Atherosclerosis is the most common condition underlying morbidity and mortality in the industrialized parts of the world. Sudden death, acute myocardial infarction, chronic heart failure and cerebral complications are the most important sequelae of the disease. The characteristic pathology is that of an elevated intimal lesion composed of a central core of lipids encased by fibrous tissue containing smooth muscle cells and inflammatory cells among which macrophages dominate. Progressive growth of such lesions may eventually lead to significant luminal obstruction, causing impairment of organ perfusion such as that seen in patients with, for instance, stable exercise related angina pectoris. On the other hand, atherosclerotic lesions may cause abrupt and often severe symptoms, which are related to an acute complication of the atherosclerotic plaque. This consists of either plaque rupture (plaque fissure) or plaque erosion. Plaque rupture consists of fissuring of the fibrous cap, which separates the lumen from the central atheroma, inducing thrombosis with or without acute luminal obstruction. Plaque erosion is the situation in which the surface lining is eroded and mural thrombus induced. It has been shown that both these complications are related to presence of a lipid-related immune mediated inflammatory process. Over the years it has been shown that atherosclerotic plaques with a large lipid core and a small often attenuated fibrous cap are prone to developing ruptures or erosions. For this reason these types of atherosclerotic plaques have been considered “at risk” or “unstable”. Plaques dominated by fibromuscular tissue, with little or no discernable lipids, are considered “safe” or “stable”. However, these observations were based on autopsy studies and, therefore, almost per definition lack the detailed precision of in vivo clinicopathological correlates. The introduction of directional coronary atherectomy (DCA) has opened gateways to this end.

DCA is an intervental cardiac procedure in which a catheter is introduced into the coronary artery carrying a cutting device on its tip. Once a culprit lesion has been identified and localized, the cutting device can be introduced and the lesion removed. The tissue can be retrieved and becomes available for microscopic evaluation. This then introduces the possibility to provide detailed correlations between the clinical state and the pathology of the lesion considered responsible for the clinical condition at the time. DCA, therefore, has contributed markedly to the understanding of the pathological mechanisms underlying acute coronary syndromes.

Patients with stable angina pectoris presented culprit lesions composed mainly of smooth muscle cells, with few inflammatory cells. On the other hand, patients with unstable angina pectoris (Braunwald class II, II) showed a significant increase in extent of inflammatory cells, closely related to the severity of the acute coronary syndrome. In fact, patients with acute myocardial infarction showed most inflammation in the culprit lesions. These studies have shown unequivocally that plaque inflammation plays a role in the onset of unstable angina and, on that basis, has initiated further investigations into the role of inflammatory mediators. One aspect distinctly revealed by DCA is that “stable” plaques may nevertheless contain foci of inflammation; an observation which has raised the question whether with time, morphologically stable plaques may turn into clinically unstable conditions. DCA procedures also have made it possible to demonstrate that interleukin-2 receptor activity on lymphocytes is significantly increased in culprit lesions of unstable angina and even more so in those obtained from patients with acute myocardial infarction, thus indicating recent onset activation. This is the most interesting since patients with acute onset of coronary syndromes have increased plasma levels of inflammatory markers, suggesting that infectious agents could play a role. All in all, DCA tissue sampling has provided the unique opportunity to study the pathology of atherosclerotic plaques in vivo. The novel observations obtained provide the impetus for additional studies into the potential relationship between acute coronary syndromes and infection.

The endothelium as a multifunctional organ: From sepsis to tumor metastasis

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The ubiquity of the endothelium makes it a prime candidate for local regulation of interfacial interactions in organ function. On the basis of this role the endothelium itself can be regarded as a multifunctional organ. Endothelial cell functions constitute a delicate balance of opposing actions, which in the physiological situation are strictly controlled. The pathobiological role of the endothelium in various disease processes is best understood by viewing the imbalance in these opposing functions. Blood contacting endothelium is nature’s most efficient anti-thrombogenic surface. However, the endothelial regulation of hemo-
stasis involves the ability to produce not only numerous anticoagulant substances, including prostacyclin, nitric oxide (NO), thrombomodulin, urokinase-plasminogen activator, and various heparan sulfate proteoglycans, but also a series of prothrombogenic molecules, such as tissue factor, the coagulation factors V and VIII, the receptors for coagulation factors IX and X, as well as plasminogen activator inhibitor-1, which under various pathological conditions become predominant. Thus, under proinflammatory cytokines and bacterial toxins procoagulant activity in endothelial cells plays a central role in the manifestations of disseminated intravascular coagulation and septic shock. Sepsis can be regarded as the extreme form of inflammation. Morphological studies have indicated that the initial pathological lesions are to be found in endothelial cells. Nevertheless, even prior to these manifestations dysfunctional endothelium is hypothesized to be instrumental in the pathogenesis of the microcirculatory dysregulation which forms the basis of multiple organ failure. In this scenario endothelial cells in tandem with plasma and blood cell mediator systems change their function from an inflammatory regulator to uncontrollable amplification of the inflammatory response. As well as procoagulant activity, endothelial cell production of cytokines [interleukin (IL)-1β, IL-6, IL-8, thrombin], as well as up-regulation of cell adhesion molecules (CAMs) is central to the clinical sequelae of sepsis and multiple organ failure, including disseminated intravascular coagulation, the interstitial fibrosis of adult respiratory distress syndrome (ARDS) and leukocyte sequestration. Evidence from our laboratory indicates that signal transduction pathways in the endothelium under these extreme proinflammatory stimuli may be distinct from the highly controlled physiological upregulation of inflammation. Thus, NF-KB-independent pathways are clearly associated with the elevated endothelial expression of CAMs relevant for monocyte and granulocyte adhesion. This could provide the molecular basis for new therapeutic concepts in multiple organ failure.

Endothelial regulation of vascular via production of vasodilators (prostacyclin, NO) and vasoconstrictors (PAF, endothelin-1) is of vital importance especially in the arterial side of the circulation. The onset of endothelial dysfunction, for example in atherosclerosis, appears to correlate with an early loss of endothelium-dependent relaxation, which accompanies down-regulation of the constitutive expression of the endothelial NO synthase gene. In atherosclerosis, as well as in other vascular disorders, numerous other features of endothelial dysfunction become operative. Among these are, for example, the effects of altered shear stress, oxidant stress from activated blood cells, oxidation metabolites of lipoproteins, advanced glycation end products (in diabetes) and antiendothelial antibodies (in vasculitis forms).

Tumor intra- and extravasation are central elements in the metastatic process and involve tumor cell interactions with the endothelium. Various tumour-dependent mechanisms, including the production of reactive oxygen species, are operative in endothelial cell damage during the process of tumor extravasation. Tumor cell adhesion to the endothelium appears to involve CAMs which are also active in the physiological inflammatory reaction. Proinflammatory cytokines, such as tumor necrosis factor-α and IL-1β, appear to promote tumor cell-endothelial cell adhesion via upregulation of these CAMs although the exact pathogenetic significance of this CAM upregulation in tumor microvessels is still controversial. One hypothesis views the increased CAM expression as favorable to tumor metastasis by facilitating tumor cell adhesion prior to extravasation. Alternatively, these CAMs could support leukocytic infiltration into the tumor and thus represent part of the immune response of the host to the tumor.

The topic of angiogenesis has entered an exponential phase in current biomedical research and is relevant not only for tumor growth but also for chronic inflammation and wound healing, including the reaction to implanted biomaterials. Various angiogenic (e.g., vascular endothelial growth factor, bFGF, IL-8, E-selectin, hyaluron oligosaccharides) and angiostatic molecules (e.g., thrombospordin, platelet factor-4, interferon-γ, angiostatin, endostatin) have been described and offer the possibility of regulating the angiogenic response. The application of angiostatic factors such as angiostatin and endostatin as an antitumor strategy has been highly successful in experimental models, but still requires as yet unforeseen modifications in the human situation. Similar strategies employing drug-delivery systems could revolutionize the field of biomaterial implantation to control the wound healing reaction. However, considerable fundamental research is still essential before this can become a reality. This applies particularly to the development of suitable models employing human primary cultivated cells to simulate the situation in vivo. In addition, of special relevance to the pathology community is the need to systematically examine explanted biomaterials with respect to the angiogenic response, which appears to differ markedly from other species, including other primates.

Finally, one of the major challenges in endothelial research remains the understanding of the molecular basis and pathobiological significance of endothelial cell heterogeneity. While the structural variability of the endothelium has been known from anatomical observations for a long time, it is only relatively recently that the extent of functional heterogeneity of the endothelium has become a focus of attention. The latter must be regarded as one of the central elements in comprehending the organ manifestations of disease, for example, the sequence and pattern of hematogenous spread of malignancy, or organ failure in sepsis. To achieve this there must be a concerted effort of modern methodology applied to human tissue specimens, combined with relevant experimental models in vivo and in vitro.

The presentation will attempt to give a concise overview of these aspects of the pathobiology of the endothelium and will also draw on experimental data from the presenter’s own laboratory.

References

Arrhythmogenic right ventricular cardiomyopathy: Pathology and genetics

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a cardiac disease of unknown etiology. It is listed among cardiomyopathies in the recent WHO classification and is characterized by transmural fatty or fibro-fatty infiltration of the right ventricle, resulting in ventricular tachyarrhythmias and high risk of unexpected cardiac arrest and sudden death, particularly in the young and in athletes.

The heart weight is usually normal and does not exceed 400 g. Right ventricular pathology is often overlooked by pathologists, despite the fact that the right side of the heart appears yellowish or whitish, suggesting a fatty or fibro-fatty infiltration of the underlying myocardium, a suspicion which is easily confirmed by cutting the right ventricular inflow-outflow, which appears lardaceous. By checking the wall transparency with a light source, the right ventricular free wall appears parchment-like. The dystrophic process of the right ventricular free wall is regional in 20% and diffuse in 80% of cases. Aneurysms of the right ventricular free wall, whether single or multiple, are reported in about 50% of cases and are considered a pathognomonic feature of ARVC. Right ventricular enlargement, whether mild, moderate or severe, is a constant feature.

The left ventricle and the ventricular septum are highly normal in the majority of cases, which explains the paradox of why these hearts are able to withstand the cardiac output of a strenuous exercise performance and at the same time are electrically vulnerable because of fibro-fatty infiltration of the right ventricle. However, following in vitro magnetic resonance and histological examination, the left ventricle appeared to be involved in nearly 50%. In hearts with end-stage disease and congestive heart failure, examined either at autopsy or at cardiac transplantation, biventricular involvement is a regular finding.

The histology of the free wall of the right ventricle clearly shows disappearance of the myocardium with transmural fibro-fatty replacement. The pathological process seems to start from the subepicardium and extend to the endocardium in a wave-front phenomenon. The dispersion of residual electrically conducting myocytes within the fibro-fatty tissue accounts for the delay of intraventricular impulse transmission and persistence of electrical depolarization during diastole (late potentials), as well as for onset of reentrant circuits with premature ventricular beats and ventricular tachycardia of left bundle-branch block morphology.

Evidence of patchy myocarditis with myocyte death and round cell inflammatory infiltrates was observed in two-thirds of specimens. Inflammatory cell infiltrates consist of CD45+ and CD43+ leukocytes and a f CD68+ macrophages. All stages of myocardial injury and repair are recognizable: acute cell death with sarcosyll and inflammatory infiltrates, subacute damage with matrix rich in fibroblasts and proteoglycans (“active fibrosis”), including dying myocytes with empty sarcomelma, lymphocytes and macrophages, or otherwise adipocytes replacing vanished myocytes, and, eventually, chronic stage with hyaline fibrous tissue and adipocytes surrounding residual surviving myocytes, of variable size and degeneration.

Endomyocardial biopsy may be of help to improve the in vivo diagnostic accuracy of ARVC because of the peculiar topographic and histological features of the disease. In young subjects, the diagnosis is based on the finding of a certain amount of fibrous and/or fatty tissue: fibrous tissue >40%, fatty tissue >3% and residual myocytes <45%.

There is now clear-cut evidence that ARVC is an acquired, non-ischemic atrophy of the right ventricular myocardium and that electrical instability appears late in childhood. Patchy cell death with inflammatory infiltrate is visible by microscope, suggesting progressive myocyte loss followed by fibro-fatty replacement. An inflammatory theory has been put forward, and infection, toxic or immune mechanisms have been postulated. An inflammatory hypothesis does not contrast with a familial occurrence, since a genetic predisposition to viral infection eliciting an immune response has been proven in animals, with a selective involvement of the right ventricle and even development of ventricular aneurysms.

According to the dystrophic theory, the progressive loss of myocardium is secondary to spontaneous myocyte death as a result of a genetic defect. Familial occurrence suggests a genetically determined myocardial atrophy with autosomal dominant transmission and variable expression and penetrance. In this regard, the term myocardial dystrophy appears more appropriate, as in Duchenne’s or Becker’s skeletal muscle dystrophies, which may also be characterized by muscular atrophy with fatty infiltration (“pseudohypertrophy”). So far, six loci have been identified, two mapping to chromosome 14, one to chromosome 1, one to chromosome 2, one to chromosome 3 and one in chromosome 17, suggesting genetic and clinical heterogeneity. The specific gene defects as well as the defective coded proteins have not yet been identified.

It has been recently suggested that the progressive myocyte death in ARVC might represent programmed cell death. Abnormal, recurrent bouts of apoptosis may lead to progressive myocardial disappearance in ARVC followed by fibro-fatty replacement of the right ventricular free wall. However, the finding of apoptosis should be viewed with caution due to autolytic phenomena or delay in fixation. We recently studied the in vivo occurrence of apoptosis in endomyocardial biopsies of ARVC patients by using both the transmission electron microscope and TUNEL method. Apoptotic myocytes were found in 35% of cases, with a mean apoptotic index of 24.4 (9.8). Apoptosis appeared to be significantly related to clinical history duration (less than 6 months) and presence of “acute” symptoms. Recent studies have demonstrated that apoptosis cannot only be triggered not the “internal clock”, it has been recently demonstrated that apoptosis may be induced by cytotoxic T lymphocytes. The “acute” symptoms and signs associated with apoptosis in ARVC patients might reflect viral infection, autoimmunity or other inflammatory noxae that trigger apoptosis.

Whatever the etiopathogenetic mechanism, recurrent bouts of apoptosis may destroy the myocardium, which is then replaced by fibro-fatty tissue, and may enhance the electrical ventricular vulnerability. Cell death and inflammation act as acute arrhythmic trigger in the setting of the chronic substrate of fibro-fatty replacement. This evidence may open new avenues not only in the understanding of the disease, but also to conceive new diagnostic and therapeutic strategies. As agents inducing apoptosis are used in tumor therapy, agents inhibiting apoptosis might also stop or slow down myocyte loss in ARVC, thus preventing the ominous electrical instability of the ventricular myocardium and eventually heart failure.
Pathology of large vessel vasculitides

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Vasculitides can be classified according to the size of the vessels involved. Large vessel vasculitides are found in Buerger’s disease, temporal arteritis, Takayasu’s disease, Behçet’s disease, infectious arteritides, rheumatologic and miscellaneous diseases. The arteri- al involvement presents pathological characteristics which con- tribute to diagnosis of the disease.

Buerger’s disease is a thrombotic arterial and venous disease which is associated with smoking in young nonatherosclerotic patients and is prominent in lower and upper limb arteries of 1-5 mm diameter. Most arterial lesions are sampled during surgical bypass surgery at the stage of nonspecific organized thrombosis. However, a characteristic pattern of Buerger’s disease is no or min- imal arterial wall damage. Venous involvement is mainly superficial thrombophlebitides.

Temporal arteritis belongs to the giant cell arteritides. It promi- nently involves the branches of the external carotid artery, mainly the temporal artery. Other sites can be involved: the aorta in about 10% of cases, and large arteries of limbs, with a risk of thrombosis, aneurysms and rupture. Temporal artery biopsy is diagnostic in less than 50% of cases. It is a focal inflammatory process with or without giant cells prominent in the internal part of the media. Steroid treat- ment does not change inflammation in biopsies for up to 4 weeks.

Behçet’s disease is based on a systemic vasculitis phenomenon. In addition to multiorgan involvement, vein involvement is frequent, and arterial lesions are observed in 2-30% according to the series. Both systemic and pulmonary arteries are involved. It is a very ag- gressive involvement destroying the vascular wall with a risk of arte- rial rupture, pseudoaneurysm and thrombosis. The inflammatory process is often massive with an infectious-like pattern.

Infectious arteritides: the most frequent concern in infectious vascular pathology is now infection after vascular surgery, mainly infection in prostheses. In native arteries, primary infectious arteritis is the bacterial involvement of a preexisting vascular lesion such as aneurysm or atherosclerosis, Gram-negative germs being frequently responsible; secondary infectious arteritis or mycotic aneurysm is now rare and depends on the septic embolism from endocarditis, Gram-positive germs being frequently responsible. It is a thrombotic and destructive phenomenon. Syphilitic and tuberculous arteritides are now very infrequent: they may be a diagnostic problem with rheumatologic disease-associated aortitis.

Rheumatologic and miscellaneous diseases: arteritis, mainly aortitis can be observed in rheumatologic or in systemic diseases such as rheumatoid arthritis, ankylosing spondylitis, Reiter’s syn- drome, relapsing polychondritis, and Cogan’s syndrome. Kawasaki syndrome is mainly responsible for coronary artery involvement.

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Takayasu’s disease, also called aortic arch syndrome or non- specific aortoaortitis, belongs to the group of giant cell arteritides. It involves mainly the aorta, but also frequently the pulmonary, the subclavian, the carotid and the renal arteries. It is characterized by a marked thickening of the arterial wall. Aneurysms and stenoses are commonly observed, In the inflammatory stage, the inflamma- tion is made of mononuclear and giant cells, and is prominent in the external part of the media where it destroys the elastic fibers. In the fibrous stage, thickening and stenosis are generated by fibrosis of the intima and mainly of the adventitia with a fibrous ring where inflammation may have disappeared.

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References

Myocarditis. How do we make a biopsy diagnosis?

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In a Swedish autopsy study the incidence of myocarditis was observed to be 1.06%, while in autopsies of children and young adults the incidence has been reported to be 17-21%. In fact, nobody knows the (true) incidence of myocarditis.

Presenting symptoms and physical examination are often non-specific. Nearly 90% of patients give a history of a flu-like syndrome, but in less than 50% is a Viral disease recognized within the preceding month. The initial presentation may be one of acute or chronic heart (allure or cardiogenic shock or symptoms mimicking acute myocardial infarction. Patients may have life-threatening arrhythmias or remain asymptomatic.

Myocarditis is best defined morphologically as an inflammatory involvement of the heart muscle characterized by a leukocytic infiltrate and resultant nonischemic necrosis/degeneration at myocytes.

With the recent onset of unexplained cardiac heart failure, chest pain, or life-threatening arrhythmias, of all patients who have endomyocardial biopsy, 5-10% have morphological myocarditis. In the 1995 Task Force of WHO/ISEC on the definition and classification of cardiomyopathies it is said that “inflammatory cardiomyopathy is defined by myocarditis in association with cardiac dysfunction. Myocarditis is an inflammatory disease of the myocardium and is diagnosed by established histological, immunological, and immunohistochemical criteria. Idiopathic, autoimmune and infectious forms of inflammatory cardiomyopathy are recognized”. In my view, this means that myocarditis and inflammatory cardiomyopathy are synonyms.

It is evident that the gap between clinical symptoms/clinal suspicion and morphological changes of myocarditis is wide ... and in fact so wide, that we should look for the missing link or reconsider our entire concept. The causes of myocarditis are summarized in Table 1.

Table 1. Summary of causes of myocarditis.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious</td>
<td>All types of microorganisms</td>
</tr>
<tr>
<td>Immune-mediated</td>
<td>Postinfectious</td>
</tr>
<tr>
<td>Toxic</td>
<td>Systemic disorders</td>
</tr>
<tr>
<td>Other/unknown</td>
<td>Drug hypersensitivity</td>
</tr>
<tr>
<td>Transplant rejection</td>
<td>Transplant rejecion</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Drug induced/toxins</td>
</tr>
<tr>
<td>Giant cell myocarditis</td>
<td>Sarcoidosis</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>Giant cell myocarditis</td>
</tr>
</tbody>
</table>

Unknown causes include special types such as sarcoid heart disease or giant cell myocarditis. However, more often one is not able to explain the process and the case is then dropped in the basket of unknown idiopathic myocarditis. This is either rational or sufficient investigations have not been carried out (or the methodology is inadequate).

What I think fit for attacking the problem when dealing with endomyocardial biopsies is the following (and of course it is my personal choice):

i) Most of the time the material is formalin fixed. The biopsies must be serially cut.

ii) Routine staining procedures include hematoxylin and eosin, elastic van Gieson’s, and trichrome reactions.

iii) Having viewed these sections, special reactions for iron, amyloid, microorganisms etc. may be undertaken.

iv) Immunohistochemical reactions presently performed on fixed tissue nearly as well as on frozen material should include char-
acterization of the mononuclear cells and some basic reactions concerning autoimmunity. There are kits for immunohistolological detection of microorganisms (cytomegalovirus, etc.) but in situ hybridization and PCR techniques on sections are also available methods in many routine labs.

v) A proper quantification of the biopsy material is important. Qualitative and semiquantitative measures are not always adequate, and correctly used morphometry is essential in this field.

vi) Most important is a meticulous correlation between clinical aspects and morphological results. This dragging exercise must never be forgotten if we want to be wiser.

Unfortunately, I know for certain that these measures are not going to solve the problem of making us understand everything about myocarditis or diagnosing it correctly—but I am convinced that we can improve the situation by using these ordinary tools and consequently reduce the gap of diagnostic frustration.