowed detection of biologic structures. Images from these sections were imported into Adobe Photoshop (version 5.5) (Adobe Systems Inc, San Jose Calif) and combined, or layered, to allow qualitative evaluation of ORP staining patterns.

Morphometric analysis. Six additional 10-μm cryostat sections prepared as above were stained with hematoxylin and eosin (H&E) and examined with light

microscopy. Digital images were collected directly by use of a Nikon microscope with a 10× objective coupled to a high-sensitivity, integrating three-chip Sony Color Video Camera (Sony Corp, Park Ridge, NJ) (700 × 600 pixels). Quantification of degree of inflammation was performed by a blinded, standard "semiautomatic" quantification technique that has been shown to be time efficient and reliable.[25] Images were imported directly to the image analysis program NIH Image (version 1.61) (US National Institutes of Health, Bethesda, Md). Each image was converted to gray-scale, and then a threshold was applied at a level that distinguished between the stained cells and the unstained background under H&E to calculate the area fraction of the inflammatory cells.[26] We further "semiautomatically" outlined the regions occupied by the smooth muscle cells and fibroblasts as shown by their characteristic appearance and then deleted this background staining from the original image. Under the gray-scale, the proportion of black to total pixels in the image was then calculated as a percentage area occupied by inflammatory cells per 100× field. Neovascularization within the aneurysm wall was also quantified in a blinded manner by counting the number of capillaries per 100× field. Capillaries were defined as tubular structures containing red blood cells and further confirmed by a positive staining to the endothelial-specific von Willebrand factor as described above. Measures were averaged from three random sites for each of the six different vessel sections per aortic sample to reduce sampling error in the quantification of both inflammation and neovascularization.

Tensile testing. Specimens designated for tensile testing were cut into long, thin circumferential strips of tissue and placed into a well-established tensile testing system as previously described.[27] The specimens were loaded along their long axis until failure while the applied force and resulting deformation were measured continuously. The mechanical stress within the specimen at any given time was calculated as previously described by normalizing the applied force by cross-sectional area.[27] The strength of each specimen was taken as the peak stress obtained before failure.

Western blotting. We obtained tissue from two additional AAA and one additional nonaneurysmal control for immunoblotting. Without dividing the specimens, they were processed as per the methods detailed above (see "Human Aortic Tissue"). Western blotting was performed as previously described.[23] In brief, tissue specimens were washed with phosphate-buffered saline solution and disrupted by use of a Polytron homogenizer in buffer containing NP-40 (1%), EDTA (5 mmol/L), phenylmethylsulfonyl fluoride (1 mmol/L), leupeptin (10 μg/mL), and aprotinin (10 μg/mL) at 4°C. After acetone precipitation, protein extracts (5 μg) were resuspended in phosphate-buffered saline solution containing NP-40 (1%) and separated in SDS-PAGE (8%). Protein content was adjusted such that approximately the same amount of α-smooth muscle actin was detected in each sample (total protein content was also comparable between samples), and the presence of immunoreactive ORP was assessed by Western blotting by use of anti-human ORP immunoglobulin G (5 μg/mL). For positive control, human cultured mononuclear cells (5×10^5 cells) were either exposed to hypoxia or maintained in normoxic condition for 24 hours, and their protein extracts (5 μg) were subjected to Western blot.

Statistical analysis. Quantitative results are presented as mean ± SEM. Comparisons of the P_{O2} measurements between the two groups of patients was made by use of the Student t test. Comparison of the paired tensile strength data and ILT thickness measures was made by use of the paired *t* test. All other quantitative results were analyzed by use of analysis of variance and the Student-Newman-Keuls method to perform ad hoc, pairwise comparisons. Analysis was performed with a statistical software package (Sigma Stat; Jandel Scientific, San Raphael, Calif). P less than .05 was considered to be statistically significant.

RESULTS

A total of 11 patients were studied for P_{O_2} measurements: 4 who provided measurements associated with a thick thrombus (group I; age 67.0 ± 5.8 years; maximum AAA diameter 5.8 ± 0.5 cm), and 7 who provided measurements associated with little or no thrombus (group II; age 67.4 ± 6.0 years; maximum AAA diameter 6.0 ± 0.7 cm). There was no significant difference in either age or maximum AAA diameter between the two groups of patients. The data revealed a significantly lower P_{O_2} in the wall of group I AAA as compared with group II AAA: 18% ± 9% versus 60% ± 6%, respectively (Fig 1, *A*; $P < .01$). We also found a marked P_{O_2} gradient through the AAA with ILT, with the P_{O_2} highest in the lumen and lowest in the AAA wall (Fig 1, *B*).

A total of seven separate patients (age 73.4 ± 2.4 years) with AAA (6.9 ± 0.7 cm maximum diameter) supplied paired wall specimens for immunohistochemical, morphometric, and tensile strength analyses. In addition, nonaneurysmal control aortic specimens were obtained from three donors (age 40.7 ± 6.8 years) for immunohistochemical and morphometric analyses. The mean thickness of the ILT attached to the group I tissue specimens was significantly greater than the mean thickness of the ILT attached to the group II tissue specimens: 16 ± 3 mm versus 6 ± 2 mm, respectively ($P < .005$).

Representative aortic sections stained for von Willebrand factor are shown in Fig 2. Fig 3 reveals a significantly ($P < .05$) greater number of capillaries per high-powered field in the group I AAA wall specimens adjacent to thick ILT (16.9 ± 1.6, n = 7) as compared with nonaneurysmal controls (8.7 ± 2.0, n = 3). There was no difference in number of capillaries in the group II AAA wall specimen adjacent to thin or no ILT (13.0 ± 2.3, n = 7) as compared with either the group I specimens or the nonaneurysmal controls.

Representative H&E-stained aortic sections are shown in Fig 4. As shown in Fig 5, we found a significantly (P < .05) greater percentage area of inflammatory cells in the group I

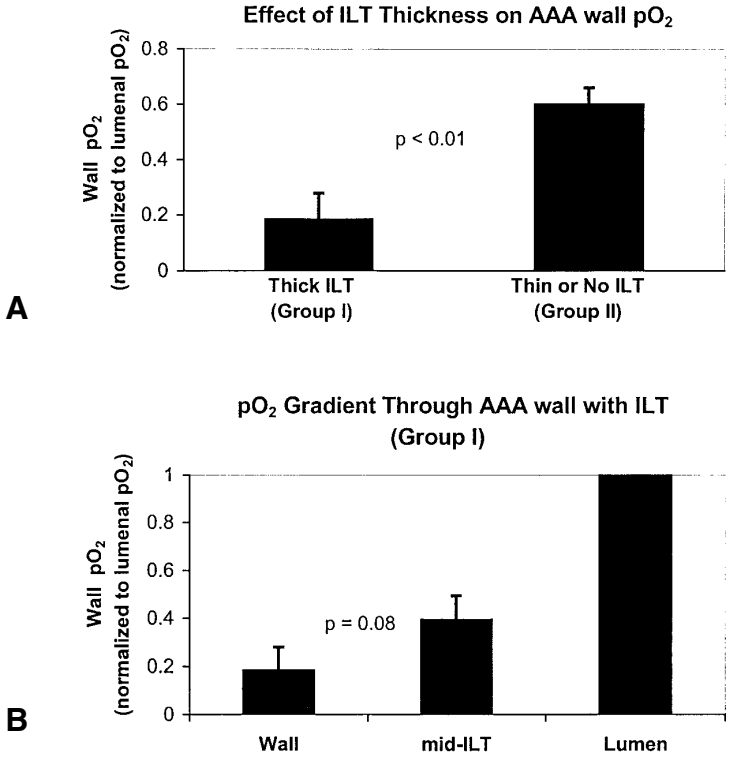

Fig 1. A, Comparison of in vivo Po_2 measurements for AAA wall adjacent to thick ILT versus AAA wall adjacent to thin or no ILT. **B,** In vivo measurements demonstrate Po_2 gradient through thickness of AAA containing thick ILT. Note: all measurements normalized to respective luminal Po_2 value.

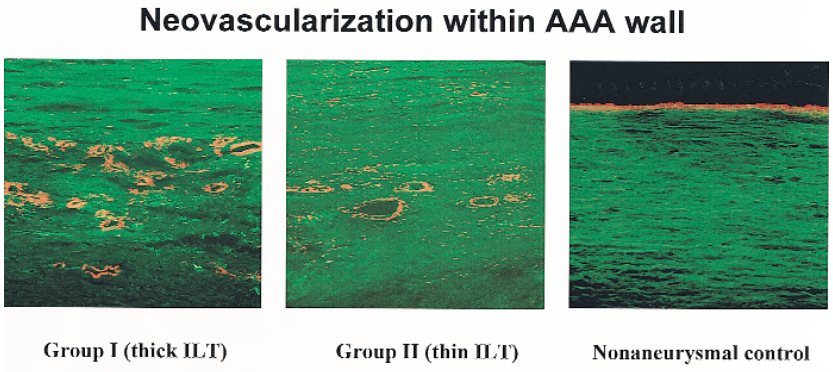

Fig 2. Representative sections demonstrate neovascularization in group I and group II AAA wall specimens and in nonaneurysmal control specimens. Capillaries are identified via positive staining for von Willebrand factor. Original magnification × 200.

AAA wall specimens adjacent to thick ILT (2.9% ± 0.6%, n = 7) as compared with both group II specimens adjacent to thin or no ILT (1.7% ± 0.3%, n = 7) and nonaneurysmal controls (0.2% ± 0.1%, n = 3). There was also a significant difference ($P < .05$) between the latter two groups.

Tissue sections stained with Hoescht stain and antibodies to ORP and CD45 are shown in Fig 6. Western blotting results for two separate group I and group II pairs of specimens, as well as nonaneurysmal, positive, and negative controls are shown in Fig 7. Both immunohistochemistry (Fig 6, *A* and *B*) and Western blotting analysis (Fig 7) suggest, qualitatively, that ORP is found in AAA sections and may be more abundantly expressed in regions with thick ILT as compared with regions with thin ILT.

Immunohistochemistry results (Fig 6, *D* and *E*) also qualitatively agree with our quantitative assessment of degree of inflammation (Fig 5).

Comparison between group I and group II tensile strength measurements is shown in Fig 8. The group I AAA wall specimens adjacent to thick ILT were significantly weaker than group II specimens adjacent to thin or no ILT (138 N/cm^2 ± 19 N/cm^2 vs 216 N/cm^2 ± 34 N/cm^2, $P < .05$).

DISCUSSION

In this article we examined the hypothesis that ILT in AAA is associated with local hypoxia of the AAA wall. We based this hypothesis on our earlier computational investigation, which suggested that ILT attenuates oxygen flow from the lumen to the AAA wall.[18] We first performed measurements within the AAA during operation by use of a Po_2 electrode, and these results support the notion that the presence of ILT is associated with lower levels of mural Po_2 (Fig 1). Vascular tissue responds to hypoxic conditions by initiating a series of events that lead to inflammation and neovascularization.[14,21] Therefore, as an indirect marker of hypoxia, we also examined the presence of inflammatory cells and neovascularizaton within the AAA wall. Although the differences that we measured with regard to neovascularization are not significant (Fig 3), we think that they demonstrate a trend that supports the overall hypothesis that there do exist differences in the pathobiologic condition of the AAA wall in the thick ILT versus thin ILT regions. Our data demonstrate a significant difference in neovascularization between group I AAA tissue and the nonaneurysmal controls. That we observed this, but not a difference between group I and group II tissue, might indicate either that we did not analyze enough samples to detect differences or that both group I and group II tissue have similar degrees of neovascularization. The inflammation measurements also support our hypothesis (Figs 4 and 5), and these data are qualitatively supported by comparison of Fig 6, *D* to *E*. As a direct marker for cellular hypoxia, we also examined qualitatively the protein expression of ORP, a hypoxia-specific polypeptide known to be expressed by various human cell types, including macrophages and smooth muscle cells.[23,24] These results also support our hypothesis that ILT is associated with hypoxia (Figs 6 and 7). A possible consequence of the hypoxia and subsequent inflammation is local wall weakening (Fig 8).

The AAA wall weakening noted in association with hypoxia might be caused by any combination of a number of factors. For example, the increased number of inflammatory cells characteristic of these regions (Figs 4 and 5) would increase the local proteolytic activity of the wall.[10,11,13] That these regions containing a large number of inflammatory cells are also exposed to hypoxia may exacerbate this situation. Macrophages exposed to hypoxia exhibit enhanced bioreactivity,[28-32] including an increase in elastase production.[33] We have recently shown that ILT contains many living cells, including macrophages and

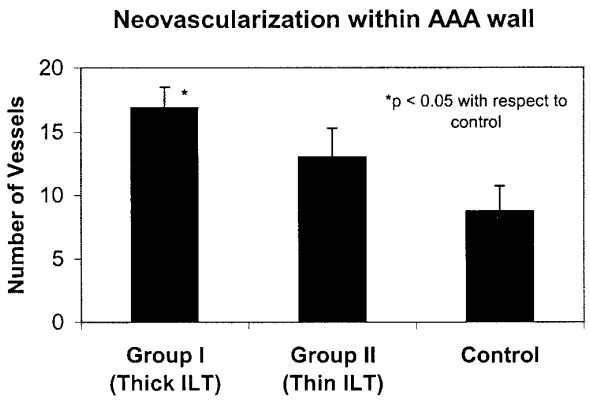

Fig 3. Quantification of neovascularization in group I, group II, and nonaneurysmal control specimens. Significant difference was noted between both group I and group II specimens and controls, but not between group I and group II.

neutrophils.[15] Therefore another possibility is that the regions of AAA wall adjacent to thicker layers of ILT are exposed to proteolytic enzymes leeching from the ILT. Indeed, Jean-Claude et al[12] found elevated levels of plasmin present in the inner layers of the AAA wall near the interface with ILT, and this may be a possible consequence of such leeching. Finally, elastin and collagen synthesis by endothelial cells, smooth muscle cells (SMCs), and fibroblasts is dependent on the availability of oxygen.[34-41] Aortic endothelial cells cultured in hypoxic conditions exhibit a decrease in collagen synthesis,[38] whereas hypoxic arterial SMCs exhibit a decrease in both collagen synthesis[39,42] and tropoelastin mRNA expression and synthesis.[35] Fibroblasts exposed to hypoxic conditions also produce less collagen.[36,40] Furthermore, the collagen that is synthesized by hypoxic cells is abnormal, because oxygen is needed for the hydroxylation of proline.[36] Therefore the extracellular matrix synthesis by the hypoxic vascular wall is likely impaired or results in abnormal matrix, and this would lead to wall weakening, which is consistent with our findings.

AAA is not the only disease process that appears to have decreased regional strength in association with local hypoxia and inflammation. Lendon et al[43] showed that the mechanical strength of atherosclerotic plaque caps is reduced with increased presence of macrophages. As with ILT, atherosclerotic plaques appear to be associated with patterns of hypoxia.[23] Therefore, as we hypothesize above for the AAA, the inverse relationship of plaque strength and number of macrophages may be due to the stimulation of these cells by the local hypoxic environment, causing them to release various proteolytic enzymes.

Our experimental in vivo measurements of Po_2 in the AAA wall are consistent with our previous estimates with a computer model. Just as we showed here (Fig 1, *B*), our previous computer model predicted a significant gradient in Po_2 through the ILT thickness, with maximum value at the luminal position, intermediate value at

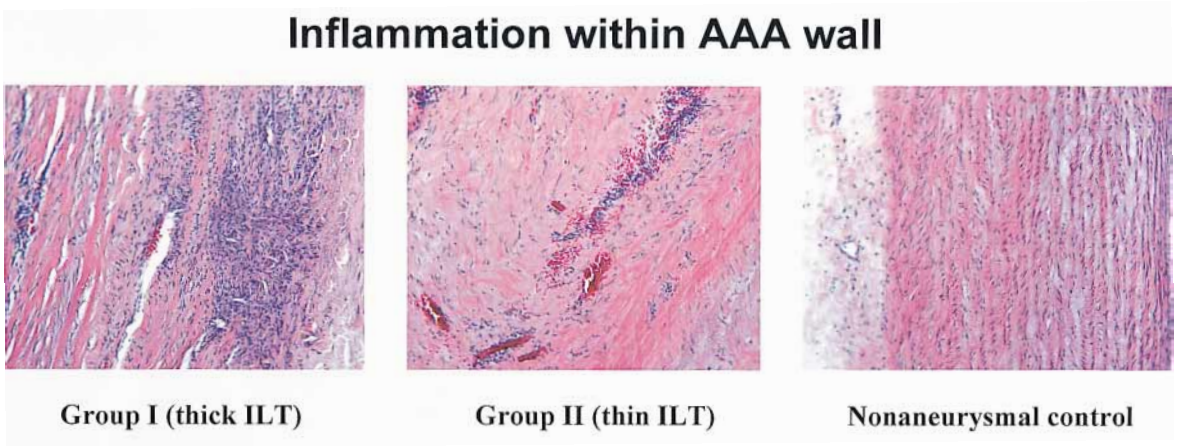

Fig 4. Representative H&E sections demonstrate inflammatory cells in group I and group II AAA wall specimens and in nonaneurysmal control specimens. Original magnification × 100.

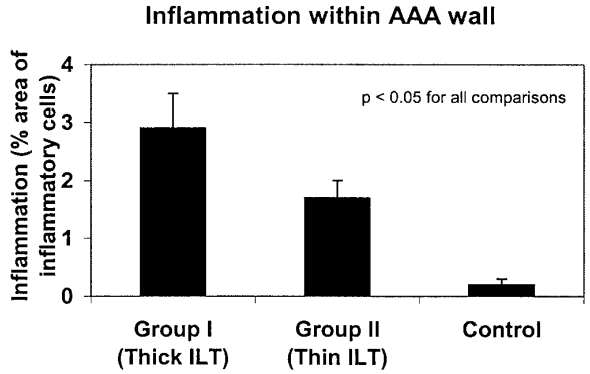

Fig 5. Quantification of inflammation in group I, group II, and nonaneurysmal control specimens. Significant difference was noted among all groups.

a medial region, and lowest value at the AAA wall.[18] Our computer model also predicted an inverse relationship of Po_2 at the AAA wall with local ILT thickness, as we demonstrated with our experimental measurements here (Fig 1, *A*).

The presence of hypoxia within the AAA wall can be intimately related to the marked inflammation and the difference in neovascularization that we also observed. A hypoxic environment has been shown to stimulate both SMCs and fibroblasts to secrete active angiogenic factors.[34,35,44] Indeed, the potent angiogenic factor vascular endothelial growth factor contains hypoxia regulatory elements in its gene and is highly stimulated by hypoxia both in vitro and in vivo.[45,46] More recently, Chiarugi et al[47] have demonstrated that hypoxia induces a variety of angiogenesis control factors, including p53, vascular endothelial growth factor, inducible nitric oxide synthase, and cyclooxygenase-2. The resultant neovascularization induced by these factors can then serve as a route for inflammatory cells to transmigrate to the media of AAA. Neovascularization may be a compensatory response to local hypoxia, serving as a route for delivery of the nutrients and oxygen to the inflamed tissue. However, the inflammatory cells may secrete proteolytic enzymes acting to locally weaken the AAA wall.

Although this study does not distinguish the primary etiologic events leading to aneurysm formation, it is clear that wall hypoxia, inflammation, and possibly medial neovascularization are closely associated in the AAA wall. Others have made similar observations of a codependency between angiogenesis and inflammation in AAA.[14,48] In addition to its primary angiogenic effect, hypoxia has been shown to stimulate monocytes and macrophages to secrete angiogenic factors.[49,50] Even in an unstimulated state, macrophages can produce a variety of cytokines and growth factors to induce angiogenesis.[14] Therefore inflammatory cell infiltration within the hypoxic AAA wall may enhance medial neovascularization.

As with all experimental work, this investigation has some limitations that must be kept in mind when the data are interpreted. For example, because the samples used for tensile testing and microscopy were obtained from open surgical resection of AAA, we were limited to tissue that was easily removed, that is, that from the anterior surface. As a result, the paired samples did not necessarily originate from regions of comparable aortic diameter. It is possible that the differences that are noted between group I and group II specimens are due to these local diameter differences. Additionally, atherosclerosis is known to be associated with each of the pathologic changes that we have noted here. However, because all AAA specimens were paired (ie, each AAA served as its own control) and assuming that both of the specimens from each pair were similarly affected by atherosclerosis, the differences that were observed between group I and group II specimens should be primarily due to the difference in ILT thickness (ie, hypoxia). Moreover, although the nonaneurysmal aortic (control) specimens were not age-matched to the AAA tissue, nearly all adult abdominal aorta has some degree of atherosclerosis. As a result, differences noted between group II AAA specimens and the nonaneurysmal aortic tissue should be primarily due to the aneurysmal disease process. Therefore, in spite of its limitations, the experi-

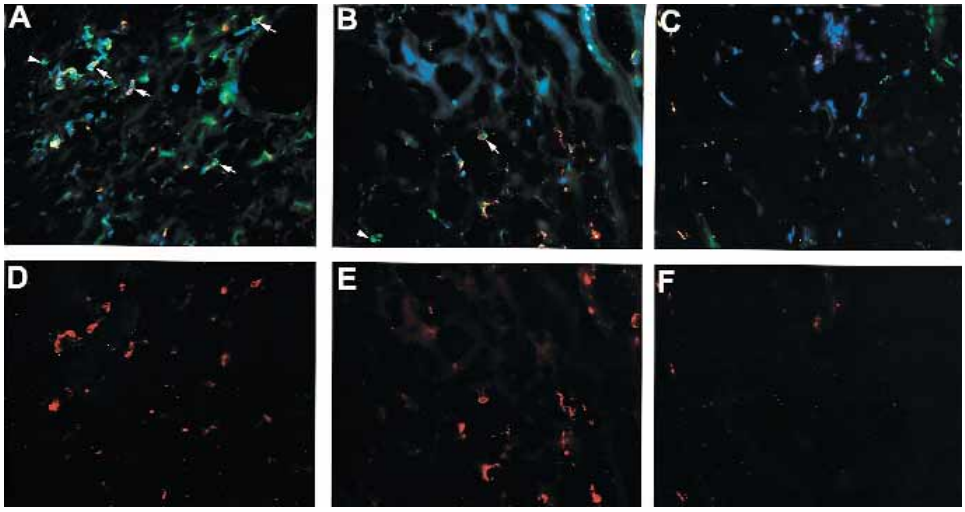

Fig 6. Serial immunohistochemistry sections for group I **(A** and **D)**, group II **(B** and **E)**, and primary-delete negative control **(C** and **F)** specimens stained with antibodies to ORP and CD45 and counterstained with Hoescht **(A-C)**, or stained with antibodies to CD45 alone **(D-F)**. Hoescht-stained nuclei appear *blue,* whereas ORP-positive cells appear *green* and CD45-positive cells appear *red*. *Long arrows* indicate representative colocalization of ORP- and CD45-positive cells, whereas *arrowheads* indicate representative cells that are ORP-positive only. Unspecific signal in negative control **(C** and **F)** is likely due to autofluorescence of elastin in tissue sections. Original magnification × 100.

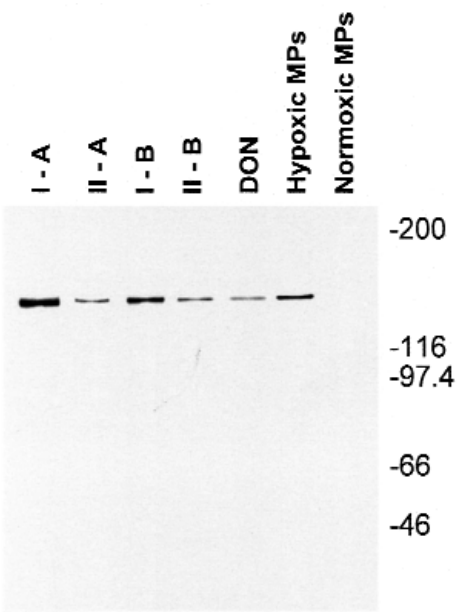

Fig 7. Western blot for 150-kDa ORP in two different **(A** and **B)** group I–group II pairs of AAA wall specimens, nonaneurysmal control (*DON*), positive control of hypoxic macrophages (*MPs*) in culture, and negative control of normoxic macrophages in culture. Molecular weight markers correspond to ovalbumin (46 kDa), bovine serum albumin (66 kDa), phosphorylase b (97.4 kDa), beta-galactosidase (116 kDa), and myosin (200 kDa). Greater signal was detected in both group I specimens as compared with their paired group II specimen, and also as compared with nonaneurysmal control specimen.

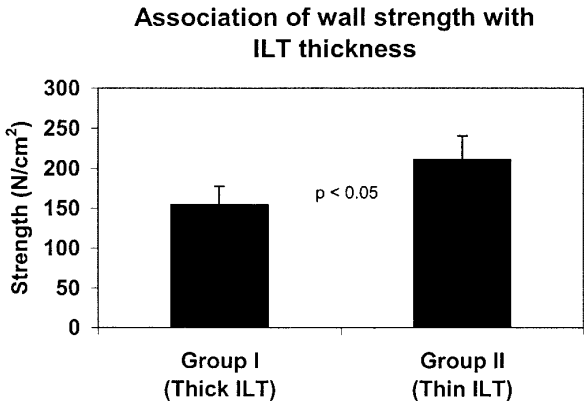

Fig 8. Comparison of tensile strength for group I versus group II specimens. Significant difference was noted by means of the paired *t* test.

mental design used in this study allowed us to evaluate AAA wall pathobiologic condition in association with ILT, which was our goal.

In summary, our results suggest that hypoxia is present in regions of AAA wall adjacent to thick ILT. This may lead to increased localized inflammation and possibly neovascularization at these sites and appears to result in localized wall weakening. We conclude therefore that ILT may play an important role in the pathology and natural history of AAA.

We thank Dr Simon Watkins and Sean M. Alber of the Center for Biologic Imaging at the University of Pittsburgh for their assistance with immunohistochem-

istry, and Dr Douglas Hamilton, Jon Mears, John Melick, Dr John Pirris, Jeff Novakoski, Sue Gleixner, and Brian Schiro for technical help. We also acknowledge Drs David Steed, Satish Muluk, Robert Rhee, and Ron Shapiro for patient recruitment, tissue harvest, and valuable insights.

REFERENCES

1. National Center for Health Statistics. Vital statistics of the United States. 1988. vol. 2, Mortality: part A. Washington, DC: Government Printing Office; 1991.
2. Vorp DA, Raghavan ML, Muluk SC, Makaroun MS, Steed DL, Shapiro R, et al. Wall strength and stiffness of aneurysmal and non-aneurysmal abdominal aorta. Ann N Y Acad Sci 1996;800:274-7.
3. Baxter BT, McGee GS, Shively VP, Drummond IAS, Dixit SN, Yamauchi M, et al. Elastin content, cross-links, and mRNA in normal and aneurysmal human aorta. J Vasc Surg 1992;16:192-200.
4. Menashi S, Greenhalgh RM, Powell JT. Collagen in abdominal aortic aneurysm: typing, content and degradation. J Vasc Surg 1987;6: 578-82.
5. Rizzo RJ, McCarthy WJ, Dixit SN, Lilly MP, Shively VP, Flinn WR, et al. Collagen types and matrix protein content in human abdominal aortic aneurysms. J Vasc Surg 1989;10:365-73.
6. Sakalihasan N, Heyeres A, Nusgens BV, Limet R, Lapiere CM. Modifications of the extracellular matrix of aneurysmal abdominal aortas as a function of their size. Eur J Vasc Surg 1993;7:633-7.
7. Verloes A, Sakalihasan N, Limet R, Koulischer L. Genetic aspects of abdominal aortic aneurysm. Ann N Y Acad Sci 1996;800:44-55.
8. Thompson RW, Parks WC. Role of matrix metalloproteinases in abdominal aortic aneurysms. Ann N Y Acad Sci 1996;800:157-74.
9. Webster MW, McAuley CE, Steed DL, Miller DD, Evans CH. Collagen stability and collagenolytic activity in the normal and aneurysmal human abdominal aorta. Am J Surg 1991;161:635-8.
10. McMillan WD, Patterson BK, Keen RR, Shively VP, Capelin M, Pearce WH. In situ localization and quantification of mRNA for 92kD type IV collagenase and its inhibitor in aneurysmal, occlusive, and normal aorta. Arterioscler Thromb Vasc Biol 1995;15:1139-44.
11. McMillan WD, Patterson BK, Keen RR, Pearch WH. In situ localization and quantification of seventy-two–kilodalton type IV collagenase in aneurysmal, occlusive, and normal aorta. J Vasc Surg 1995;22: 295-305.
12. Jean-Claude J, Newman KM, Li H, Gregory AK, Tilson MD. Possible key role for plasmin in the pathogenesis of abdominal aortic aneurysms. Surgery 1994;116:472-8.
13. Newman KM, Jean-Claude J, Hong L, Scholes JV, Ogata Y, Nagase H, et al. Cellular localization of matrix metalloproteinases in the abdominal aortic aneurysm wall. J Vasc Surg 1994;20:814-20.
14. Thompson JF, Mullee MA, Bell PR, Campbell WB, Chant AD, Darke SG, et al. Intraoperative heparinization, blood loss and myocardial infarction during aortic aneurysm surgery: a joint vascular research group study. Eur J Vasc Endovasc Surg 1996;12:86-90.
15. Adolph R, Vorp DA, Steed DL, Webster MW, Kameneva MV, Watkins SC. Cellular content and permeability of intraluminal thrombus in abdominal aortic aneurysm. J Vasc Surg 1997;5:916-26.
16. Harter LP, Gross BH, Callen PW, Barth RA. Ultrasonic evaluation of abdominal aortic thrombus. J Ultrasound Med 1982;1:315-8.
17. Vorp DA, Gorcsan J III, Mandarino WA, Webster MW. The potential influence of intraluminal thrombus on abdominal aortic aneurysm as assessed by a new noninvasive method. Cardiovasc Surg 1996;4:732-9.
18. Vorp DA, Wang DHJ, Webster MW, Federspiel WJ. Effect of intraluminal thrombus thickness and bulge diameter on the oxygen flow in abdominal aortic aneurysm. J Biomech Eng 1998;120:579-83.
19. Cappeller WA, Engelmann H, Blechschmidt S, Wild M, Lauterjung L. Possible objectification of a critical maximum diameter for elective surgery in abdominal aortic aneurysms based on one- and three-dimensional ratios. J Cardiovasc Surg 1997;38:623-8.
20. Krupski WC, Bass A, Thurston DW, Dilley RB, Bernstein EF. Utility of computed tomography for surveillance of small abdominal aortic aneurysms. Arch Surg 1990;125:1345-50.
21. Satta J, Laara E, Juvonen T. Intraluminal thrombus predicts rupture of an abdominal aortic aneurysm. J Vasc Surg 1996;23:737-9.
22. Wolf YG, Thomas WS, Brennan FJ, Goff WG, Sise MJ, Bernstein EF. CT scan findings associated with rapid expansion of abdominal aortic aneurysms. J Vasc Surg 1994;20:529-38.
23. Tsukamoto Y, Kuwabara K, Hirota S, Ikeda J, Stern D, Yanagi H, et al. 150-kD oxygen-regulated protein is expressed in human atherosclerotic plaques and allows mononuclear phagocytes to withstand cellular stress on exposure to hypoxia and modified low density lipoprotein. J Clin Invest 1996;98:1930-41.
24. Kuwabara K, Matsumoto M, Ikeda J, Hori O, Ogawa S, Maceda Y, et al. Purification and characterization of a novel stress protein, the 150 kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. J Biol Chem 1996;271:5025-32.
25. Kraan MC, Haringman JJ, Ahern MJ, Breedveld FC, Smith MD, Tak PP. Quantification of the cell infiltrate in synovial tissue by digital image analysis. Rheumatology 2000;39:43-9.
26. Furness PN, Rogers-Wheatley L, Harris KP. Semiautomatic quantitation of macrophages in human renal biopsy specimens in proteinuric states. J Clin Pathol 1997;50:118-22.
27. Raghavan ML, Webster MW, Vorp DA. Ex-vivo biomechanical behavior of abdominal aortic aneurysm: assessment using a new mathematical model. Ann Biomed Eng 1996;24:573-82.
28. Albina JE, Henry WL Jr, Mastrofrancesco B, Martin BA, Reichner JS. Macrophage activation by culture in an anoxic environment. J Immunol 1995;155:4391-6.
29. Compeau CG, Ma J, DeCampos KN, Waddell TK, Brisseau GF, Slutsky AS, et al. In situ ischemia and hypoxia enhance alveolar macrophage tissue factor expression. Am J Respir Cell Mol Biol 1994;11:446-55.
30. Melillo G, Taylor LS, Brooks A, Cox GW, Varesio L. Regulation of inducible nitric oxide synthase expression in IFN-gamma-treated murine macrophages cultured under hypoxic conditions. J Immunol 1996;157:2638-44.
31. Scannell G, Waxman K, Kaml GJ, Ioli G, Gatanaga T, Yamamoto R, et al. Hypoxia induces a human macrophage cell line to release tumor necrosis factor-alpha and its soluble receptors in vitro. J Surg Res 1993;54:281-5.
32. West MA, Li MH, Seatter SC, Bubrick MP. Pre-exposure to hypoxia or septic stimuli differentially regulates endotoxin release of tumor necrosis factor, interleukin-6, interleukin-1, prostaglandin E2, nitric oxide, and superoxide by macrophages. J Trauma 1994;37:82-90.
33. Campbell EJ, Wald MS. Hypoxic injury to human alveolar macrophages accelerates release of previously bound neutrophil elastase: implications for lung connective tissue injury including pulmonary emphysema. Am Rev Respir Dis 1983;127:631-5.
34. Berse B, Hunt JA, Diegel RJ, Morganelli P, Yeo K, Brown F, et al. Hypoxia augments cytokine (transforming growth factor-beta (TGF-beta) and IL-1)-induced vascular endothelial growth factor secretion by human synovial fibroblasts. Clin Exp Immunol 1999;115:176-82.
35. Durmowicz AG, Badesch DB, Parks WC, Mecham RP, Stenmark KR. Hypoxia-induced inhibition of tropoelastin synthesis by neonatal calf pulmonary artery smooth muscle cells. Am J Respir Cell Mol Biol 1991;5:464-9.
36. Herrick SE, Ireland GW, Simon D, McCollum CN, Ferguson MW. Venous ulcer fibroblasts compared with normal fibroblasts show differences in collagen but not fibronectin production under both normal and hypoxic conditions. J Invest Dermatol 1996;106:187-93.
37. Hunt TK, Pai MP. The effect of varying ambient oxygen tensions on wound metabolism and collagen synthesis. Surg Gynecol Obstet 1972;135:561-7.
38. Levene CI, Kapoor R, Heale G. The effect of hypoxia on the synthesis of collagen and glycosaminoglycans by cultured pig aortic endothelium. Atherosclerosis 1982;44:327-37.
39. Pietila K, Jaakkola O. Effect of hypoxia on the synthesis of glycosaminoglycans and collagen by rabbit aortic smooth muscle cells in culture. Atherosclerosis 1984;50:183-90.
40. Steinbrech DS, Longaker MT, Mehrara BJ, Saadeh PB, Chin GS, Gerrets RP, et al. Fibroblast response to hypoxia: the relationship between angiogenesis and matrix regulation. J Surg Res 1999;84: 127-33.
41. Uitto J, Prockop DJ. Synthesis and secretion of underhydroxylated

procollagen at various temperatures by cells subject to temporary anoxia. Biochem Biophys Res Commun 1974;60:414-23.
42. Stavenow L, Berg AL. Effects of hypoxia and other injurious stimuli on collagen secretion and intracellular growth stimulating activity of bovine aortic smooth muscle cells in culture. Artery 1987;14: 198-208.
43. Lendon CL, Davies MJ, Born GVR, Richardson PD. Atherosclerotic plaque caps are locally weakened when macrophages density is increased. Atherosclerosis 1991;87:87-90.
44. Sakuda H, Nakashima Y, Kuriyama S, Sueishi K. Media conditioned by smooth muscle cells cultured in a variety of hypoxic environments stimulates in vitro angiogenesis: a relationship to transforming growth factor-beta 1. Am J Pathol 1992;141:1507-16.
45. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997;18:4-25.
46. Minchenko A, Salceda S, Bauer T, Caro J. Hypoxia regulatory elements of the human vascular endothelial growth factor gene. Cell Mol Biol Res 1994;40:35-9.
47. Chiarugi V, Magnelli L, Chiarugi A, Gallo O. Hypoxia induces pivotal tumor angiogenesis control factors including p53, vascular endothelial growth factor and the NFkappaB-dependent inducible nitric oxide synthase and cyclooxygenase-2. J Canc Res Clin Oncol 1999;125:525-8.
48. Satta J. Expansion and rupture of abdominal aortic aneurysms. Ann Chir Gynaecol 1998;87:63.
49. Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science 1983;221:1283-5.
50. Jackson JR, Seed MP, Kircher CH, Willoughby DA, Winkler JD. The codependence of angiogenesis and chronic inflammation. FASEB J 1997;11:457-65.

Submitted May 24, 2000; accepted Jan 5, 2001.

CALL FOR NOMINATIONS
EDITOR
JOURNAL OF VASCULAR SURGERY

On December 31, 2002, the second and final 3-year term of the current Editors of the *Journal of Vascular Surgery* will be completed. In anticipation of this, the Joint Council has instructed the Publications Committee (Hugh H. Trout III, MD, Chair) to conduct a broadly based academic search for a successor or successors to be presented to the Joint Council for consideration during the last quarter of this year and the first half of 2002. This will allow time for review of applications, interview of leading candidates, negotiation of subsequently proposed budgets, and final Joint Council action on the Publications Committee's recommendations by the June meetings in 2002. An orderly transition can occur during the last half of 2002.

Our two societies have been most fortunate to have the energetic, scholarly, and skillful leadership of Drs Johnston and Rutherford as Editors of the Journal. They have continued and expanded on the previous efforts of Drs DeBakey, Thompson, Szilagyi, Ernst, and Stanley, in establishing the *Journal of Vascular Surgery* as a preeminent international scientific publication.

The Publications Committee now invites nominations, including self-nominations, of individuals or teams of individuals for the position of Editor. If submitting a team, please designate which person shall serve as the Editor in Chief.

The criteria for selection include an established reputation in clinical vascular surgery and clinical or basic research, a reputation for fairness, evidence of scholarly activity in an editorial role, and demonstrated management skills. The individual(s) must be an Active or Senior Member(s) in the Society for Vascular Surgery or the American Association for Vascular Surgery. The current co-Editors indicate they each devote approximately 20 hours per week to their positions and rely on significant input from two Associate Editors.

The institution in which the main JVS office will be located should supply adequate space for conducting the editorial activities. The current editorial office consists of 650 square feet, but additional space may be needed because of expanding workload. The Joint Council reimburses the institution for support of office personnel, telephone, and postage and has purchased the office equipment necessary to conduct the affairs of the Editors. Previous budgets are available.

Additional information regarding budgets and details of current management can be obtained by contacting the current editors with operational questions. Nominations should be accompanied with a current curriculum vitae, a summary of the candidate's (candidates') editorial experience, other experience that would reflect on the position, and an outline of plans for both continuing and changing the present management and orientation of the Journal and should be submitted by **October 1, 2001,** to the following address:

Chairman, Publications Committee
Joint Council, SVS/AAVS
13 Elm Street
Manchester, MA 01944-1314
Fax: 978-526-4018
email: jvs@prri.com