Upregulation of endothelial adhesion molecules in hearts with congestive and ischemic cardiomyopathy: immunohistochemical evaluation of inflammatory endothelial cell activation

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Abstract

Objective: In recent years many data emphasized, that inflammatory reactions seem to be involved in the pathogenesis of ischemic (ICM) and congestive (CCM) heart disease. Since, it is well known that endothelial adhesion molecules play a pivotal role in the initiation and maintenance of inflammatory reactions we therefore, evaluated the endothelial expression of a wide variety of different adhesion molecules in hearts suffering from ICM and CCM. Methods: Tissue samples from coronary arteries, and left and right ventricle myocardium originating from heart with ICM and CCM were evaluated. Tissue samples from healthy human donor hearts, which were not transplanted, served as controls. Evaluated adhesion molecule expression:
selectin-family ELAM-1, CD62, immunoglobulin-supergene-family PECAM-1, ICAM-1, VCAM-1, integrin-family VLA-1,-2,-3,-4,-5, and -6, complementary-adhesion-molecules CD34, CD44 and the von-Willebrand-factor (vWF). Results: While endocardial surfaces and coronary arteries revealed only little differences when comparing tissue samples originating from healthy donor hearts and those suffering from ICM and CCM, significant differences were found within the myocardial microvasculature. Both kinds of diseased hearts showed stronger expressions for CD62, ELAM-1, ICAM-1 and VCAM-1 (only CCM) than controls. More and above, integrin molecules showed differential expressions too. Whereas, VLA-1 showed stronger expressions in diseased hearts, VLA-3, -5, and -6 were expressed much weaker in those hearts. Complementary adhesion molecules (CD34/CD44) did not show significant differences and the vWF was not found in any sample. Conclusions: Inflammatory reactions play a pivotal role in the propagation and maintenance of both these cardiac deteriorating diseases.

1. Introduction

Congestive cardiomyopathy (CCM) is an etiological heterogeneous disease, the leading indication for cardiac transplantation and a significant cause of death. Specific effective therapeutic interventions have been hampered by incomplete understanding of the pathophysiological mechanisms leading to this situation. However, in recent studies, some authors describe that degenerative processes of contractile components of the cytoskeletal apparatus in myocytes is one aspect leading to functional deterioration and final failure of these diseased hearts [1]. More and above, it was noted that CD3 positive T-lymphocytes as well as other inflammatory mediators are present in CCM hearts and thus, pointing to low-grade chronic inflammation.

Over the years it becomes clear that inflammatory reactions also play a pivotal role for the degeneration and progression of atherosclerotic plaques in ischemic heart disease (ICM) as well [2,3]. Therefore, it was supposed that increased activities of adhesion molecules on endothelial cells, as well as on platelets and leukocytes are associated with the propagation of myocardial ischemia, damage during re-perfusion, healing and scare formation [4].

Here we evaluated the endothelial expression of a wide variety of different adhesion molecules in hearts suffering from ischemic and congestive cardiomyopathy. Potentially existing differential expression patterns should be documented and discussed in front of their diagnostic and therapeutic relevance.

2. Patients and methods

Tissue samples originated from human hearts suffering from ischemic and congestive cardiomyopathy (left ventricular ejection fraction <40%). Native human donor hearts, which could not be transplanted due to acute instability of the prospective recipient (e.g. sudden death during transport to hospital), or which were only dispensed for heart
valve banking procedures by the patient’s will, served as controls (Table 1). Samples were taken intraoperatively, were immediately snap frozen and stored in liquid nitrogen (−193 °C) until further use. Blood analyses as well as macro- and microscopic evaluations performed to exclude located/ generalized inflammatory reactions or other pathological changes, showed results within the normal range in all native donor hearts. For immunohistochemical examination, samples were cut into 3-5 μm sections and stored at −20 °C until staining. Due to temperature-dependent antibody kinetics, a slightly modified alkaline phosphatase/anti alkaline phosphatase (APAAP) staining method previously described elsewhere was applied [5]. Negative and positive controls were performed in parallel to all staining series and found valid in all cases. More and above, for further validation of staining results, all tissue samples were evaluated by two independent observers, who did not get any information about tissue origin and used antibodies.

2.1. Investigated endothelial adhesion molecules

All primary antibodies, except the one against the von Willebrand Factor, which was polyclonal, were monoclonal (mAB). CD62 (P-Selectin) (H18/7), PECA1 (5.6E), ICAM-1 (B4H10), VCAM-1 (1G11), VLA-2 (G9), VLA-3 (M-KID2), VLA-4 (HP2/1), VLA-5 (SAM1), VLA-6 (Go H3), CD34 (QBEND 10) and CD44 (J-173) were purchased from IMMUNOTECH S.A.(Marseille, France), and ELAM-1 (H18/7) from Becton Dickinson (Singapore). Von Willebrand Factor (A 082), APAAP-bridge and APAAP-complex were purchased from DAKO A/S (Glostrup, Denmark), VLA-1 (TS2/7) from T CELL Diagnostics (Cambridge, MA).

All monoclonal antibody dilutions were titrated in separate staining series to determine optimal staining results. For visualization of staining results light microscopy was used.

2.2. Qualitative analysis

Using endocardial surfaces and coronary arteries, we established a semiquantitative grading system for the expression of proteins. Staining intensities were graded as follows: negative (−), slightly positive (+), moderate (+++) and very intense (+++). A negative staining was defined as no expression (−). A weak positive staining (slightly positive stain) was defined as a weak positive staining (++). A positive staining (+) was defined as a positive staining (++). A strongly positive staining (+++) was defined as a strongly positive staining (+++). A very strong positive staining (++++) was defined as a very strong positive staining (++++)

2.3. Quantitative analysis

In order to analyse the myocardial microvasculature, we established a method for quantitative analysis. Using three randomly chosen microscopic high power fields of each tissue section, the number of positive stained vessels of all specimens of the same vascular location (e.g. left ventricle myocardium) were added and the mean was calculated. In order to compare single staining results of different endothelial adhesion molecules, all results were compared to those obtained by PECA1 (CD31) staining, which is known to be a highly sensitive endothelial marker [6]. Using the formula given below, absolute numbers were transferred to percentage values in order to make comparisons less difficult [7]:

\[
\text{mAB} – \text{positive vessels} \times 100 \\
\text{PECA1} – 1 \text{positive vessels}
\]

Statistical analyses were performed by using the ANOVA test modified by Tukey. Results were judged significant, when \( P < 0.005 \). Normal distribution as fundamental prerequisite for this test was assumed to be given because of the biological determination of adhesion molecule expressions, but was also tested mathematically and found valid in all cases.


3. Results

3.1. Endothelial adhesion molecule expression on endocardial surfaces and coronary arteries

Light microscopic evaluations of conventional (HandE) stained tissue samples from ICM and CCM hearts did not
reveal major conformational changes of their endocardial surfaces. However, minor alterations consisted of a few smaller discontinuations of the endocardial endothelial cell monolayers, which were located directly above areas of myocardial fibrosis.

Coronary arteries obtained from hearts with ICM and CCM revealed physiological wall structures, composed of the typical three-layer architecture. Pathological changes like inflammatory and/or degenerative processes could only be observed in those arteries which originated from ICM hearts. Intimal calcifications reaching from mild to subtotal stenosis, as well as unspecific plaque depositions characterized these arteries.

### 3.1.1. Selectins

ELAM-1 presented with comparable staining results in all evaluated tissue samples. Coronary arteries showed moderate, left and right ventricle endocardium weak expressions. Thus, major differential expression patterns between CCM, ICM and donor hearts were not observed. CD62 (P-selectin), which did not stain any endocardial surface, showed weak expressions on coronary arteries originating from donor hearts and moderate expressions on those obtained from ICM hearts. CCM hearts did not stain for CD62 (Table 1).

### 3.1.2. Immunoglobulin—supergenes

Endocardial surfaces of all tissue samples revealed identical staining results. PECAM-1 showed strong, ICAM-1 and VCAM-1 weak expressions. Comparing staining intensities on coronary arteries, more differential staining results were found. ICAM-1 stained moderate in coronary arteries originating form donor and CCM hearts and weak on those obtained form ICM hearts. VCAM-1 revealed weak staining on donor coronary arteries and moderate expressions on CCM and ICM hearts (Table 1).

### 3.1.3. Integrins

Integrin proteins, which predominately serve as endothelial anchorage towards extracellular matrix proteins showed comparable staining results on left and right ventricle endocardium. VLA-1, -2, -4, and -6 revealed moderate expressions in both ventricles. VLA-5 showed moderate staining results on endocardial surfaces of chronic diseased hearts and weak staining in donor hearts. VLA-3 was strongly expressed on endocardial surfaces obtained from chronic diseased hearts and moderately in donor hearts. Donor and CCM coronary arteries revealed strong, those obtained from ICM moderate expressions for VLA-1. VLA-3 showed strong expressions on donor and ICM coronary arteries and moderate staining for DCM. Evaluating VLA-5 staining results strong expressions were observed for donor coronary arteries, moderate staining for those obtained from CCM and weak for ICM. VLA-6 was observed only on donor and ICM coronary arteries. VLA-4 showed weak expressions on donor coronary arteries, VLA-2 was not detectable in any sample (Table 1).

### 3.1.4. Complementary adhesion molecules and vWF

All molecules evaluated here (CD34, CD44 and von Willebrand factor) showed identical staining. CD34 stained moderate on left and right ventricle endocardium, strong in coronary arteries. CD44 revealed strong expressions in all cases and von Willebrand factor was detected in any samples (Table 1).

### 3.2. Endothelial adhesion molecule expression in the myocardial microvasculature

Light microscopic evaluations of conventional stained myocardial structures obtained from CCM hearts revealed disseminated small areas of fibrosis, which were surrounded by hypertrophic and atrophic myocytes. Areas of fibrotic changes could also be observed in hearts suffering from ischemic heart disease. However, here those areas were predominately located in subendocardial regions.

#### 3.2.1. Selectins

Statistical analyzes revealed that both, ELAM-1 and CD62, were significantly stronger expressed in the left ventricle microvasculature of ICM and CCM hearts than in those obtained from donor hearts. However, microvascular regions of right ventricles did not show significant differences between samples (Table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Adhesion molecule expression in the myocardial microvasculature</th>
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<tr>
<td></td>
<td>LV-Donor (n=6/4) %</td>
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<tr>
<td>ELAM-1</td>
<td>5</td>
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<tr>
<td>CD62</td>
<td>2</td>
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<tr>
<td>PECAM-1</td>
<td>100</td>
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<td>ICAM-1</td>
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<td>65</td>
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<td>VWF</td>
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*(n=x/y)*, number of samples/patients; *; significant difference in comparison to same native vascular area; %, expression in relation to PECAM-1; Donor, donor heart; CCM, congestive cardiomyopathy; ICM, ischemic cardiomyopathy; LV, left ventricle; RV, right ventricle; VLA, very late antigen (Integrin-proteins); vWF, von Willebrand Factor.
3.2.2. Immunoglobulin—supergenes
ICAM-1 was significantly stronger expressed in the microvasculature of left ventricles originating from ICM and CCM hearts. Significant differences for VCAM-1 were only found for CCM hearts. Significant differences in right ventricle myocardial regions were found only for ICAM-1 in ICM hearts, which revealed weaker expressions (Table 2).

3.2.3. Integrins
Significant weaker expressions for VLA-3, -5, and -6 were found in all samples obtained from chronic diseased hearts. However, statistical stronger expressions were found for VLA-1 in CCM and VLA-4 in ICM hearts. Right ventricle myocardium revealed weaker expressions for VLA-1 and VLA-3 in ICM heart (Table 2).

3.2.4. Complementary adhesion molecules and vWF
CD34, CD44 and von Willebrand factor expressions did not reveal statistical differences (Table 2, Figs. 1–6).

4. Discussion
One fundamental supposition to analyze endothelial expression patterns is the characterization of endothelial cells themselves. Because of its well known high endothelial specificity [6], we like many others before [7,8], chose PECAM-1 to confirm endothelial cell origin. The molecule showed a very intense expression at all vascular sites investigated.

In recent years many data suggested, that inflammatory reactions play a pivotal role in the pathogenesis and progression of atherosclerotic lesions [2,3]. Furthermore, it was postulated that phenotypic endothelial alterations resulting in differential endothelial adhesion molecule expression patterns, exist in hearts suffering from congestive heart disease [9]. In concert with observations of reduced endothelial dependent dilation and functional alterations within the microvasculature of these hearts [10–12] and, i.e. in front of endothelial significance for the regulation of vascular tonus and contractility, these further underscores the central physiological and pathophysiological role of endothelial cells.

Comparing chronic diseased and native donor hearts for endothelial adhesion molecule expressions on endocardial surfaces and coronary arteries, almost no differences could be observed. However, statistical analyses of expression patterns within the myocardial microcirculation revealed stronger expressions for ELAM-1, CD62 (P-selectin), ICAM-1 and in the case of CCM hearts additionally VCAM-1.
A first step in the cascade of immunological interactions between endothelial and immunocompetent cells is established by selectin proteins. Expressions of these molecules lead to the initial adherence of leukocytic cells on endothelial surfaces and initiate the so-called 'rolling' of these cells [13]. Thus, stronger staining for ELAM-1 and CD62, which are known to be inducible under conditions of inflammation [5], point to an increased readiness for these cellular interactions. VCAM-1, which through interaction of leukocytic integrins (VLA-4) further strengthens the adherence of immunocompetent cells, underscores this thesis. More and above, examining VCAM-1 expression during periods of graft rejection, some authors reported the protein to be markedly expressed only after a certain period, shortly before reaching maximal immunological reactions [14]. Thus, stronger expressions of this protein point to the presence of maximal (acute) immunological reactions. Other authors described increased VCAM-1 and MHC-DR antigen expressions on atherosclerotic plaque endothelial cells and smooth muscle cells [15–17]. Thus, although observations made in this study point to the presence of low-grade rather than acute inflammatory reactions [18], it becomes evident, that periods of acute inflammations may occur in hearts with cardiomyopathies as well. Another immunological relevant adhesion molecule is ICAM-1. The immunoglobulin-supergene protein was observed to be markedly expressed within the myocardial microcirculation and thus, further point to immunological reactions too [16]. Analysing all these data, some authors reported that persistent ICAM-1 expression is characteristic for chronic inflammatory disorders, whereas parallel expressions of ELAM-1 and VCAM-1 indicate endothelial cell damage and acute inflammation [18].

Furthermore it was noticed, that not only different periods but also different quantities of inflammation may occur in patients with ischemic heart disease. Vassi and co-workers underscored this hypothesis in that they observed that patients suffering from coronary artery disease show increased values for TNF-α and IFN-γ [19]. Since these cytokines play an important role for the induction and regulation of adhesion molecules it becomes clear, that inflammatory reactions undergo dynamic rather than static regulations. Periods of chronic reactions can be followed by acute inflammation and vice versa. Some authors speculated that vascular stenosis leads to a decrease of blood flow velocity and shear stress and an increase of inflammatory mediators [20,21]. Variable quantities of these plasma soluble factors may reveal various quantities and qualities of adhesion molecule expressions and thus, lead to different degrees of inflammatory reaction. Tousoulis and co-worker underscored this hypothesis in that they observed that plasma levels of VCAM-1 correlate with the severity of heart failure, although they found plasma VCAM-1 levels unrelated to the presence or absence of angiographically demonstrable atherosclerotic coronary artery disease [22].

Although it was postulated that most endothelial adhesion molecules exhibit generalized expressions within the entire heart [23], comparisons between microvascular areas of healthy donor hearts and those originating from ICM hearts revealed, that ICAM-1 showed only significantly different expression patterns when comparing native tissue samples with those obtained from left, but not right ventricles. This may have some important implications for our pathophysiological understanding and could shows that inflammatory reactions found in one cardiac ventricle are not necessarily present in the other as well.

### 4.1. Diagnostic considerations

Endomyocardial biopsy is a commonly performed procedure used for the evaluation of myocardial tissues. Biopsies may be used to monitor transplant rejection, but they have many other applications as well, such as the evaluation of myocarditis, cardiomyopathy, drug toxicity, chest pain, arrhythmia and cardiac involvement by systemic diseases or neoplasms [24].

However, unfortunately and for multiple reasons, the histological standard analysis delivers very limited results. One reason is that structural alterations within suspected inflammatory diseased hearts are still classified by the 'Dallas-criteria'. This method does not fulfill the sensitivity and specificity requirements imposed for the diagnosis of inflammatory cardiomyopathy for that it judges cellular infiltrates without really distinguishing between inflammatory and non-inflammatory reactions. Another important

![Image](https://academic.oup.com/ejcts/article-abstract/27/1/122/342256/fig-6-native-right-ventricle-myocardium-original-magnification-100x-apaap-stain-sample-stained-against-icam-177-of-all-vessels-stained-positive)
reason is based on the lack of standardized histological criteria, which often leads to errors between different observers. All this underscores the necessity to establish new methods for a more differential diagnosis and subsequent therapy of chronic cardiac diseases.

Since cellular infiltrates are often focally restricted to small tissue regions, the solely identification and quantification of cellular infiltrates is an inappropriate method in the diagnosis and characterisation of inflammation [25]. Mahon and co-workers who tried to differentiate native hearts from those suffering from dilated cardiomyopathy observed CD3+ leukocytic infiltrates in only 21% of diseased tissue samples [17]. Although this approach is a much more sensitive diagnosis than characterizing cellular infiltrates by conventional histology solely, Mahon as we do, proposes, that a combinative diagnostic approach characterizing and quantifying both endothelial and leukocytic adhesion molecule expressions may result in the most sensitive diagnosis of inflammatory and non-inflammatory cardiomyopathies.

5. Conclusion

The study shows that chronic low grade inflammation occurs in cardiac tissue from congestive and ischemic heart disease. Thus it can be concluded that inflammatory reactions are of etiological importance in these pathologies. They contribute to the structural and functional deterioration of failing hearts and thus, propagate the maintenance and progression of ischemic and congestive heart disease.

References