New combined treatments avoided transplantation in a child with severe pulmonary hypertension

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Increased serum concentrations of soluble CD95/Fas and caspase 1/ICE in patients with acute angina


Coronary artery disease is associated with systemic immune responses and signs of chronic inflammation. Apart from the well-known traditional risk factors, seroepidemiological studies have suggested a link between atherosclerosis and microbial infections. The atherosclerotic lesions contain large numbers of immune cells, particularly monocytes, macrophages, and T cells. Most T cells are CD3+, CD4+, and T cell receptor zβ+. They are largely T helper cell 1 subtype, which secrete interferon γ, interleukin 2, and tumour necrosis factor (TNF) α. These proinflammatory cytokines, as well as interleukin 1, interleukin 6, interleukin 8, and C reactive protein, cause activation of T cells, macrophages, and vascular endothelial cells. They also augment expression of adhesion molecules, chemoattractants, and other cell surface markers.

The maintenance of T cell homeostasis and peripheral tolerance to self-antigens following repeated cell stimulations is regulated mainly by apoptosis. Apoptosis, or programmed cell death, is initiated by death-inducing receptors. These are transmembrane receptors that include the TNF receptors and a variety of immune cell receptors (CD95, CD40, CD30, and CD27) and their corresponding ligands. It has been previously shown that interaction between CD95 (Fas; apolipoprotein 1) and CD95 ligand (CD95L; Fas ligand, CD178) induces apoptosis in human plaque derived vascular smooth muscle cells. Activated cytotoxic CD4+CD161+ T cells can lyse capillary endothelial cells in the presence of C reactive protein at concentrations frequently found in patients at risk for coronary heart disease.

In addition, patients with unstable angina have increased concentrations of shed membrane microparticles, originating from activated CD3+ cells, endothelial cells, and macrophages, hence indicating increased intravascular peripheral apoptotic turnover.

It is not well known whether patients with acute coronary syndrome have increased concentrations of soluble CD95 (sCD95) and sCD95L, soluble TNF receptor type 1 (sTNFR1), and interleukin 1β converting enzyme (ICE; caspase 1). Therefore, we investigated comparatively the concentration of these molecules, which are involved in the apoptotic pathways, in the serum from patients with stable and unstable angina.

**METHODS**

**Study group**

Eighty consecutive patients admitted to our institution for the assessment of angina chest pain were studied and had undergone coronary angiography. Table 1 summarises demographic and some baseline clinical characteristics. Stable angina (n = 40) was defined by typical exertional chest pain angina relieved by rest, glyceryl trinitrate administration, or both with positive responses to exercise ECG stress testing and ≥ 50% diameter stenosis in ≥ 1 coronary arteries at catheterisation. Unstable angina (n = 40) was defined according to Braunwald. All patients with unstable angina class IIIB had diagnostic ST segment changes, T wave inversion, or both, as well as negative cardiac enzymes. No patient included in the study had evidence of ongoing systemic or cardiac inflammatory processes as defined by clinical history. Blood samples were taken at the time of admission into hospital. The investigation conformed to the principles outlined in the Declaration of Helsinki.

**Angiographic analysis**

Coronary angiography was carried out according to the Judkins technique. Images of the coronary tree were obtained in routine, standardised projections with standard equipment (Siemens BICORTOP, Erlangen, Germany). To assess the extent of atherosclerotic disease the coronary artery tree was...
assessed according to vessel score, stenosis score, and extension score.13

**Laboratory analysis**

Serum samples were obtained after clinical assessment grading the patient affected with stable or unstable angina and before cardiac catheterisation. Serum was then aliquoted and kept frozen. Circulating sCD95 and sCD95L were measured by a commercial enzyme linked immunosorbent assay (ELISA) with polyclonal antibodies against human TNF receptor type 1 (TNFR1) and TNF with polyclonal antibodies against human CD95 (Bender MedSystems, Vienna, Austria). The sensitivity of the ELISA was 20 pg/ml for sCD95 and sCD95L, 3 pg/ml for (R&D Systems Inc, McKinley, Minnesota, USA). ICE was measured in the serum by ELISA with monoclonal antibody to ICE 1 antibody (Bender MedSystems, Vienna, Austria). The sensitivity of the ELISA was 20 pg/ml for sCD95 and sCD95L, 3 pg/ml for sTNFR1, 80 pg/ml for TNFα, and 5 pg/ml for human ICE. The analytical unit of the biochemistry department following standard methods carried out all other biochemistry measurements.
suggests that increased apoptosis specific markers are associated with clinical instability. We have also found that the serum concentrations of TNF-α, sTNFRI, and sCD95L were not significantly different in the study cohorts. These results indicate that patients with unstable angina may be in a paradoxical state of increased apoptotic turnover, as indicated by increased secretion of sCD95 and ICE/caspase 1.

Apoptosis is a highly organised, controlled, and energy dependent mechanism whereby a cell commits suicide. Distinct morphological features such as cell shrinkage, chromatin condensation, DNA fragmentation, membrane blebbing, and formation of apoptotic bodies are characteristic of this cell death mechanism. A family of cysteine proteases, termed caspases, homologue to the Caenorhabditis elegans cell death gene CED-3[14], play a pivotal part in apoptosis, particularly caspase 8, which initiate the caspase activation cascade through caspase 3 and caspase 9.[7–15] Caspases are present in the cell in an inactive form and are activated by autoproteolytic processing, oligomerisation, and heterocleavage by other caspases or granzyme B. Activation of caspase requires transduction of a signal from cell membrane receptors, such as CD95 and TNFFR1. Binding of ligand to death inducing receptors leads to trimerisation and cross linking of the aggregated cytoplasmic domain of the receptor complex and recruitment of the trimerisation and cross linking of the aggregated cytoplasmic domain of the receptor complex and recruitment of the

The role of ICE in apoptosis and its potential to interfere in the activity of atherosclerotic lesions and plaque stability is not well understood. It has been shown that ICE cleaves the 31 kDa interleukin 1β precursor protein at Asp116-Ala117, whereby it creates the mature biologically active interleukin 1β, a key mediator of inflammation.[16] Interestingly, ICE can also cleave an inactive precursor of interferon γ inducing factor or interleukin 18[18]; thus, ICE regulates production of interferon γ and subsequently T cell activation. Although vascular smooth muscle cells express ICE, an ICE inhibitor suppresses the release of mature interleukin 1β.[19] This may be a novel regulatory mechanism in vascular biology and pathology.

The cell death machinery is tightly controlled by Bcl-2, a member of a large gene family encoding proteins that can either promote (for example, Bax, Bak, Bid, and Bad) or inhibit (for example, Bcl-2 and Bcl-H1) apoptosis.[17] The nuclear factor κB pathway is a further key component of the cellular response to a variety of stimuli. The multiplicity of mechanisms whereby the transcription factor nuclear factor κB serves the pro-apoptotic or anti-apoptotic function is complex and may be determined by the nature of the death stimulus rather than by the tissue origin.[20] Other important regulators of apoptosis act at the level of caspase. Such proteins include cellular FADD-like inhibitory protein and the inhibitor of the apoptosis family, which counteracts the effect of the caspase inhibitors themselves.[21] The roles of sCD95 and sCD95L as pro-apoptotic and anti-apoptotic molecules are not clearly defined. The CD95 antigen is expressed on T and B cells, granulocytes, monocytes, and natural killer cells, particularly on activated cells. CD95 is cleaved by proteolytic enzymes produced by inflammatory cells and sCD95 is generated by alternative mRNA splicing. It has been previously shown that supernatants containing sCD95 can inhibit anti-CD95 antibody induced apoptosis, hence indicating that sCD95 may block signal transduction and apoptosis.[20] Overexpression of sCD95 protects the integrity of the vessel wall from immune injury and attenuates transplant arteriosclerosis.[23] Furthermore, an increased concentration of sCD95 has been regarded as a predictor of atherothrombosis in patients with end stage renal disease.[24] CD95L is expressed on activated T cells, natural killer cells, and vascular endothelial cells.[25] CD95L is cleaved from the cell membrane by a metalloproteinase-like enzyme. sCD95L may be detected in the serum[26] and considered to be a marker of atherosclerosis.[27] The coexistence of increased apoptotic turnover and secretion of the apoptosis prohibiting sCD95 molecule may be a desperate attempt by the immune system in unstable angina to overcome lifelong and constant triggering of autoreactive T cells by way of the T cell receptor. We are tempted to believe that sCD95, sCD95L, sTNFRI, and ICE are serological markers that may help to discriminate between stable and unstable angina. We conclude that our results showing increased concentrations of sCD95 and ICE in unstable angina may merely reflect inflammatory cell activation and the role in relation to apoptosis specific immune activation remains to be determined. However, to elucidate the role of these molecules in the pathogenesis, disease progression and stabilisation, diagnosis, and prognosis of acute coronary syndromes further clinical studies as well as new experimental designs are needed.

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REFERENCES

Images in Cardiology

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Therapeutic options for the management of pulmonary hypertension have been limited for many years. However, recent progress in the understanding of its pathology have led to the introduction of new treatments that have changed its prognosis, such as the continuous infusion of prostacyclin. Several other treatments (oral, inhaled, or subcutaneous prostacyclin, phosphodiesterase inhibitors, and endothelin receptor blockers) have shown beneficial effects in recent trials. An 8-year-old boy with severe primary pulmonary hypertension showed a dramatic improvement following combined treatment with inhaled iloprost (a prostacyclin analogue) and oral bosentan (a dual endothelin receptor blocker). In the six minute walk test he increased the distance from 295 m before bosentan to 400 m after three months of combined treatment. Dramatic changes were observed at echocardiography. Echocardiographic short axis views were taken at the same time in the cardiac cycle before combined treatment (panel A) and three months after combined treatment of iloprost and bosentan (panel B). There was a significant reduction in right ventricular (RV) size and changes in interventricular septal curvature (IVS), which went from flat before treatment (panel A) to normal left to right on combined treatment (panel B). Echocardiography showed a decrease in right ventricular surface, from 39 cm² to 34 cm². The left ventricle (LV) is squeezed by the dilated right ventricle before (panel A) and is almost normal after three months (panel B). This allowed the lung transplantation to be postponed and improved the patient’s quality of life.

This case shows that combined therapies may offer further improvement in the therapeutic options for the treatment of pulmonary hypertension. Appropriate clinical trials should be performed to confirm this beneficial result.

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