Isolation, Characterization, and Localization of Cardiac Collagen Type VI

Associations With Other Extracellular Matrix Components

Reza I. Bashey, Antonio Martinez-Hernandez, and Sergio A. Jimenez

We have isolated and characterized collagen type VI from murine, canine, and nonhuman primate hearts. In the three species studied, collagen type I was the major collagenous component of the cardiac interstitium (80% of total collagen), whereas collagen type VI represented ~5% of total collagen. To define the exact distribution of collagen type VI and its possible interactions with other components of the cardiac extracellular matrix, collagen types I, III, IV, and VI, laminin, and fibronectin were localized in the rat myocardium by immunohistochemistry, using monospecific antibodies. In the rat myocardium, collagen type VI was prevalent in the media and adventitia of muscular arteries, in fine connective tissue septa, in the area surrounding capillaries, and in the delicate endomysium in proximity to myocardial cells. When compared with the immunohistochemical localization of collagen types I, III, and IV, laminin, and fibronectin, the continuity and hierarchical organization of the cardiac extracellular matrix became apparent. The matrix forms a continuous network extending from the pericardium to the endocardium. Furthermore, there is an arborecent hierarchy in the system such that collagen type I is more prevalent in the wider septa, collagen type III being more obvious in medium-sized branches, and fibronectin and collagen type VI prevailing in the terminal (pericellular) aspects of the network. In this pericellular location, fibronectin and collagen type VI, by means of specific interactions, may act as anchor components linking the myocardial cell basement membranes not only to the extracellular matrix but also to the cardiac interstitial cells. This continuity, organization, and coupling of the cardiac extracellular matrix appears well suited to integrate and distribute the physical stress generated by the continuous contraction and relaxation of the myocardium. (Circulation Research 1992;70:1006–1017)

KEY WORDS • collagen • extracellular matrix organization • fibronectin • immunohistochemistry • laminin • dogs • macaques • rats

Recent studies are beginning to unravel the organization and characteristics of the cardiac extracellular matrix (ECM).

An intimate association between matrix components and myocytes has been demonstrated, and it has been suggested that the ECM plays an important role in normal cardiac function. Collagen appears to be the most abundant component of this ECM, and types I, III, IV, and V have been identified in the myocardium. Although extensive studies have been performed regarding some collagens (types I, III, IV, and V), little is known about collagen type VI in the myocardium. Collagen type VI was first identified by Chung et al as a highly disulfide-bonded complex present in pepsin digests from the interstitial layer of human blood vessels. Subsequently, it has been demonstrated that this collagen is an abundant component of most soft connective tissues. Collagen type VI is a glycoprotein composed of three distinct polypeptide chains (α1, α2, and α3) that are encoded by separated genes. Two of the α chains have molecular masses of 150,000 d (α1) and 140,000 d (α2). The α3 chain was recently shown to be present in avian and human tissues as a ladder of polypeptides ranging in molecular mass between 180,000 and 260,000 d. This variability was interpreted as indicative of either partial in vivo processing or partial degradation during isolation. The three α chains contain collagenous domains of 335– or 336–amino acid residues with one or two interruptions and large N- and C-terminal globular domains. The monomers assemble into filamentous structures through the formation of dimers and subsequent formation of tetramers. Protein and cDNA sequencing have revealed the presence of many cysteine residues within the N- and C-terminals near the ends of the triple-helical domains. It has been suggested that these cysteines contribute to the high thermal stability of nonreduced monomers through interchain disulfide bonding. Although the size of the triple-helical domain of collagen type VI is only about one third of that of type I collagen, its denaturation temperature is ~40°C, which is very close to that of type I collagen. Despite the wealth of biochemical and molecular information obtained for collagen type VI over the past several years, its physiological role is still not clear. In vitro studies have suggested that it may be involved in

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cell adhesion probably through Arg-Gly-Asp sequences.\textsuperscript{15,19,20} Morphological studies suggest that collagen type VI may act as a connecting unit linking different ECM components.\textsuperscript{8,21,22} Although peptin digestion under conditions that preserve the native triple helical conformation of collagen has been successfully used for extraction of collagen types I, II, and III from various tissues, this method is not useful for the isolation of intact collagen type VI molecules because the disulfide bonds in and near their helical domains render them resistant to proteolytic digestion even under denaturing conditions.\textsuperscript{9} Higher yields of collagen type VI were obtained when the tissues were first extracted with a dissociative chaotrop agent, followed by digestion with pepsin.\textsuperscript{9} In the present report we have used this procedure to isolate and partially characterize cardiac collagen type VI from three mammalian species.

The morphology and organization of the myocardial connective tissue has been described in detail.\textsuperscript{35-26} By analogy with skeletal muscle, it is considered to be organized on three interconnected levels\textsuperscript{26,27}: epicardium, the layer that encloses the entire muscle; perimysium, the septa that define major muscle bundles; and endomysium, the delicate tracts associated with groups of cells. In the endomysium, scanning electron microscopy studies have demonstrated a complex system of filaments, fibrils, and fibers bridging the lateral surface of myocytes and becoming interconnected. By analogy with engineering structures, they have been termed struts, pericellular fibers, surface cables, and weaves.\textsuperscript{26,27} In recent years, the distribution of some individual ECM components, such as collagen types I and III, within the myocardial connective tissue has been studied\textsuperscript{26,23,27,30}; however, an exact understanding of the composition and organization of cardiac ECM has yet to emerge. To define the distribution of collagen type VI in the cardiac ECM and its associations with other components of the ECM, we examined the immunolocalization not only of collagen type VI but also of five other ECM components (collagen types I, III, and IV, laminin, and fibronectin). With this combined approach, a picture of the hierarchical organization of the cardiac ECM has been obtained.

Materials and Methods

**Animals**

The following species were used: adult male Wistar rats weighing 200–250 g, mongrel dogs weighing 18–22 kg, and long-tailed macaques (Macaca fascicularis) weighing 4 kg. The macaque hearts were available through the courtesy of Dr. K.T. Weber, University of Missouri, Columbia. All animals used in these studies were housed in facilities supervised by a veterinarian, and all experimental protocols were approved by the Institutional Research Committee.

**Extraction and Quantitation of Interstitial Collagens**

An aliquot of the lyophilized myocardial tissues was hydrolyzed in 6N HCl at 110°C for 18 hours, and hydroxyproline was measured to determine total collagen content of the myocardium as described previously.\textsuperscript{4,31} Interstitial collagens present in the hearts of rats, dogs, and macaques were examined using previously described procedures.\textsuperscript{4,31-36} The lyophilized tissues were subjected to sequential extraction under nondenaturing conditions.\textsuperscript{31,33} The peptin extracts that contained >70% of the collagen were used for determination of the relative proportions of various collagens using interrupted sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) to separate the α chains of collagen types I, III, and V.\textsuperscript{37} This procedure has been successfully used by us to quantitate interstitial collagen types in a variety of vascular tissues including myocardium.\textsuperscript{31,35} Table 1 indicates the relative effectiveness of the extraction procedure. Briefly, total collagen was precipitated from the peptin extracts by adding NaCl to 5% final concentration at 4°C and collected by centrifugation. The collagen precipitate was resolubilized in 0.5 M Tris-HCl (pH 7.4). The relative proportions of types I, III, and V collagens were next determined on these samples using SDS-PAGE as described previously.\textsuperscript{4,31,35} After electrophoresis, the gels were stained with Coomassie blue and scanned at 580 nm, and the recordings of the areas under each peak were determined using a planimeter. The areas representing α chains were used to calculate the relative percentage of types I, III, and V collagen.

**Isolation and Purification of Collagen Type VI**

Collagen type VI was isolated from the myocardium of adult rats, dogs, and macaques. The animals were anesthetized and killed, the hearts were rapidly dissected, and the atria were removed and discarded. The ventricular myocardium was rinsed in buffered saline.
blotted, weighed, and lyophilized. The lyophilized tissues were suspended in cold 50 mM Tris-HCl buffer (pH 7.4) containing 1 M NaCl and a mixture of protease inhibitors described previously31,34 and extracted in the same buffer at 4°C, under constant agitation, for 48 hours. After removal of the soluble proteins by centrifugation at 20,000g, the insoluble residue was extracted twice with 6 M guanidine-HCl in 50 mM Tris-HCl buffer at pH 7.4 and at 4°C for 24 hours. The guanidine extracts were separated by centrifugation, pooled, and dialyzed exhaustively against 0.5 M acetic acid. The extracts were then subjected to limited trypsin digestion (0.5 mg/ml extract) for 3 days at 4°C and salt-fractionated to isolate collagen type VI as described by Trueb et al.9,38 The purified collagen type VI was suspended in a small volume of deionized water and dialyzed against 0.15 M NaCl in 50 mM Tris-HCl buffer (pH 7.4 at 4°C) for subsequent analyses.

**SDS-PAGE**

Aliquots of the samples were analyzed by SDS-PAGE39 in 8% slab gels under reducing and nonreducing conditions. Gels were stained with Coomassie blue and destained with ethanol and acetic acid.

**Western Blot Analysis**

Goat antibodies to pepsinized human and bovine collagen type VI were purchased from Southern Biotechnology Inc., Birmingham, Ala. For immunoblotting, the samples were electrophoresed in SDS slab gels as described above and transferred onto nitrocellulose filters using a buffer containing glycine, Tris-HCl, and methanol.40 The nitrocellulose membrane was blocked in defatted powdered milk at 4°C and probed with the collagen type VI antibodies at 1:500 dilution, and the membrane was reacted with alkaline phosphatase-labeled rabbit anti-goat immunoglobulin G. The phosphatase activity was developed with NBT/BCIP (Promega Biotech) as directed by the manufacturer. Prestained molecular-sized standards (Bethesda Research Laboratory) and standard human collagen type VI were simultaneously electrophoresed on the gels.

**Quantitation of Collagen Type VI**

Two methods were used, depending on the availability of the purified collagen type VI: 1) Aliquots of purified collagen type VI were hydrolyzed in 6N HCl at 100°C for 18 hours, and total hydroxyproline was determined according to previously published methods.4 The collagen content (type VI) was calculated assigning a 6% hydroxyproline content.41 2) Aliquots of partially purified samples were electrophoresed under reducing conditions, and after staining with Coomassie blue, the areas corresponding to the α chains of collagen type VI were determined by scanning densitometry and planimetry. The values were established by comparison with similar scans of known amounts of standard collagen type VI. Quantitation of type VI collagen was based on the assumption that all of the type VI collagen was solubilized by extraction with 6 M guanidine-HCl. To assure a complete extraction of the type VI collagen, the extraction with 6 M guanidine-HCl was repeated once. The residue remaining after the guanidine extractions was tested for the presence of nonextracted type VI collagen by Western blot. This analysis yielded negative results, indicating the solubilization of essentially all of the type VI collagen by the guanidine extraction. Because the total collagen content of these samples was known before extraction with guanidine, the percentage of type VI collagen could be calculated.

**Immunohistochemistry**

The immunohistochemical procedures were those we have previously described,42,43 Briefly, under ketamine anesthesia, rat hearts were removed from the thoracic cavity and immediately immersed in phosphate buffer (0.1 M, pH 7.4) containing 4% formaldehyde. While immersed in fixative, each heart was cut transversely into 2-mm slices and fixed for 1 hour. After fixation, the tissues were rinsed in buffer, dehydrated in graded ethanol, and embedded in paraffin via xylene. Five-millimeter sections were deparaffinized in xylene and sequentially treated as follows: digested with pepsin (0.5 mg/ml) in 0.5% acetic acid for 30 minutes; reduced with sodium borohydride (1 mg/ml); reacted with normal goat serum, primary antibody (or control serum), biotinylated secondary antibody, peroxidase-streptavidin complex, and H2O2, diaminobenzidine. The hearts from six animals, with a minimum of two blocks per animal, were studied with all antibodies.

**Antibodies**

The following antibodies were used: anti-collagen type I elicited in rabbits against rat-tail collagen type I31,44,45; monoclonal anti-collagen type III (Heyl Laboratories); anti-collagen type IV elicited in rabbits against collagen type IV from mouse EHS tumor10,21; rabbit anti-human, pepsinized collagen type VI (Heyl Laboratories); anti-laminin elicited in rabbits against laminin purified from rat ED-PYS carcinoma46,47; and anti-fibronectin elicited in rabbits against bovine plasma fibronectin.48–50 These antibodies are routinely used in our laboratory. The monospecificity of the antibodies has been previously established by electroimmunoblotting and by electron immunohistochemistry localization.10,21,22,43,44,51 Thus, there was no cross-reactivity between the antibodies to any of the various collagen types (I, III, IV, and VI) nor any cross-reactivity

### Table 2. Collagen Concentration and Types in Left Ventricle of Rat, Dog, and Long-Tailed Macaque

<table>
<thead>
<tr>
<th>Collagen Type</th>
<th>Rat mg/100 mg dry wt</th>
<th>Dog mg/100 mg dry wt</th>
<th>Macaque mg/100 mg dry wt</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>91±3</td>
<td>89</td>
<td>85±5</td>
</tr>
<tr>
<td>III</td>
<td>6±3</td>
<td>9</td>
<td>11±4</td>
</tr>
<tr>
<td>V</td>
<td>3±1</td>
<td>2</td>
<td>3±3</td>
</tr>
<tr>
<td>VI</td>
<td>5%</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

Ten rats were used for determination of collagen concentration, and four rats were used for determination of the relative proportion of each type of interstitial collagen. Two dogs were used for determination of collagen concentration and of the relative proportion of each type of interstitial collagen. Macaque data were adapted from Weber et al.4 Type VI collagen was purified from 6 M guanidine extracts of the myocardium of rat, dog, and macaque, and a minimum of two samples was used. The relative proportion of type VI collagen was determined as described in "Materials and Methods.”
between these and antibodies to the other ECM components (fibronectin and laminin) examined in this study. Biotinylated goat anti-rabbit immunoglobulin G, biotinylated goat anti-mouse immunoglobulin G, and streptavidin-peroxidase complex were obtained from Vector Laboratories, Inc., Burlingame, Calif.

Results

Collagen Extraction

A sequential extraction procedure was used for solubilization of myocardial collagen from the three species. More than 75% of the total collagen was solubilized by this procedure (Table 1). There were no substantial differences in the patterns of myocardial collagen solubilization between the species, and in all cases the pepsin extracts contained most of the collagen. These extracts were pooled and used for determination of collagen types I, III, and V in each of the species.

Quantitation and Ratios Among the Collagens

Table 2 shows the concentration of collagen present in the left ventricle of the three species. The greater amount of collagen shown in macaque myocardium may be species or age related, or it may be due to the fact that the samples of lyophilized macaque myocardium were defatted before determination of collagen content. The relative ratios of the three interstitial collagens were similar in the three species. In all cases, collagen type I was the major collagen type, representing >85% of total collagen, whereas type III represented 6–11%. A small proportion of type V (2–3%) was also found in the three species.

Table 2 also shows the relative proportion of collagen type VI present in the myocardium of the three species. Because the guanidine extracts contained only a small proportion of the total myocardial collagen and because some losses during the purification steps were unavoidable, it was not possible to quantitate precisely the type VI present in the myocardium. Nevertheless, of the three species examined, rat myocardium had the highest content of collagen type VI (~5% of total collagen), whereas this content was lower in dog and macaque myocardium.

Characterization of Collagen Type VI

When isolated collagen type VI from myocardium of the three species was electrophoresed under nonreducing conditions and visualized by Coomassie blue, the majority of the material barely penetrated the gel. The band observed near the top of the gels in the absence of reducing agents represents the disulfide-linked aggregates of collagen type VI. The α-chains of pepsin-digested (p) collagen type VI are identified.

FIGURE 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cardiac collagen type VI. Aliquots of purified collagen type VI (pepsinized) were electrophoresed in an 8% polyacrylamide gel under reducing (+) and nonreducing (−) conditions. Lane 1 contains globular protein standards; lanes 2, 3, and 4 contain unreduced collagen type VI from rat, dog, and macaque, respectively; lanes 5, 6, and 7 contain reduced collagen type VI from rat, dog, and macaque, respectively. The band at the top of the gel (arrowhead) represents disulfide-linked aggregates of collagen type VI. The α-chains of pepsin-digested (p) collagen type VI are identified.

FIGURE 2. Immunoblot of cardiac collagen type VI. Aliquots of purified collagen type VI were electrophoresed in an 8% sodium dodecyl sulfate gel, transferred to nitrocellulose, and reacted with polyclonal antibodies monospecific for collagen type VI. Lanes 1 and 2 contain unreduced (−) and reduced (+) rat collagen type VI, respectively. The band at the top of the gel (arrowhead) represents disulfide-linked aggregates of collagen type VI. The α-chains of pepsin-digested (p) collagen type VI are identified. Globular protein standards are indicated at the left.
weaker than the α2(VI) and α3(VI) chains, and in some preparations, it was even difficult to identify any α1(VI) chains.

The nature of these bands was further confirmed as being collagen type VI by immunoblotting. In Figure 2, it can be seen that under nonreducing conditions (lane 1) the band barely penetrating the gel reacted with the anti-collagen type VI antibodies. Furthermore, under reducing conditions murine collagen type VI yielded the three α chains. Here again, the intensity of the top band corresponding to α1(VI) chain was less than that of the α2 and α3 chains.
FIGURE 4. Photomicrographs showing localization of collagen type III in rat myocardium. Panel a: Compared with collagen type I, type III is less prevalent in larger septa but more abundant in smaller ones. Myocardium was lightly counterstained with hematoxylin. Magnification, ×37.5. Panel b: Detail of collagen type III distribution is shown. Type III is present within the media and in the adventitia of a medium-sized muscular artery (A), in the connective tissue surrounding smaller vessels (arrows), and even in the surrounding capillaries (arrowheads). The myocardial cells are not surrounded by a continuous layer of type III collagen. Myocardium was lightly counterstained with hematoxylin. Magnification, ×150.

Immunohistochemistry

Collagen type I. Collagen type I was abundant in the epicardium and in large connective tissue septa (perimysium) but was less prominent in the delicate septa (endomysium) separating small groups of myocardial cells (Figure 3). It was found in the adventitia of medium-sized coronary arteries (Figure 3b) but not in the interstitium surrounding capillaries.

Collagen type III. Collagen type III displayed some similarities in distribution with type I but also some
differences. It was less prominent in the epicardium and perimysium but more prominent in the endomysium (Figure 4). It was found in the media of medium-sized coronary arteries (Figure 4b) and in the connective tissue surrounding large and small blood vessels.

Fibronectin. This glycoprotein was less prominent than collagen types I and III in the epicardium and perimysium; however, it was more prevalent in the endomysium, often surrounding small groups of myocardial cells (Figure 5). It was not prominent in the adventitia of medium-sized coronary arteries, but it was often found in the connective tissue surrounding capillaries (Figure 5).

Collagen type VI. Collagen type VI had a distribution somewhat similar to that of fibronectin but appeared to be more prevalent. It was not prominent in the epicardium or perimysium, but it was abundant in the finer connective tissue septa (endomysium) around small groups of myocardial cells (Figure 6). Occasionally, it was found partially surrounding individual myocardial cells (Figure 6b). It had a prominent perivascular distribution, being found in the adventitia of large and medium-sized arteries and surrounding capillaries (Figure 6).

Laminin and collagen type IV. These two basement membrane components were restricted to these structures, codistributing in all cardiac basement membranes including those of myocardial, endothelial, and smooth muscle cells (Figure 7).

The pattern and distribution of the six cardiac ECM components studied is depicted in tabular form in Table 3 and in diagrammatic form in Figure 8.

Discussion

We report the isolation and partial characterization of cardiac collagen type VI from rat, dog, and macaque. To accomplish the isolation of this collagen required an initial tissue extraction with guanidine buffer, followed by pepsin digestion and salt fractionation. Pepsin-extracted collagen type VI was identified by its behavior on SDS-PAGE under reducing and nonreducing conditions. In the absence of reduction, collagen type VI barely entered 8% polyacrylamide gels and appeared as a single band near the top. When the same samples were electrophoresed under reducing conditions, three distinct bands corresponding to each of the collagen type VI α chains were recognized. The mobility of the α chains of cardiac type VI was similar to that of standard collagen type VI (obtained commercially) from bovine and human sources. Further confirmation of the presence of collagen type VI in the guanidine-extracted pepsin-digested samples was obtained by immunoblot analysis using monospecific antibodies.

Immunohistochemical studies of collagen type VI in the murine myocardium yielded intense staining, suggesting that this collagen may be more prevalent than other minor collagen types. Therefore, attempts were made to quantify this collagen in the myocardium. Our results indicate that collagen type VI constitutes ~5% of total myocardial collagen. However, this figure is probably only an approximation, because of the following limitations: Only the guanidine extracts were used for isolation and purification, and it is conceivable that a certain proportion of collagen type VI may not be extractable with guanidine. However, boiling the guanidine-insoluble residue in SDS and submitting this extract to Western blotting failed to demonstrate any collagen type VI epitopes (data not shown). A second consideration is that some losses during the salt fractionation and subsequent purification steps are unavoidable.
able. Nevertheless, it is clear in the three species that \(~5\%\) of the total myocardial collagen was collagen type VI.

In agreement with our previous studies,\(^2,3,5\) we found that collagen represented only a minor fraction (\(2-5\%\)) of the total dry weight of the heart. Type I was the major collagen, representing \(~80\%\) of total collagen. Type I collagen has been shown to play a crucial role in maintaining structural and functional integrity of heart along with collagen type III.\(^4\) To define the relations...
among the different components of the cardiac ECM, the distribution of six of these components was studied by immunohistochemistry in the rat myocardium. The monospecificity of the antibodies used had been previously established; furthermore, the unique and distinctive patterns obtained with each one of the antibodies in the myocardium demonstrate the absence of cross-reactivity. Several findings deserve consideration. The nonquantitative nature of immunohistochemistry was clearly demonstrated in these studies; although collagen types I and VI represent ~80% and 5%, respectively, of the total murine cardiac collagen, comparison of sections reacted with antibodies against these collagen types (Figures 3 and 6) would suggest a similar cardiac content or even a prevalence of collagen type VI. Probably, staining intensity is a better reflection of the surface containing the antigen rather than of the actual concentration. The orderly distribution of the different ECM components studied is noteworthy. The cardiac ECM seems to have a remarkable continuity from epicardium to endocardium. However, this ECM, in addition to being continuous, appears to be system-
that larger epimysial septa do not contain type VI but simply that, as a generalization of the overall organization, collagen type I is more prevalent in the thicker connective tissue structures and collagen type VI and fibronectin are more prevalent in the more delicate ECM framework. It is conceivable that the myocardial basement membranes are connected to fibronectin and/or collagen types V and VI; these three ECM components are, in turn, connected to collagen type III that is itself connected to collagen type I. These associations among ECM components have been demonstrated by electron microscopy immunohistochemistry in other organs. The organization seems particularly well suited to distribute the changes in shape and

![Photomicrographs showing localization of collagen type IV and laminin in rat myocardium. Panel a: Collagen type IV is absent from connective tissue septa but is present in all endothelial, smooth muscle, and myocardial basement membranes. Myocardium was lightly counterstained with hematoxylin. Magnification, ×37.5. Panel b: Detail of the distribution of collagen type IV in all basement membranes is shown. The endothelial and smooth muscle cell basement membranes of this artery (A) contain collagen type IV. Notice that the adventitial connective tissue (arrows) is devoid of collagen type IV. The capillary endothelial and myocardial basement membranes also contain this collagen, forming a continuous layer. Myocardium was lightly counterstained with hematoxylin. Magnification, ×150. Panel c: Detail of laminin distribution is shown. Laminin had the same distribution as collagen type IV, being present in all cardiac basement membranes. Notice that each myocardial cell is surrounded by a continuous laminin (basement membrane) layer. Myocardium was lightly counterstained with hematoxylin. Magnification, ×150.](http://circres.ahajournals.org/)

<table>
<thead>
<tr>
<th></th>
<th>Pericardium</th>
<th>Large septa (perimysium)</th>
<th>Small septa (endomysium)</th>
<th>Pericellular area (myomysium)</th>
<th>Vascular media</th>
<th>Vascular adventitia</th>
<th>Basement membranes</th>
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<td>+++</td>
<td>+++</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Laminin and collagen type IV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>+++</td>
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</table>

The relative staining intensity of the studied antigens is based on an arbitrary scale, from no demonstrable antigen (−) to maximum intensity (+++). Because of the nonquantitative nature of immunohistochemistry, the staining intensity reflects neither amount nor concentration of the antigens.

**TABLE 3. Distribution of Extracellular Matrix Components in Rat Myocardium**
stress that continuously take place throughout the myocardium. The filaments of fibronectin and collagen types V and VI, which are probably more pliable, could absorb most of the shape changes and transform them into tension that is transmitted to the stiffer types III and I. It can be speculated that the endomysial struts elegantly described by scanning electron microscopy\cite{24,27} may correspond in great part to collagen type VI and fibronectin; either one of these glycoproteins may be tightly bound to some of the basement membrane components (laminin, entactin, collagen type IV, and perlecanc). Because some of these basement membrane components are bound to the cell surface by interaction with specific integrins, this system provides a mechanism for signal transduction between myocytes and the interstitium. Furthermore, the presence of Arg-Gly-Asp sequences in collagen type VI could allow binding of this collagen to the cell surface of cardiac fibroblasts and the subsequent exchange of stress information between myocytes and fibroblasts. Such a system may explain the excellent correlation between myocardial overload, hypertrophy, and increased ECM deposition.

Several studies have described changes in the morphology and the phenotype of the collagen matrix in cardiac hypertrophy.\cite{2,4,24,28,55,56} The alterations that have been observed vary with the animal model and the experimental manipulation required to induce cardiac hypertrophy (e.g., pressure versus volume overload). In spontaneously hypertensive rats, although no changes in the myocardial collagen content were noted, there was a shift in collagen phenotype with an increment in the proportion of collagen type III.\cite{30} In contrast, in our previous study in the macaque with pressure-overload hypertension, we found an increment in both collagen concentration and the proportion of type III collagen in the early phase of hypertrophy.\cite{4} To date, there are no descriptions of the organization of the normal cardiac extracellular matrix that simultaneously examine the six components described in the present study. Given the exquisite order and hierarchy of the cardiac ECM demonstrated here, it will be important to determine whether pathological conditions, such as hypertension and chronic ischemia, cause alterations in the organization of the cardiac ECM rather than simple increases in quantity. Furthermore, it will be of interest to determine if such changes may be responsible for the decreased efficiency and failure of the cardiac pump. Additional studies will be necessary to establish a definite relation between alterations in the content, phenotype, and structural organization of myocardial collagen and the mechanical properties of the heart.

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References


FIGURE 8. Diagram of the distribution of the six cardiac extracellular matrix components studied. Collagen type I forms the major structural buttress of the myocardium, being present in large septa spanning from the epicardium to the endocardium. Collagen type III is also found in the large septa, but it is less prevalent than type I. Unlike type I, type III extends to form a major part of the finer septa and is prominent in the vascular adventitia. Collagen type VI and fibronectin extend from these finer septa to the connective tissue surrounding capillaries and partially to myocardial cells. In this pericellular location, they are likely to bind to basement membrane components (laminin and collagen type IV). This organization suggests that the cardiac extracellular matrix is organized, resembling an architectural system in which load and stress are distributed in a hierarchical manner. The hierarchy in order of decreasing tensile strength and increasing pliability is as follows: type I→type III→type VI and fibronectin (and perhaps type V)→basement membranes. In this manner, changes induced by contraction and relaxation of myocardial cells can be distributed throughout the heart.
Isolation, characterization, and localization of cardiac collagen type VI. Associations with other extracellular matrix components.

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