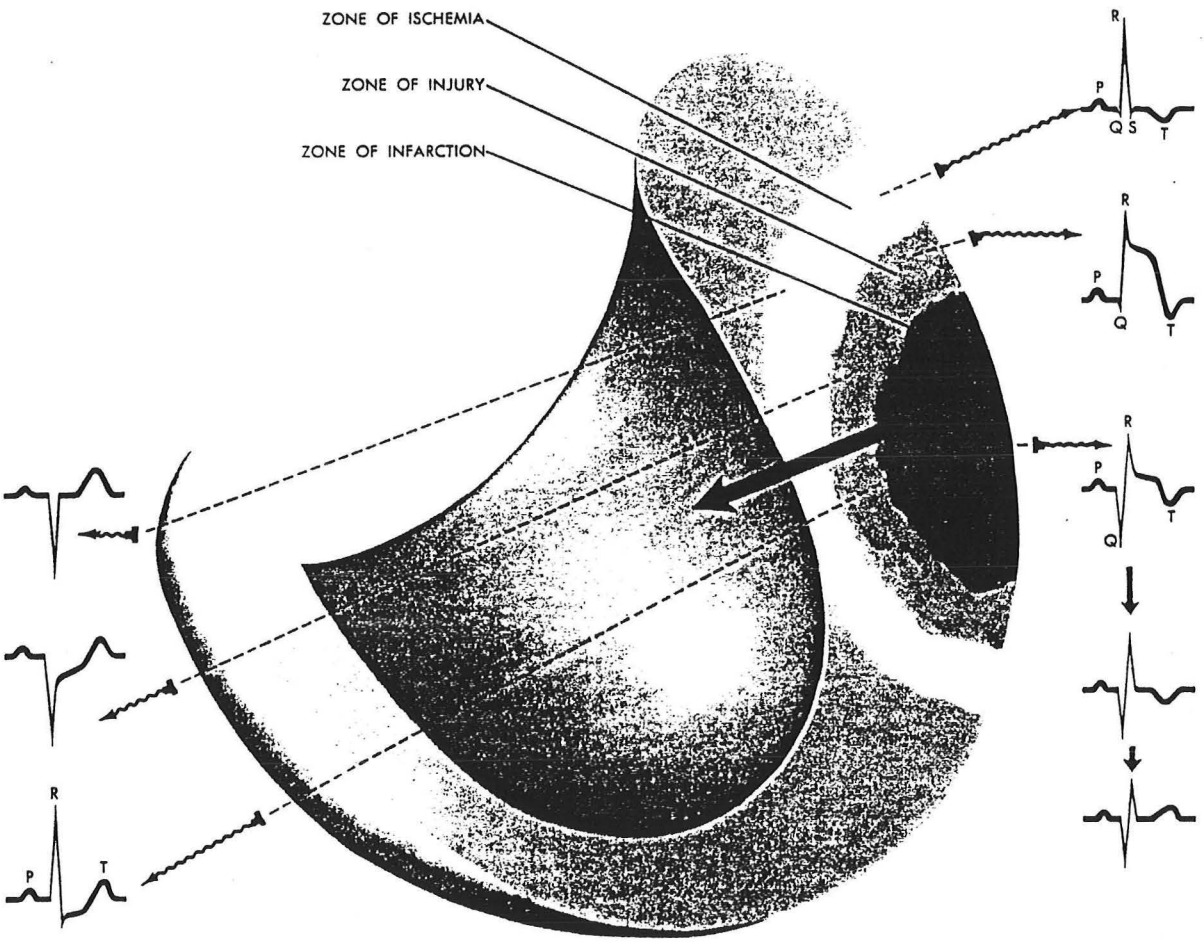
FRONTIER STRATEGIES FOR PROTECTION AFTER ACUTE MYOCARDIAL INFARCTION



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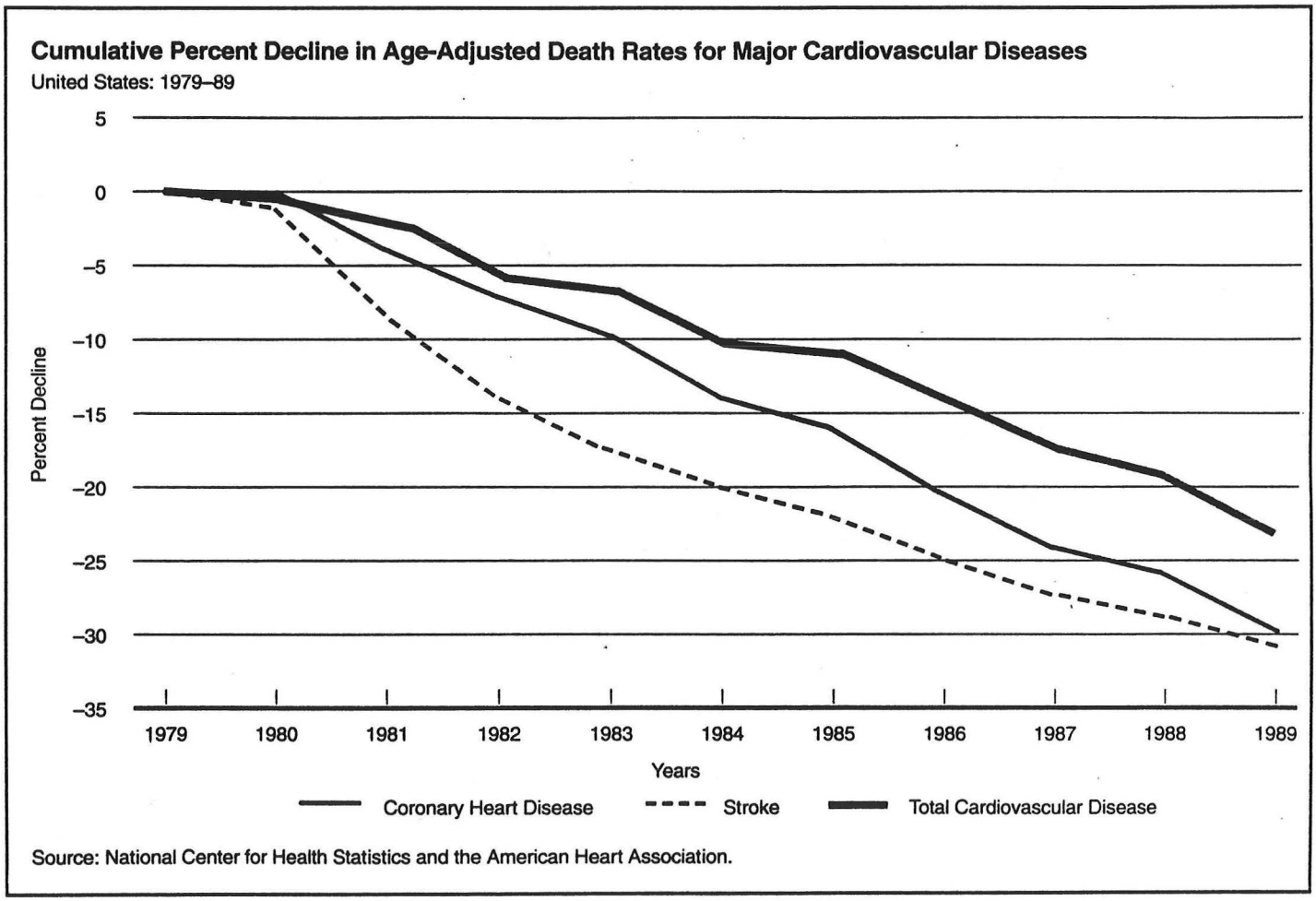
INTRODUCTION

Major progress has been made in the past decade to reduce the mortality of coronary heart disease ever since the introduction of thrombolytic therapy along with alternative methods of coronary revascularization, beta-blocker therapy, aspirin and other preventive measures now in standard practice, Figure 1 (NCHS & AHA, 1992). Despite these advances, cardiovascular disease remains the leading cause of morbidity and mortality afflicting 1.25 million heart attack victims yearly, 25% of whom die before reaching the hospital. In survivors, the residual myocardial function is dependent upon the extent of myocardial necrosis sustained after an infarction and is the major determinant of a patient's prognosis. Therefore, efforts to understand the mechanisms by which cells are damaged during ischemia and to identify compensatory or adaptive responses that may augment cell survival have major clinical importance.

Figure 1



Mechanism of Ischemic Cell Death

Prolonged occlusion of a major coronary artery, as commonly occurs with acute thrombosis of an ulcerated atherosclerotic plaque, will cause myocardial infarction if blood flow is not restored in a timely manner. Several factors appear to influence the size of the accompanying infarct including the duration of ischemia, the size of the myocardium at risk, and how promptly blood supply is restored to the ischemic region (Jennings and Reimer, 1988; Yusuf et al, 1990).

Figure 2

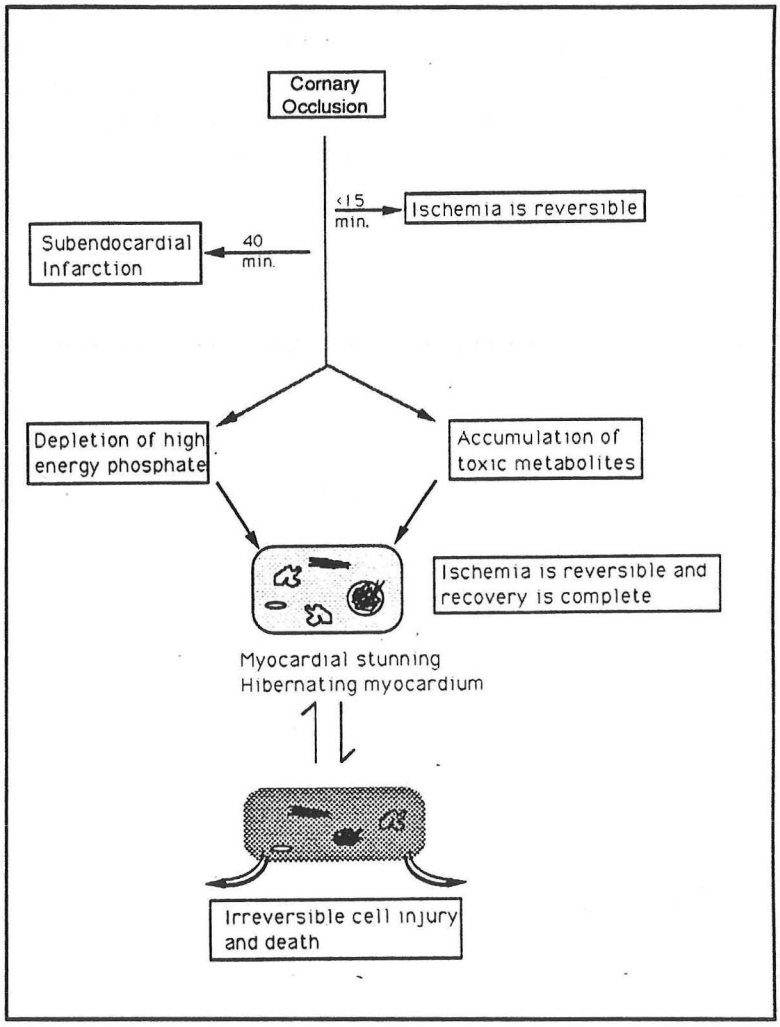


Figure 2 is a schematic in general terms of the distinctive features of this process leading to irreversible cell death, the precise mechanism of which remains incompletely understood. Studies, primarily in experimental animals, have established that the ischemic injury from occlusion of a major coronary artery for fifteen minutes is reversible and recovery is complete (Reimer et al, 1981). However, prolonged occlusion beyond forty minutes causes subendocardial infarction, which is dependent upon the amount of collateral blood flow to the ischemic bed, but averages about twenty-five percent of the ischemic area at risk.

The major factors implicated in ischemic cell death include progressive depletion of high energy phosphates which are required for vital metabolic processes and/or the accumulation of toxic metabolites, secondary to ischemic metabolism. The terminal events likely to precipitate irreversible cell death presumably occur when the cellular electrochemical gradient is disrupted resulting in loss of the integrity of the cell membrane and disruption of critical functions of organelles such as the mitochondria.

Cell death during the severe ischemic period is not instantaneous as the proven benefit of thrombolytic therapy is still achievable up to six hours after onset of an acute myocardial infarction. Extensive studies, primarily from experimental animals, have revealed several phenomena associated with reperfusion of the myocardium after a prolonged ischemic episode and will be briefly reviewed here.

Ischemic preconditioning is a phenomenon in which prior sublethal episodes (five minutes) of ischemia are apparently protective to a later lethal challenge of ischemia (Reimer and Jennings, 1992). This enhanced viability to a potentially lethal episode of ischemia is associated with reduced rates of adenosine triphosphate (ATP) utilization and anaerobic glycolysis, as reflected by the slower accumulation of lactate (Murray, et al, 1990). The precise mechanism of ischemic preconditioning is not known but has recently been proposed to be mediated through activation of A_1 adenosine receptor via adenosine produced during the ischemic episode (Thornton, et al, 1990).

Myocardial stunning refers to the post-ischemic ventricular dysfunction that is seen after reperfusion in the absence of demonstrable evidence of irreversible damage (Kloner and Braunwald, 1983; Bolli, 1990). Conceptually, it may be viewed as the mechanical dysfunction seen after a sublethal ischemic episode leading to the preconditioning effect. In practice, however, efforts to make this distinction are arbitrary, since the clinical presentation of acute myocardial infarction in fact is not well-demarcated in regions of ischemic, injured, and necrotic myocardium (cover).

My major goal in today's grand rounds is to discuss the pathophysiology of myocardial ischemic injury by focusing on recent developments that provide insights into possible strategies for myocardial salvage. I have outlined my grand rounds to cover the following major areas. I will first discuss the acute inflammatory response and explore how our understanding of leukocyte-adhesion interactions might lead to possible therapeutic intervention. I will then examine the role of endogenous defenses such as the role of stress proteins as cytoprotective agents to limiting myocardial damage. Then, I will discuss the role of the coronary collateral circulation by focusing on the role of peptide growth factors in mediating angiogenesis or new blood vessel formation as a strategy for myocardial salvage. Last, but not least, I'll review how the clinical recognition of infarct expansion and ventricular remodeling led to the recent impact of ACE inhibitors in reducing the morbidity and mortality after acute myocardial infarction.

ACUTE INFLAMMATORY RESPONSE

Longstanding efforts to limit infarct-size have existed since the recognition of the lymphocytic infiltrate after an infarction over fifty years ago (Mallory, et al, 1939). In their classic study on the time course of healing after myocardial infarction Mallory GK, White PD and Salcedo-Salgar J (1939 observed that neutrophils accumulate in the region of infarct as early as 12 hours after infarction and peaks in five to seven days at the site of tissue damage. This is illustrated in figure 3 at seven days after an acute myocardial infarction. An intense inflammatory infiltrate is present in the middle with darker staining dead cells on the left (figure 3A), while on the right there is interstitial edema and a few viable myocytes.

Figure 3A

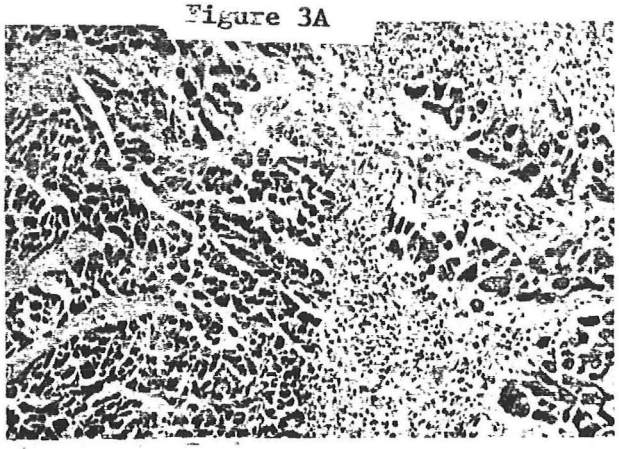
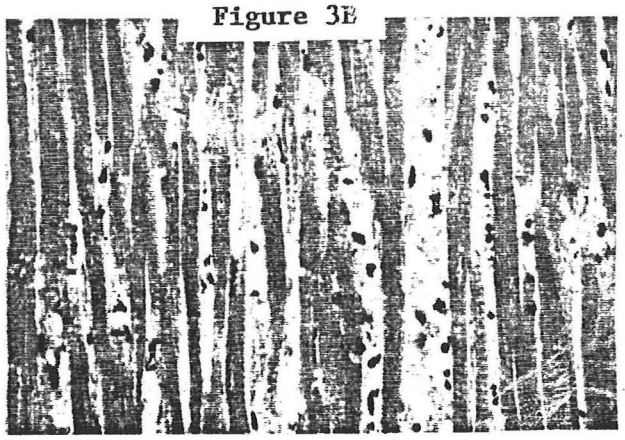


Figure 3B



Likewise Figure 3B also shows a characteristic feature of ischemic injury in which muscle cells are devoid of the central staining nuclei. The essential role of the neutrophil during the healing process was demonstrated in an early clinical trial, where high-doses of prednisone were used as a non-specific anti-inflammatory agent in patients with acute myocardial infarction. The trial was prematurely terminated because of the high incidence of aneurysm formation and ventricular rupture, thereby, underscoring the hazards of indiscriminate interventions (Roberts et al, 1976). Alternatively, some studies in animals, have convincingly shown experimental infarction is reduced in animals depleted of neutrophils (Jolly et al 1984). Furthermore, studies of experimental canine myocardial infarction have demonstrated that the largest influx of neutrophils occurs within the first 2 hours after reflow but continues for up to 48 hours (Dreyer et al 1991; Entman et al, 1991). Because thrombolytic therapy and percutaneous angioplasty are now standard interventions during acute ischemic syndromes, interest has heightened on the role of neutrophil in the pathogenesis of "reperfusion injury" (Dreyer et al, 1991) Thus, the inflammatory response of acute myocardial infarction begins within minutes of tissue injury and is mediated by a variety of factors released from endothelial cells, vascular smooth muscle cells, lymphocytes, monocytes, and platelets. Recent studies at the cellular and molecular levels have identified specific cell surface receptors on effector cells, which appear to influence their recruitment and mobilization at the sites of tissue injury.

Table 1. Families of adhesion molecules and their counter-receptors

Adhesion molecule	Counter-receptor
Ig superfamily	
CD2	LFA-3
LFA-3[a] (CD58)	CD2
ICAM-1[b] (CD54)	LFA-1, Mac-1
ICAM-2, ICAM-3	LFA-1
VCAM-1[c]	VLA-4
NCAM[d] (CD56)	NCAM, heparan sulfate
PECAM-1[e] (CD31)	Unknown
Integrin family	
LFA-1 (CD11a/CD18)	ICAM-1, ICAM-2
Mac-1[f] (CD11b/CD18)	ICAM-1, C3b, factor X, LPS
p150,95 (CD11c/CD18)	Unknown
VLA-1[g] (CD49a/CD29)	Laminin, collagen
VLA-2 (CD49b/CD29)	Laminin, collagen
VLA-3 (CD49c/CD29)	Laminin, collagen, fibronectin
VLA-4 (CD49d/CD29)	Fibronectin, VCAM-1
VLA-5 (CD49e/CD29)	Fibronectin
VLA-6 (CD49f/CD29)	Laminin
LPAM-2[h] (CD49d/CD$^-$)	Unknown
GpIIb/IIIa (CD41/CD61)	Fibrinogen, factor VIII
CD51/CD29	Fibronectin
CD51/CD61	Vitronectin, factor VIII, fibrinogen, thrombospondin
Selectin family	
E-selectin	Sialyl-Lewis^X antigen
L-selectin	Vascular addressins
P-selectin	Unknown
Cadherin family	
Uvomorulin	Homophylic
LCAM[i]	Homophylic
E-cadherin	Homophylic
N-cadherin	Homophylic
P-cadherin	Homophylic
Unclassified	
CD44	Hyaluronic acid
Sialylated CD15	E-selectin

[a]LFA: lymphocyte function associated antigen. [b]ICAM: intercellular adhesion molecule. [c]VCAM: vascular cell adhesion molecule. [d]NCAM: neural cell adhesion molecule. [e]PECAM: platelet/endothelial cell adhesion molecule. [f]Mac: macrophage-1. [g]VLA: very late antigen. [h]LPAM: lymphocyte-Peyer's patch adhesion molecule. [i]LCAM: liver cell adhesion molecule.

Mechanisms of Leukocyte-Mediated Myocardial Injury

At least three pathophysiologic mechanisms have been proposed for neutrophil-mediated ischemic injury. These include 1) the mechanism by which leukocytes are activated by specific cell surface molecules for adhesion to endothelium and emigration to the extravascular space 2) specific processes by which leukocytes in the extravascular space secrete proteolytic enzymes and the release of oxygen-free radicals, implicated in the pathogenesis of reperfusion injury and myocardium stunning, and 3) the process of capillary plugging by adherent leukocytes during the no reflow phenomenon, which is applicable in the post-angioplasty setting.

Neutrophils are confined primarily within the intravascular circulation and their recruitment at sites of inflammation into the extravascular space requires leukocyte-endothelial cell interaction as the first step in mediating this process. Leukocyte adhesion is facilitated by an array of specialized cell surface molecules, as shown in Table 1 on page 4. Unstimulated leukocytes form low avidity interactions to remain within the intravascular space predominantly, except for periods through the lymphoid areas and other peripheral tissues. Another complex network of cell surface molecules participate in their primary missions during immune surveillance and specialized effector functions during inflammation (Parti et al, 1992). My discussion will focus on the mechanisms for adhesion and migration of leukocytes during ischemic myocardial injury.

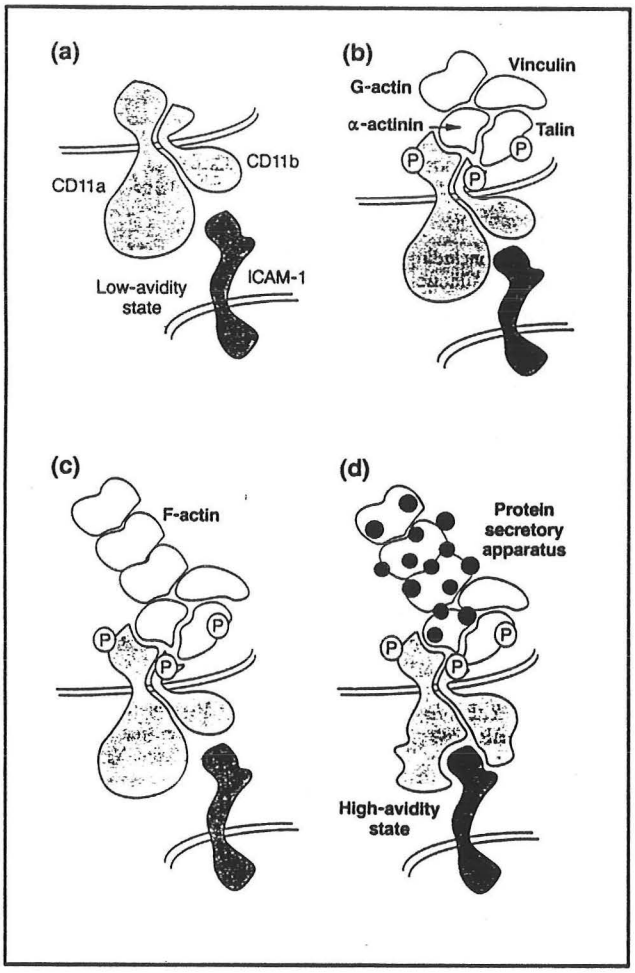
Evidence is now available to show leukocyte adhesion is a tightly regulated process and is facilitated by membrane receptors involved in cell-cell interactions and cell matrix recognition. These specialized receptors have been subgrouped into the Ig super family integrin, selectin, cadherin families and their accompanying ligand receptors are shown in Table 1. In addition, multiple factors from antigen-presentation and effector cells along with matrix proteins control the activation of leukocyte mobilization. The class of integrin molecules has received the most attention and what is known about their role in ischemic injury will be discussed here.

Lymphocyte Function - Associated Antigen I (LFA-1)

LFA-1 is a cell surface protein, which is expressed widely on hematopoietic lineage cells. It consists of heterodimer of α and β subunits of 180,000 and 95,000 kD (CD-11A and CD-18, respectively) as shown in figure 4. LFA-1's function was elucidated by the ability of anti-LFA-1 monoclonal antibodies to block adhesion-dependent leukocyte interaction. Even more dramatic are studies of patients with an inherited defect in cell surface expression of CD-11 and CD-18 to be discussed below (Anderson and Springer, 1987). The participation of LFA-1 in other immunological and inflammatory processes, as well as its structural relationship to other receptors which subserve intercellular adhesion makes it a bona fide member of the integrin family.

Lymphocytes can be stimulated to acquire adhesion properties independent of antigen presentation has led to the current view that under resting conditions, leukocytes have low avidity properties, but are triggered by the appropriate external stimuli for adhesion as shown in figure 4 (Parti et al, 1992). Recent work illucidated the process by which LFA-1 mediates intercellular interaction and has identified the intercellular adhesion molecule-1 (ICAM-1) as an intercellular ligand for LFA-1.

Figure 4



Intercellular Adhesion Molecule-1 (ICAM-1)

This cell surface glycoprotein of 90,000 kD is more widely distributed on a variety of cell types including lymphocytes, monocytes, fibroblasts, as well as cardiac myocytes (Mullane and Smith, 1990). Evidence that ICAM-1 is a bona fide ligand for LFA-1 was shown by anti-ICAM-1 antibodies which inhibit adhesion of T-lymphocyte adhesion to fibroblast and endothelial cells; (2) high levels of ICAM-1 are found at inflammatory sites and (3) ICAM-1 is induced by inflammatory cytokines (Dustin, et al, 1986; Cotran, et al, 1987). Definitive evidence for ICAM-1 and LFA-1 interaction was shown in which ICAM-1 binding into artificial planer membranes was strictly dependent on the presence of LFA-1 expressed on B-lymphoblastic cells, but not from similar cells of patients, without LFA-1 and leukocyte adhesion deficiency (Marlan and Springer, 1987). The specificity of binding was dependent on divalent cations (Mg^{2+} & Ca^{2+}) and required metabolic energy production. Interestingly, unlike other members of the integrin family, whose ligands contain the sequence RGD (Arg-Gly Asp), ICAM-1's adhesion appears to be independent of the tripeptide.

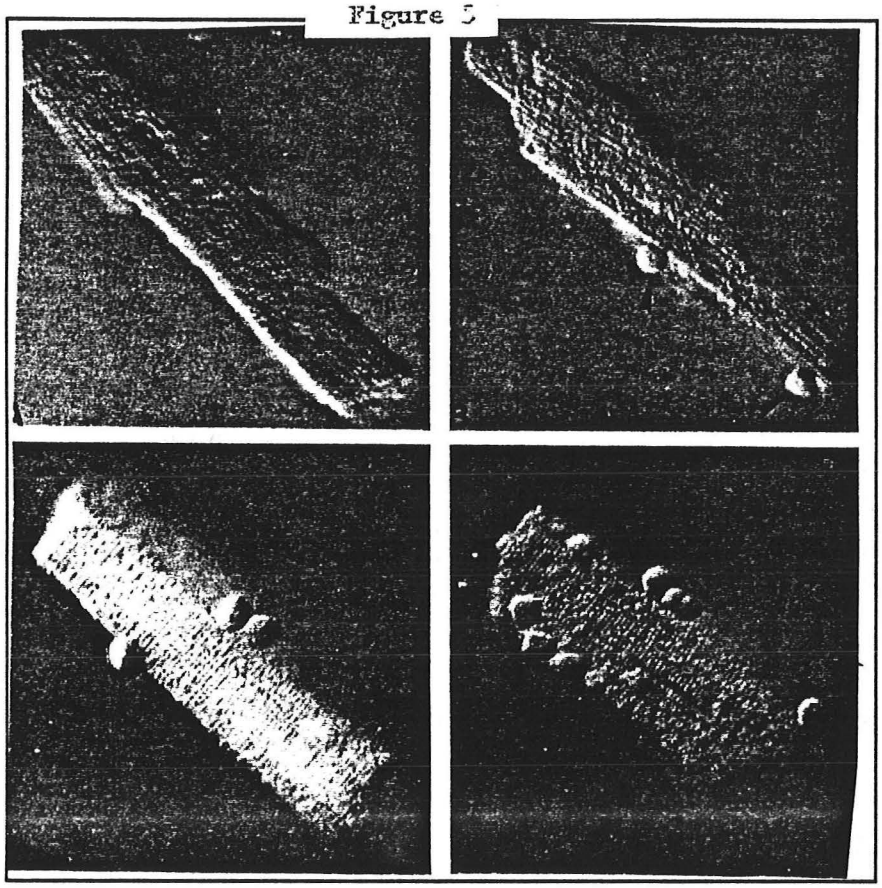
The Role of Cytokines in Lymphocyte-Mediated Ischemic Injury

Cytokines are soluble polypeptides, primarily non-immunoglobulin in nature, that are released by living cells in sub-nanomolar concentrations to mediate and regulate cellular functions (Nathan and Sporn, 1991). Among the diverse roles suggested for cytokines is a central one in remodeling of tissues which which occur during infarct expansion. Several cytokines including IL-1, IL-2, IL-6, TNF and many others have been shown to stimulate interaction between cell type during ischemic response. For example, the cytokine TNFα acts as a secretagogue from neutrophils, a condition which is dependent upon neutrophil adhesion to matrix proteins via the integrins (Nathan and Sanchez, 1990). Cytokines are also implicated during angiogenesis to stimulate proliferation and differentiation of endothelial cells.

The requirements for interaction of neutrophils and cardiac myocytes have been elegantly shown by Entman and colleagues by in vitro studies, and illustrated in figure 5A, which is an interference contrast image of isolated canine myocytes with typical appearance of rod shaped cell in the absence of neutrophils or cytokines. Figure 5B - 5D show the attachment of neutrophils after myocytes were stimulated with interleukin 1 and neutrophils with the chemoattractant. Note in 5D the contracted appearance of the myocyte with numerously attached neutrophils (Entman et al, 1990). Entman and colleagues recently reported that the observed interaction of activated myocytes and stimulated neutrophil is associated with a neutrophil respiratory burst of oxidants and neutrophil-induced myocyte injury (1992).

Further evidence has shown that this activation of cardiac myocytes requires new protein synthesis of IL-1 or IL-2 mediated ICAM-1 expression (Smith et al, 1991), when cardiac myocytes were stimulated by other cytokines, such as IL-1 (TNα) or IL-6 or the post ischemic cardiac lymph. There was similarly expression of ICAM-1, to further support the notion that ischemic tissues release chemoattractant and effector molecules that participate in the leukocyte-mediated adhesiveness.

Figure 5



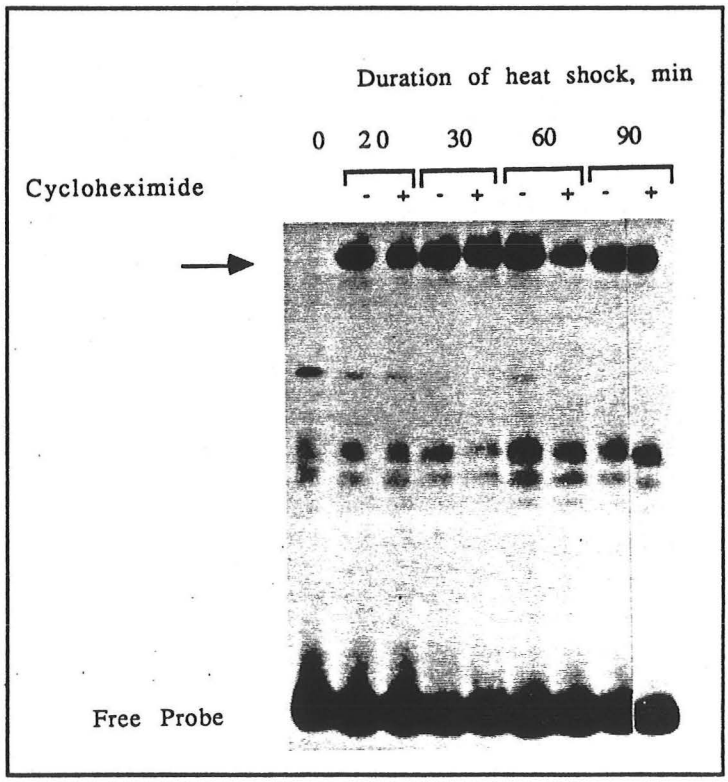
Role of endogenous host defenses

The heat shock (stress) response, defined as the rapid synthesis of a set of highly conserved proteins after environmental stresses, is implicated in cellular adaptation (Lindquist, 1986; Morimoto, 1990; Pasini et al, 1991). The observation that prior sublethal exposure facilitates tolerance to an otherwise lethal challenge (i.e. thermotolerance) has now been observed in essentially all living organisms (Lindquist, 1986). Recent studies have established an essential role for stress proteins in cellular processes such as macromolecular transport (Morimoto et al, 1990), organelle assembly and protein folding (Gething and Sambrook, 1992) however, **the physiological importance of these proteins in providing cytoprotection during myocardial ischemia remains to be established** (Williams and Benjamin, 1991; Yellon and Latchman, 1992). The central hypothesis is that member(s) of the heat shock (stress) protein multigene family attenuate damage to myocardium during ischemia and reperfusion.

Heat shock proteins are molecular chaperones. Members of the stress protein family are present in every major intracellular compartment and in the cytosol of eukaryotic cells (Craig & Lindquist, 1988; Morimoto et al, 1990). A picture is now emerging that stress proteins serve as "molecular chaperones" and are essential for normal growth during unstressed conditions. Stress proteins interact noncovalently with newly synthesized polypeptides; participate in folding, assembly, and oligomerization of substrate polypeptides; permit polypeptides to remain competent for translocation across membranes, and, finally, facilitate either the refolding of abnormal proteins or their degradation (Gething and Sambrook, 1992). Thus, it is speculated that the preferential synthesis of stress proteins is to either stabilize and/or even refold denatured proteins during or subsequent to ischemic stress as part of the cell's homeostatic and cytoprotective responses (Pelham, 1986).

Transcriptional Regulation of the HSP70 Promoter. As a well-studied inducible-promoter, the functional HSP70 promoter requires a cis-acting upstream heat shock element (HSE), located 80-150 bp from the transcriptional start site, and consists of two or three of the five-bp repeating units NGAAN or NTTCN which serve as the binding site for the heat shock transcription factor (HSF) upon its conversion from an inactive, unbound factor to its active bound form as shown in Figure 6 by heat stress (reviewed in Morimoto et al., 1990; Benjamin et al, 1990). However, not all transcription from the HSP70 promoter requires the HSF as exemplified by the adenovirus early gene product, E1A, which induces HSP70 expression through the basal promoter complex, presumably by protein-protein interactions (Simon et al, 1988; Morimoto et al, 1990).

Figure 6



Stress Proteins are induced in the myocardium by diverse stimuli, including ischemia. Induction of stress proteins has been demonstrated after several physiological stresses including pressure overload (Delcayre et al, 1988; Izumo et al, 1988) coronary occlusion (Currie et al, 1988; Mehta et al, 1988), treadmill exercise isoproterenol (White and White, 1986) and hypoxia (Howard and Geohegan, 1986). As illustrated in Figure 7 studies by Currie et al (1988) in the isolated rat heart showed that prior hyperthermia of animals, which induced increased HSP70 expression, was associated with improved functional indices and reduced infarct size after reversible ischemia. Recently, similar findings have been corroborated by studies in an infarct reperfusion model of the rat using whole animals rendered thermotolerant by prior hyperthermia as seen in Figure 8 (Donnelly et al, 1992).

Figure 7

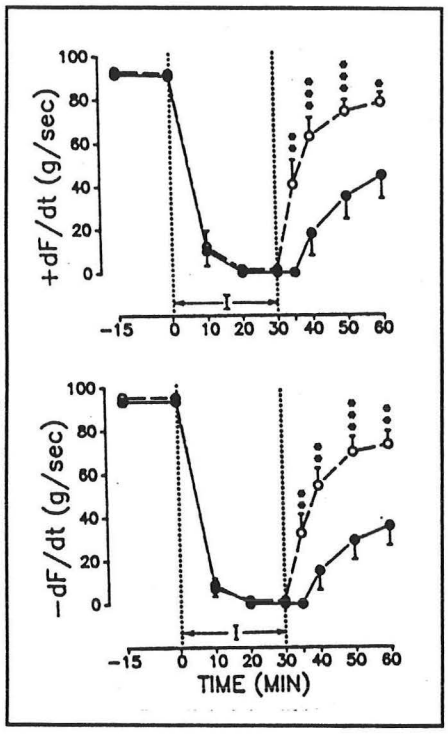
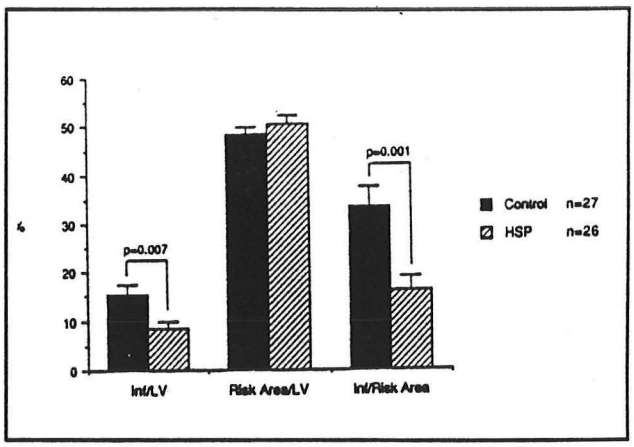
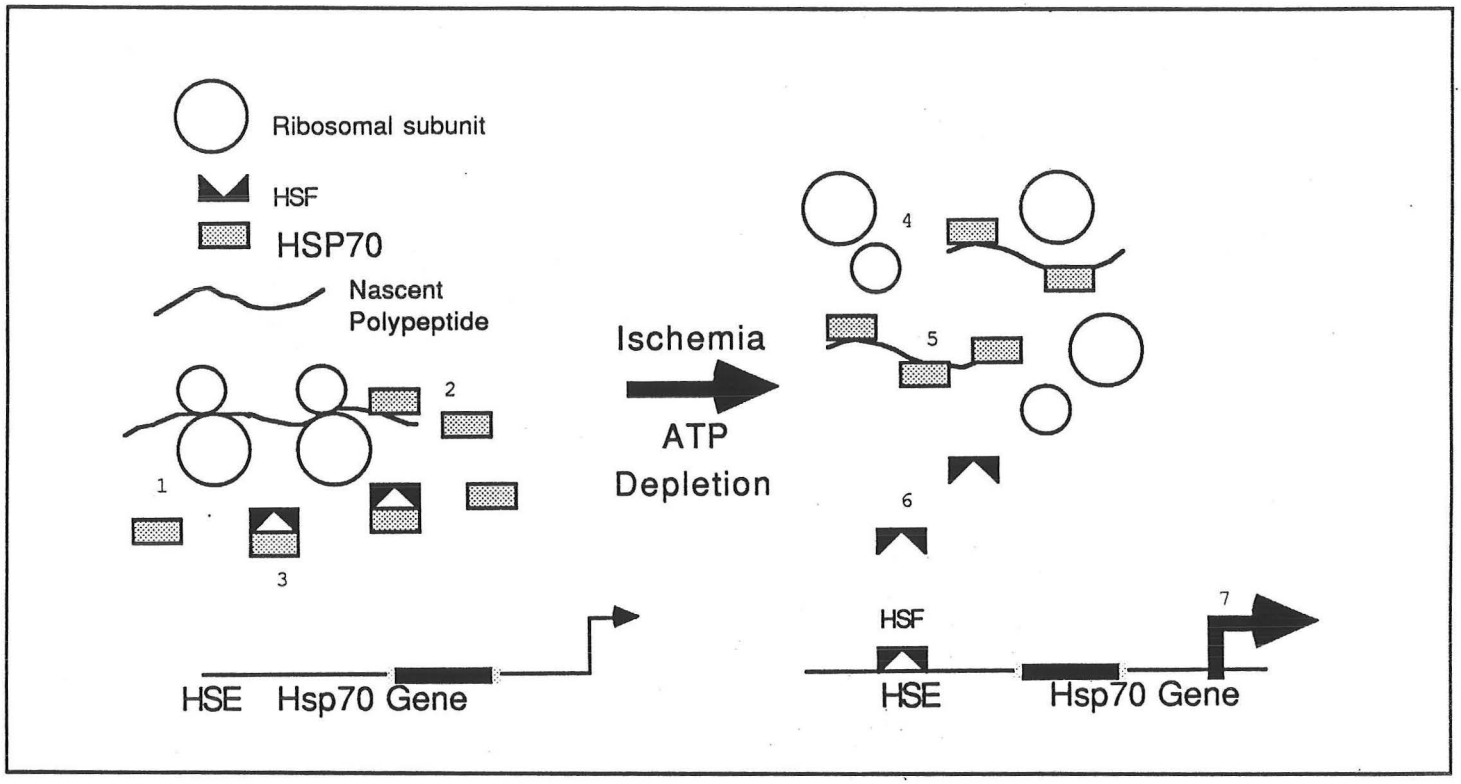


Figure 8



What are the molecular mechanisms by which HSF are activated during ischemia? This is illustrated by the following steps in Figure 9. An existing pool of heat shock proteins, step 1, serve as chaperones by binding newly sythensized nascent polypeptides, step 2. We and others propose that the DNA binding domain of HSF is masked in unstressed cells by complex formation with HSP70, step 3. As a consequence of ATP depletion or ischemia, HSP70 proteins complexed to nascent, step 4, or unfolded protein, step 5 cannot be recycled, thereby reducing the free pool of HSP70 available to complex with HSF, step 6. In addition, ATP depletion also may augment the intracellular load of denatured and/or unfolded proteins, thereby increasing the demand for HSP70 and further depleting the pool of free hsp70. Finally, if the affinity of HSP70 for HSF is lower than its affinity for unfolded proteins, the depletion of free HSP70 will release HSF for nuclear translocation, DNA binding and transcriptional activation, step 7, (Benjamin et al, 1992).

Figure 9



ANGIOGENESIS

Coronary Collateral Circulation

The first recognition of the capillary circulation was made over a century ago (Travers 1844 in Hudlicka, 1982)). Examination of the coronary circulation and attempts to define the significance of collateral anastomosis in humans has been studied for over fifty years (Blumgardt, et al, 1940; Fulton, et al, 1963). In the early sixties, Folkman and colleagues performed the first studies that demonstrated the growth of tumors in isolated perfused organs was inhibited when the diameter exceeded a few millimeters (Folkman, et al, 1963). Conversely, when the tumors were placed in donor mice, the growth now exceeded one cm^3 and became vascularized. Progress moved slowly until the mid-seventies when the purification of angiogenic factor from tumors rapidly led to the identification of several others from vascular endothelial cells of the aorta and capillaries.

The coronary collateral development is dependent upon the pressure gradient across the coronary bed and the time course during which this process occurs. It has long been appreciated that the extent of collateral formation is highly variable among species, including humans. The use of percutaneous transluminal coronary angioplasty (PTCA) in the epicardial coronary vessel has facilitated analysis of the appearance or development of coronary collaterals (Sabri et al, 1991). The following has been observed: Recanalization of a coronary vessel with antegrade flow is associated with the appearance of collaterals; when there is reclosure, for example after unsuccessful PTCA, there is recruitment of collaterals; in some instances the presence of collaterals prevents myocardial infarction during coronary artery stenosis (Sabri et al, 1991). Nonetheless, the questions persist whether the appearance of collateral vessels is the result of recruitment of existing coronary vessels or is a consequence of neovascularization.

Recent attention has focused on the role of several angiogenic factors in the formation of new blood vessels in the vascular bed in a variety of tissues and organs (Folkman and Klagsburn, 1987). Before discussing the therapeutic potential and strategies for neovascularization in the coronary bed after myocardial infarction, I will review the biology of angiogenesis as we know it today.

Cardiac myocytes contribute to most of the myocardial mass (75 percent), but constitute only 30% of the total cell numbers in the adult heart (Zak, 1973). Recent attention has focused on the non-myocyte population that consists predominantly of mesenchyma cells that include fibroblast, endothelial cells,. and vascular muscle cells. The plasticity of the myocardium in response to injury and infarction is characterized by myocyte hypertrophy, mesodermal differentiation, cellular proliferation, and vascular growth (Weiner and Swain, 1989) and how these pleiotropic responses are mediated by peptide growth factors will be reviewed (Kardami and Fandrich, 1989).

Acidic and Basic Fibroblast Growth Factor (aFGF, bFGF)

Fibroblast growth factors avidly bind heparin, a property that facilitated their easy purification by affinity chromatography for subsequent characterization. Basic FGF was the first of these factors to be identified from brain endothelial cells (Gospodarowicz, et al, 1978). Acidic and basic fibroblast growth factos share fifty-five percent sequence homology and similar spectrum of biological activity (Esch et al, 1985; Gospodarowicz, et al, 1987).

Figure 10

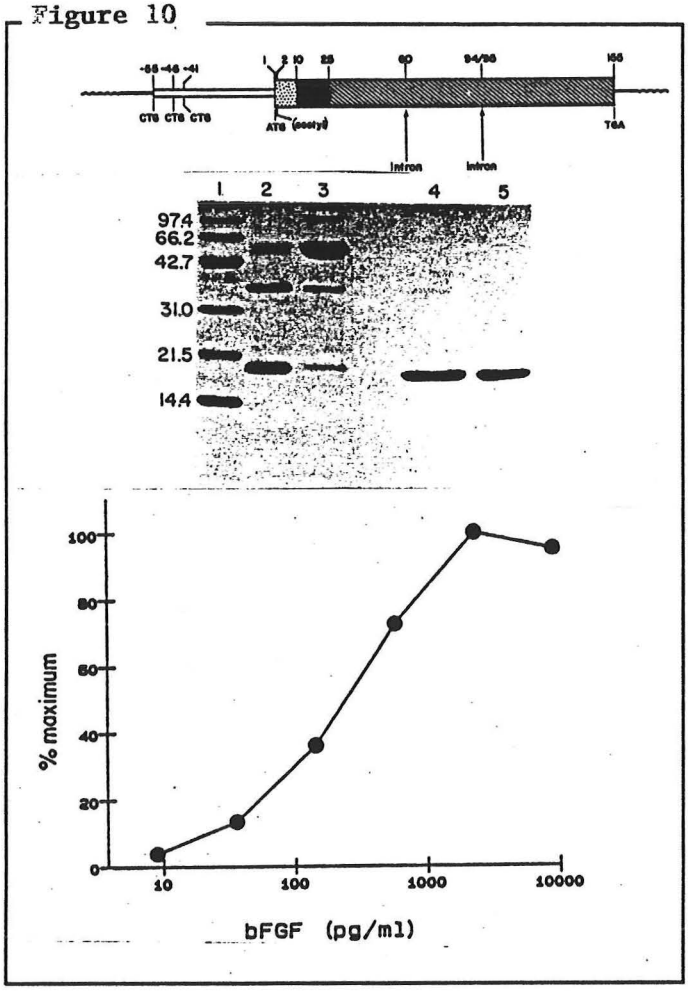


Figure 10 shows the 155 amino acid residue sequence of basic FGF, the recombinant expressionin E. Coli is a monomeric 18 kD polypeptide and its mitogenic property for endothelial cells growth in culture. Acidic FGF has a pI 5-7 (MW 15,000 and 18,000 D) while basic FGF has pI 8-10 (16,000 and 18,500 D) (Folkman and Klagsburn, 1987; Thomson, et al, 1991) and Figure (10).

Receptors for the acidic FGF have been identified by cross linking studies of capillary endothelial cells, with molecular weight ranging from 135,000 to 150,000 and are post-translationally modified by tyrosine phosphorylation. The distribution of basic FGF receptor appears to be much wider and ranges in molecular weight for 125,000 to 145,000 kD. The functional role of these receptors and the tissue specific distribution remains to be completely elucidated. However, FGFs are also under tight regulation, are found either in the cell or, upon secretion, are complexed to the extracellular proteoglycans to facilitate rapid release after cell damage at the site of tissue repair. Among several biological activities demonstrated for bFGF is the stimulation and propagation of vascular endothelial cells and induction of plasminogen activator as part of its biological role in promoting capillary growth or angiogenesis and tissue healing.

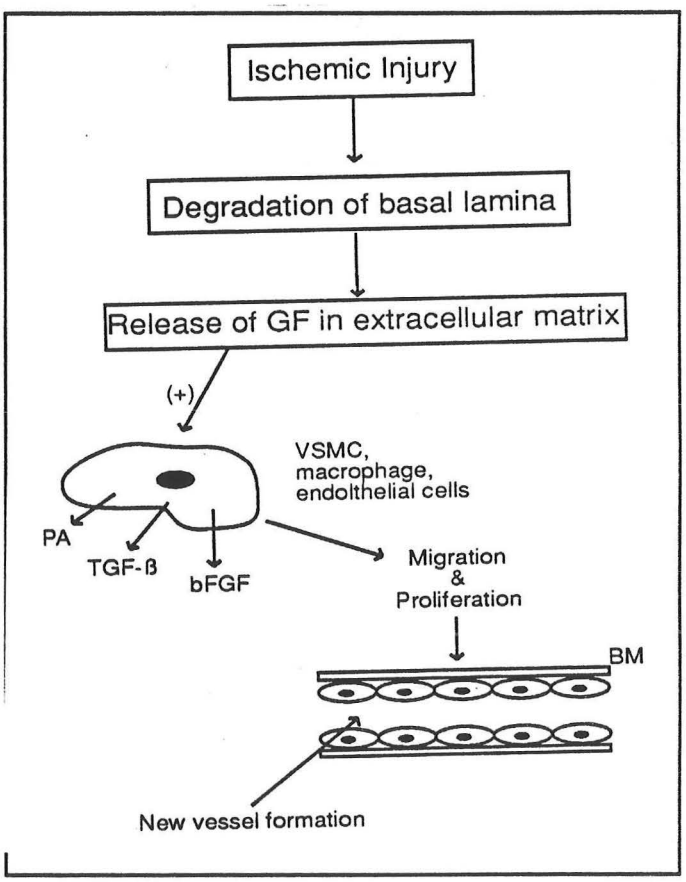
Transforming growth factor-β

The TGF-βs (1-5) are a family consists of at least five related low molecular weight (25-kD) polypeptides which are secreted by endothelial cells and are active inhibitors of endothelial cell proliferation, migration and protease synthesis (Heimark et al, 1986 Roberts and Sporn, 1990; Flaumenhaft et al 1992). Latent TGF-βs are secreted as part of a macromolecular complex (180-210 kD) and is noncovalently associated along with a 75-kD latency-associated protein which, in turn, is disulfide-linked to a larger 125-190 kD latent TGF-β binding protein (Miyazono et al, 1988; Flaumenhaft et al, 1992). Activation of latent, biologically inactive TGF-β is achieved after cleavage of the amino-terminal propeptide by plasmin and other proteases and the active form is released from its complex for binding to its cell surface receptor to elicit its biological response (Pircher et al, 1986; Sato et al 1990; Flaumenhaft et al, 1992).

Further studies have revealed that TGF-β1-3 are found primarily in mammalian tissues such as the extracellular matrix of the myocardium where its primary role is regulation of bFGF (Roberts and Sporn, 1990). More direct evidence for this notion is taken from observations after surgical brain injury in rats where expression of TGF-β as well as nerve growth factor from microglial/brain macrophages are increased are inhibitory for astrocyte proliferation and, thereby, attenuate the amount of scar formation (Lindholm et al, 1992).

The angiogenic effect of bFGF is inhibited by TGF-beta

Figure 11



The effects of the peptide growth factors on angiogenesis is known to be regulated depending on the cell type and pathophysiological setting (Folkman and Klagsburn, 1987; Flaumenhaft et al ,1992). For example, it has been suggested that bFGF production from cardiac myocytes plays a role during embryonic development since cardiac muscle differentiation is an early event that is influenced by bFGF. In turn, bFGF is released by cardiac myocytes to promote formation capillaries, blood vessels and specialized cells of the conduction system (Schubert et al, 1987; Walicke, 1988).

The angiogenic process is initiated by degradation of the basement membrane and release of bFGF for interaction with its cell surface receptor. As illustrated in Figure 11, urokinase type 1 plasminogen activator is induced and released into the extracellular matrix where it converts the precursor plasminogen to its active form. Plasmin activates TGFB by proteolytic clearage. Ultimately, reduced levels of plasmin serves to inhibit latent bFGF activity as a potential mechanism to restore homeostasis (Flaumenhaft et al , 1992). Thus, an intricate biological process has evolved which is responsive to the demands of tissue injury but is also tightly regulated to readily reverse the degree and time course of bFGF stimulation.

TGF-β is essential for new blood vessel formation

Several lines of investigations have converged to show how TGF-β might modulate the invasiveness of angiogenesis and formation of new capillaries. This shown schematically in the next diagram (Fig 11). First, TGF-β acts to inhibit the proliferation, migration and synthesis of proteases to limit the invasiveness of angiogenesis. Second, new extracellular matrix such as fibronectin and heparan sulfate proteoglycan are synthesized by TGF-β stimulation of endothelial cells (Madri et al, 1989; Newton et al, 1990). Last but not least, TGF-β might participate in new capillary formation by mediating changes in ECM and cell-cell junction formation (Merwin et al, 1990).

Fibroblast Growth Factor in the Heart

Both acidic and basic FGF have been identified in isolated cardiac myocytes as well as the intact myocardium (Weiner and Swain, 1989; Sasaki, et al, 1989; Kardami and Fandrich, 1989; Casscells, et al, 1990). The most comprehensive studies to date have been performed by Kardami and Fandrich who examined the distribution and proliferative properties of basic FGF in the myocardium.

Basic FGF is present in both the atria and the ventricle but predominantly in the atria, a finding that has now been observed in several mammalian species, along with the demonstration that extracts from the respective regions were mitogenic for cellular growth of skeletal myocytes. Basic FGF is more abundant in atrial tissue by indirect immunofluorescence although the physiological importance is presently unknown. This distribution was not limited to myocytes, since very high content of FGF was demonstrated in blood vessels and connective tissue of the non-myocyte population, as shown in Figure 11. The predominance of atrial bFGF distribution has been suggested to play a role in the proliferative and regenerative properties of atrial myocytes, however, definitive evidence in support of this proposal is still lacking.

Localization of bFGF in cardiac tissue

Immunofluorescence with highly specific anti-[1-24]bFGF antibodies were used in studies of cardiac muscle which was identified with a triple-staining method for bFGF, myosin and muscle nuclei (Kardami & Fandrich, 1989). Both muscle and nonmuscle cells are immunoreactive indicating synthesis for bFGF; pericellular localization of bFGF is greater in atria than in ventricle; intense staining of intercalated discs region is present in both cardiac chambers, Figure 12; longitudinal sections of blood vessels especially in association with the heparan-sulfate -rich extracellular matrix were expectedly positive for specific fluorescence as shown here in Figure 13 (heavy arrow). These findings are shown in Figure 13 in this low power micrograph of bFGF immunostaining with the characteristic pericellular localization, in the extracellular matrix, of bovine atria on your right in comparison to the more central staining of the ventricular myocytes on your left.

Figure 12

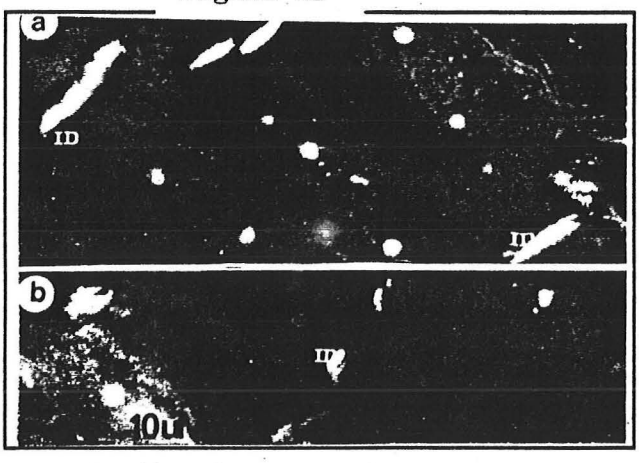
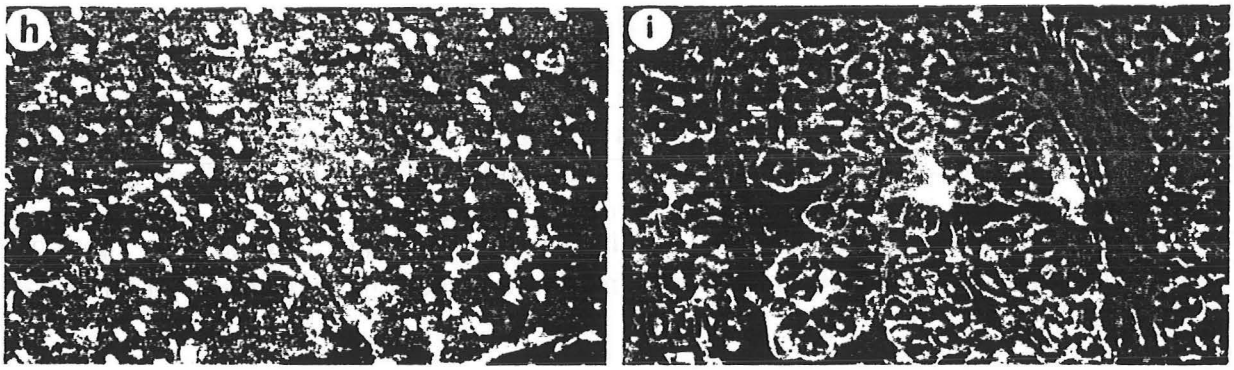


Figure 13



bFGF stimulates collateral formation and reduces infarct size

Recently, evidence to test the hypothesis that regeneration of blood vessels is beneficial in myocardial salvage was provided in a canine model of myocardial infarction (Yananagisawa-Miwa et al, 1992). Experimental myocardial infarction in the dog was produced by insertion of an artificial thrombus into the left anterior descending artery previously made stenotic by laser ablation. An infusion of 10 μg human recombinant bFGF over 1 minute was made 30 minutes after occlusion into the left circumflex artery and was repeated at six hours. Figure 14 shows that the control animals (open circle) (n=5) as well as treated animals (closed circle, N=5) had similar decreases in the left ventricular ejection fraction (LVEF) at 30 minutes; however, while the untreated group had further decreases in LVEF, there was almost complete restoration to pretreatment levels at one week (96 % of control as seen in Figure 14, upper panel). Likewise, the infarct size expressed as a ratio of infarct weight to LV wall weight was significantly lower in the treated group. Further analysis of the coronary vasculature from left circumflex artery distribution revealed that with bFGF treatment, the percentage of small arteries greater than 100 μm as well as capillaries and arterioles (10 to 50 μm) is increased significantly (Figure 15, Yanagisawa-Miwa et al, 1992).

These dramatic findings must be confirmed in other experimental models before clinical trials are even contemplated. Among several questions to be resolved are: 1) Is the local administration of bFGF confined to the immediate environment?: 2) What is the duration of the biological activity of the recombinant bFGF?; and 3) Are there any adverse effects of exogenous bFGF in the myocardium? The latter question has been partially answered since small quantities of recombinant bFGF used in these studies appeared to have avoided intimal thickening caused by proliferation of VSMC and fibroblasts (Pasyk et al, 1991; Yanagisawa-Miwa et al , 1992). In addition, the proliferative effects of bFGF on specific cell types are tightly regulated by the inhibitory efforts of TGF-β.

Figure 14

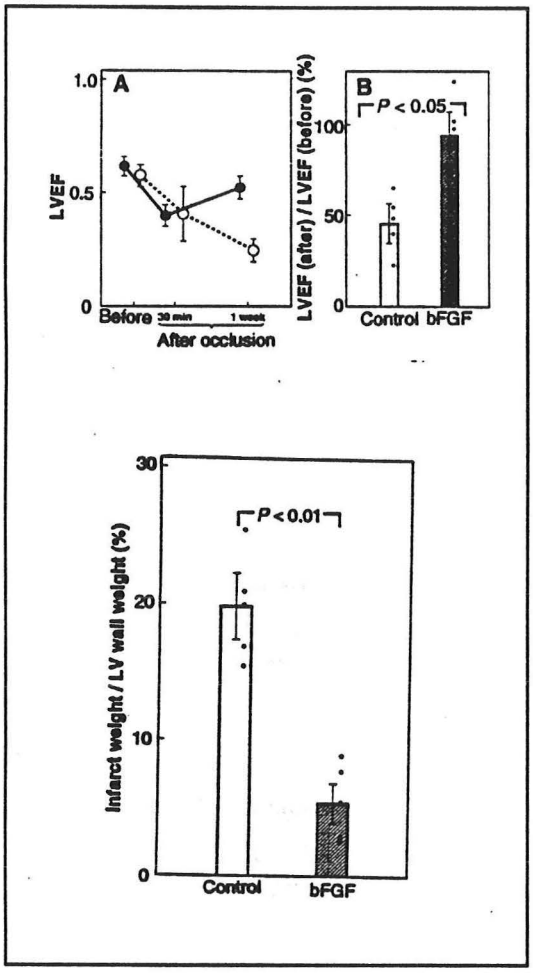
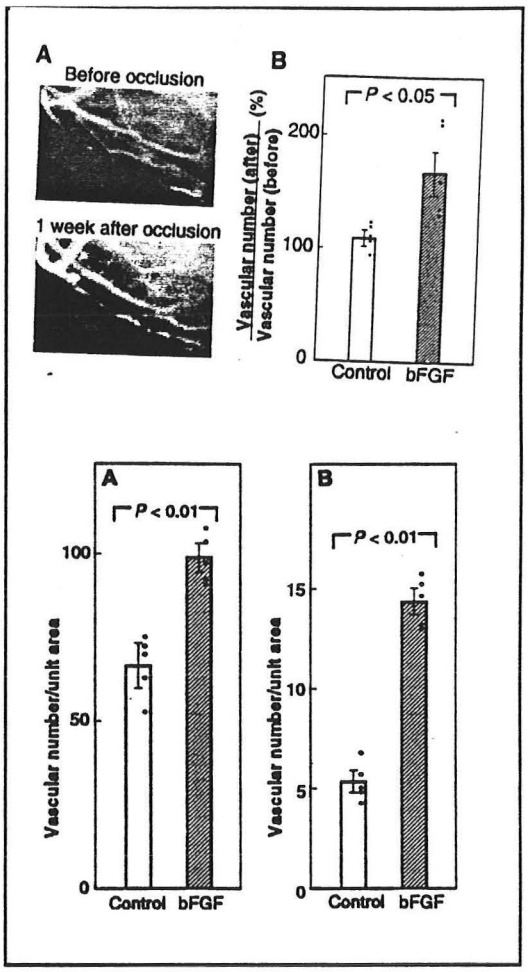


Figure 15



Infarct Expansion and Ventricular Remodeling

Much progress has been made in the past 14 years since the term "infarct expansion" was coined by Hutchins and Bulkley in 1978 to describe the clinical importance about alterations of the ventricular architecture after acute myocardial infarction. (Hutchins and Bulkley,1978). The major concepts of infarct expansion, also referred to as "ventricular remodeling," can be examined by the following major categories illustrated in Figure 16. These investigators distinguished infarction extension, defined histologically as "more recent foci of contraction band necrosis" and seen in a small proportion of 13 infarcts (17%), from "infarction expansion," described as "the acute dilatation and thinning of the area of infarction not explained by additional myocardial necrosis" and seen in the majority or approximately 60% of cases in their landmark study of myocardial infarcts identified at autopsy (Hutchins and Bulkley, 1978). The subgroups with more severe expansion had marked thinning of the ventricular wall before demise as shown in Table 2. A larger series of 205 patients by Pirolo and colleagues reported that 50 % of infarct expansion was observed in the distribution of the left anterior descending artery that supplies the region with greater curvature; more endocardial thrombus and fibroelastosis were associated with infarcts and the degree of preexisting hypertrophy appeared to be protective (Pirolo et al, 1986).

Anatomical Features of Infarct Expansion

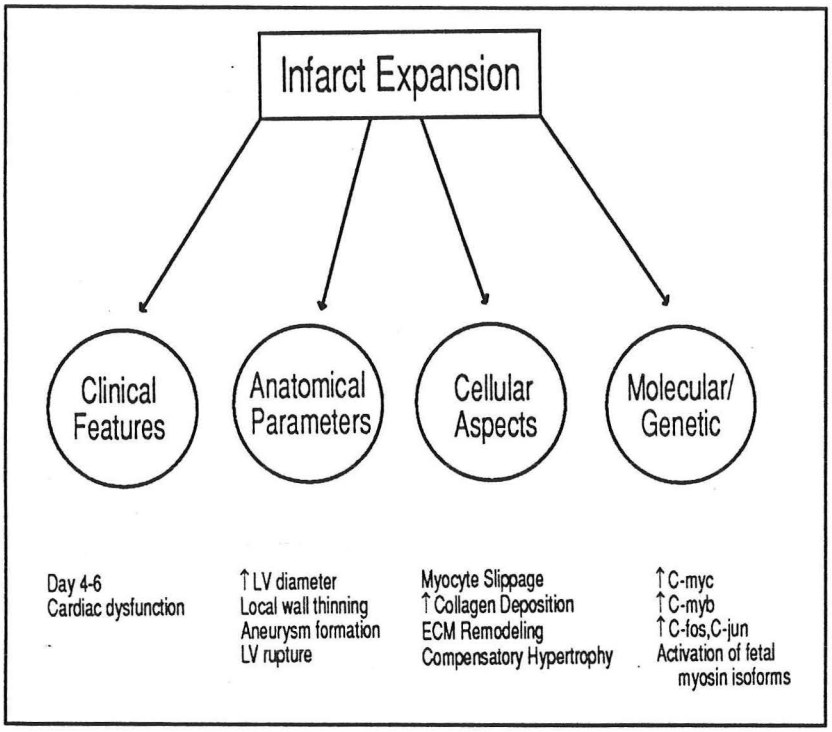
Male/Female	29/27
Age	23-86 (mean 63)
Survival Time	3.5 hours - 30 days (median 7d)

Expasion Grade	Patients	Infarct: Normal Wall Thickness, cm	Infarct: Age Days
None	31	0.98 ± 0.04	6 ± 7
Slight	22	0.79 ± 0.1	5 ± 4
Moderate	13	0.62 ± 0.13	8 ± 7
Severe	10	0.52 ± 0.1	12 ± 7

TOTAL: 76

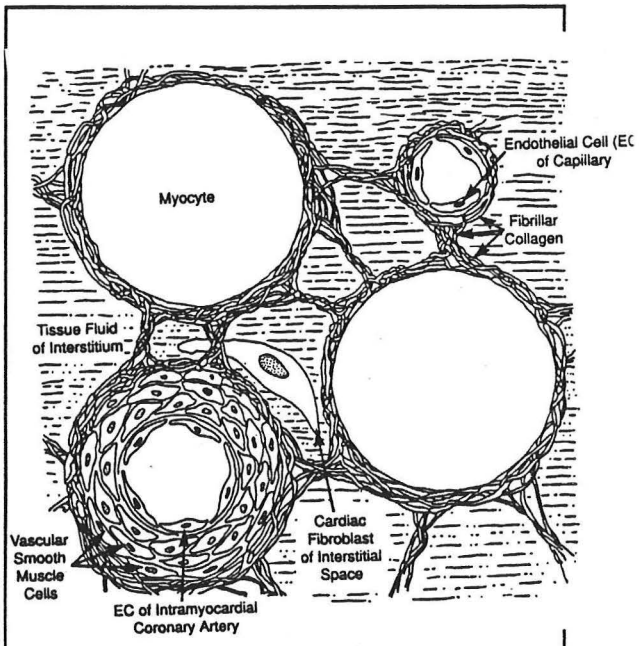
Hutchins and Bulkley, *Am J Card.* 41:1127, 1989.

Figure 16



Thus, the identification of infarct expansion as a common clinical complication led them to their prediction that "although little can be done to prevent or to treat infarct extension, pharmacologic and surgical interventions might be useful in the management of infarct expansion. Thus, extension and expansion are two complications of acute myocardial infarcts that should be distinguished because of their differing pathophysiologic features and therapeutic implications". Their finding set in motion a decade of research, first in experimental animals, specifically aimed to the reduction of infarct expansion by pharmacologic measures and validated in a recent large scale randomized clinical trial (Pfeffer et al, 1992).

Figure 17



From a clinical point of view, infarct expansion is seen most frequently in large transmural infarctions and is initiated within hours of an acute myocardial infarction in patients who manifest severe left ventricular dysfunction, most notably congestive heart failure, pulmonary edema and systemic hypotension. In a series from the Johns Hopkins Hospital, Silverman and Hutchins identified the most adverse clinical outcome or "the second event " after an acute MI at Day 4-5 as aneurysmal dilatation and ventricular rupture (Silverman and Hutchins, 1980). Morphologically, there is an increase in radius of curvature and LV diameter, an alteration in the spatial arrangements of anterior and posterior muscles and local wall thinning which is recognized noninvasively by echocardiography (Eaton et al, 1980).

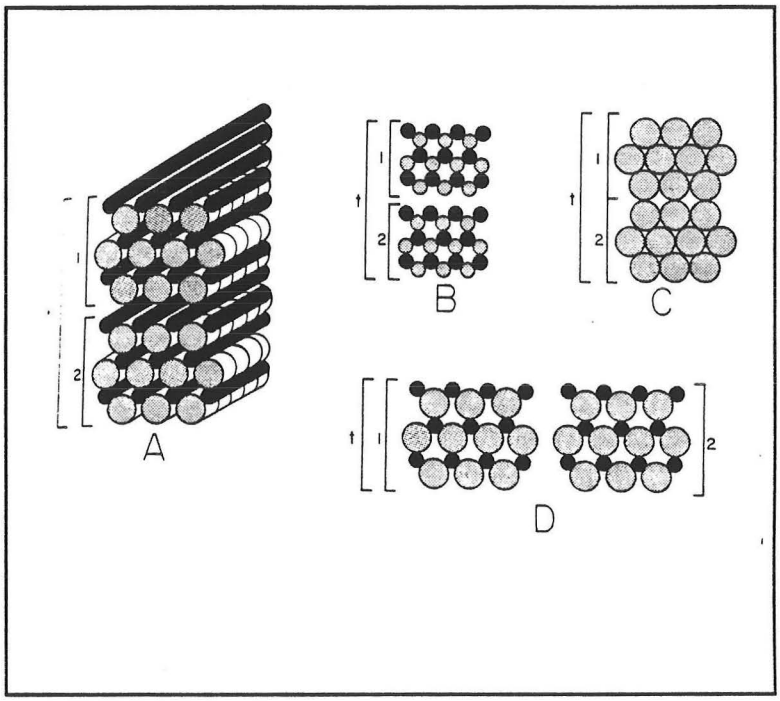
Structural Organization of The Normal Myocardium

The normal myocardium is comprised of many different cell types, as shown in Figure 17 (Weber and Brilla, 1991). Since cardiac myocytes occupy over 75% of the ventricular space but constitute about a third of the cell population (Zak 1983), the remaining cells which form the cardiac interstitium consist of 1) endothelial cells, which form the lining of capillaries and coronary vasculature 2) vascular smooth muscle cells, which are present in epicardial and intramyocardial coronary arteries and arterioles 3) fibroblasts, which are collagen producing cells and, finally, 4) macrophages and mast cells for immune surveillance. Recent investigations by Weber and others on the altered structural organization of the interstitium in disease

states have pointed to the integral role of the collagen network which provide the tensile strength between cardiac myocytes to the other essential structures of the myocardium, namely the capillary and other vessels of the vasculature (Weber and Brilla, 1991).

Within hours of an acute myocardial infarction there is disruption of this relationship because of tissue injury and the release of collagenases such that there is disruption of myocytes from the supporting structures as determined by electromicrographic studies (Weisman et al, 1991). Further analysis has revealed that the predominant mechanism for wall thinning is myocyte slippage or reduction of myocyte per unit area as illustrated in Figure 18.

Figure 18



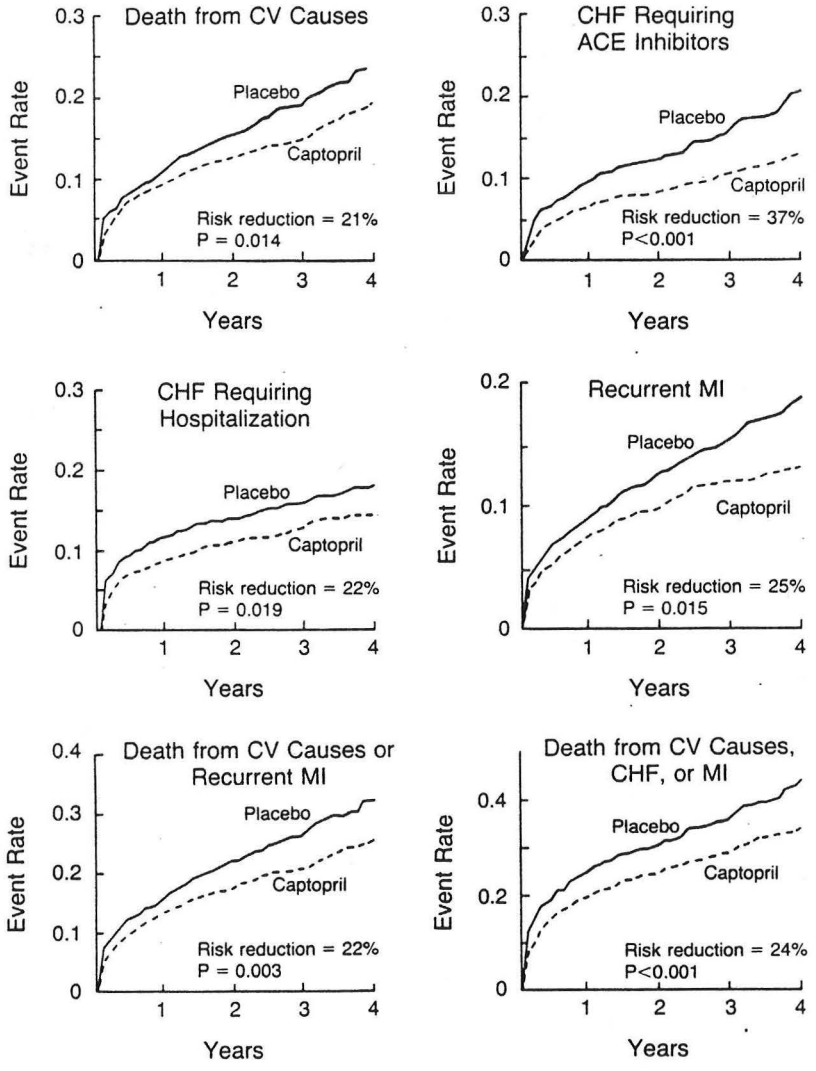
Two additional important functional consequences are noteworthy. During the acute phase, infarct expansion increases ventricular volume and the radius of curvature such that thinning of the ventricular wall will increase wall tension and hence the vulnerability of these patients to ventricular dilatation and occasionally rupture (Silverman and Hutchins, 1980; Pfeffer and Braunwald, 1990). Longitudinal studies of infarct expansion in experimental models have indicated that ventricular enlargement increases well beyond the healing phase and is dependent on the size of the myocardial infarction (Pfeffer et al, 1985; Jugdett 1985 & McKay et al, 1985).

In summary, the major determinants of myocardial remodeling is secondary to infarct expansion are illustrated previously in Figure 16 and include 1) anatomically acute dilatation and thinning of the region within the infarct, 2) alterations of ventricular dimensions, primarily expansion of a kinetic and dyskinetic segments (McKay, et al, 1986), slippage of myocytes bundle at the cellular level (Weisman, et al, 1988). Concomitantly are the molecular signals for hypertrophic growth in non-infarcted segments, collagen deposition, and release of collagenoses, as well as endogenous activation of the RAS in the myocardium (Weber, et al, 1992).

The survival and ventricular enlargement (SAVE) trial was initiated in patients with depressed left ventricular function (LVEF < 40%) but without overt heart failure to test the hypothesis that long-term therapy with an angiotensin-enzyme converting enzyme inhibitor could further improve survival by attenuating progressive left ventricular dysfunction (Pfeffer et SAVE Investigators, 1992). Enrollment was initiated in-hospital with low-dose captopril (12.5 mg three times daily) and was increased to the maximum dose 50 mg three daily. Over 1100 patients were randomized to either placebo or treatment arms and were followed for an average of 42 months. Total mortality was reduced by 19% as seen in Figure 19. Likewise, these were significant reductions in 1) death from cardiovascular causes (21%), development of severe congestive heart failure (37%), recurrent hospitalizations for CHF (22%) and a 25 % reduction in recurrent myocardial infarction. These benefits were observed even in patients receiving thrombolytic therapy, aspirin or beta-blockers.

While these results will impact directly the management of patients with impaired ventricular function after myocardial infarction, evidence is emerging at the cellular level that ACE inhibition may have beneficial effects within the ischemic milieu of the myocardium. As seen in Figure 20, Yamada and colleagues used quantitative in vitro autoradiograph and an ^{125}I angiotensin radiolabelled ligand to identify angiotensin converting enzyme in the myocardium of the rat, predominantly localized in the atria and the ventricles as well (Yamada et al 1990). Interestingly, this study which is the first of its kind, could not demonstrate ACE within the specialized conduction tissues. Other studies have not only demonstrated generation of local angiotensin I to angiotensin II conversion in the myocardium but this conversion is blocked by ACE inhibition (Linz et al, 1986)

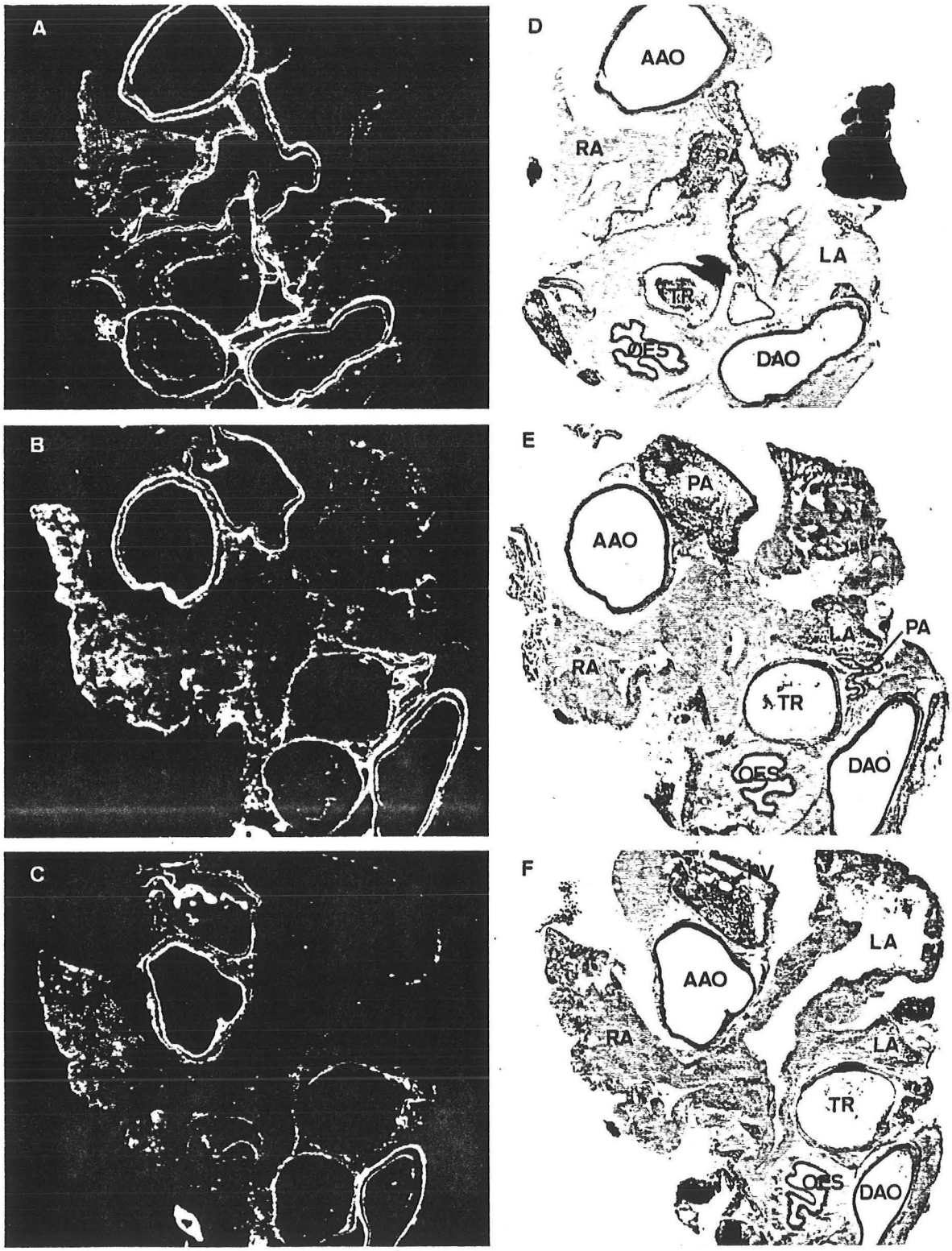
Figure 19



Life Tables for Cumulative Fatal and Nonfatal Cardiovascular Events.

CV denotes cardiovascular, CHF congestive heart failure, and MI myocardial infarction. The bottom right panel shows the following events: death from cardiovascular causes, severe heart failure requiring angiotensin-converting–enzyme inhibitors or hospitalization, or recurrent myocardial infarction. For all the combined analyses, only the time to the first event was used.

Figure 20



Yamada, et al, Circ Res 68: 141, 1991

Figure 21

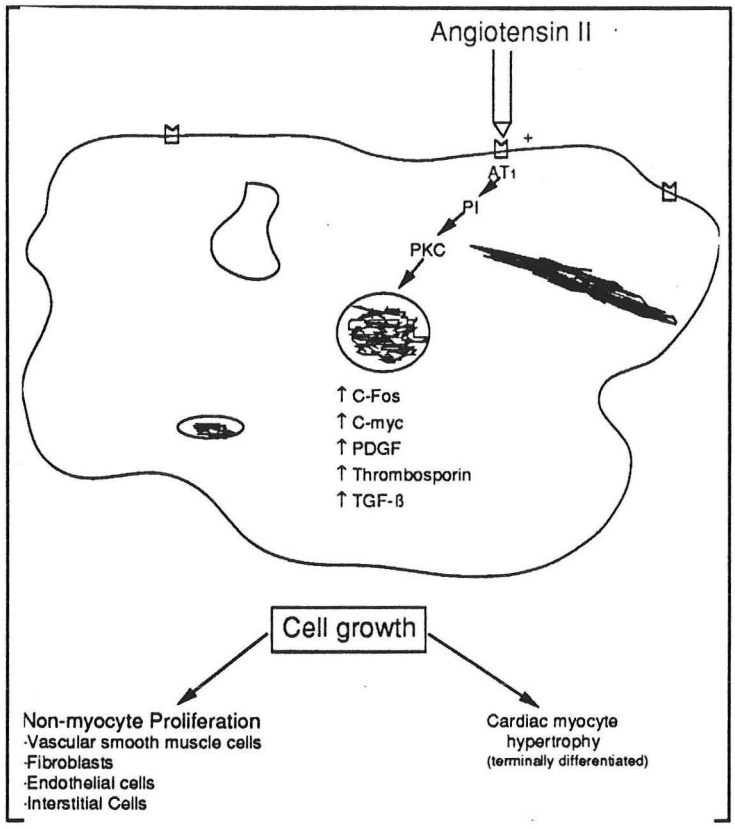
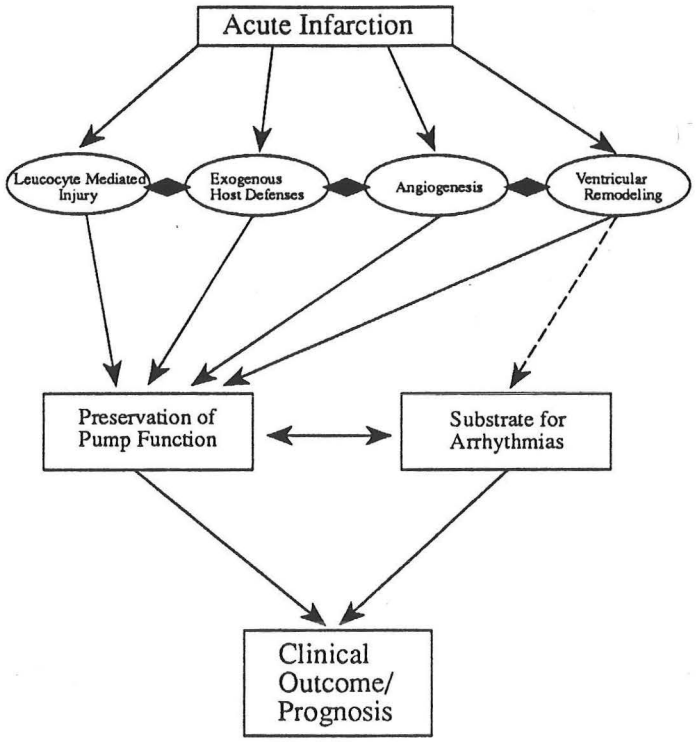


Figure 21 is a schematic diagram which summarizes the evidence for an independent renin-angiotensin system, and likely pathophysiologic role in the heart. Intrinsic renin and angiotensin are present within the myocardium for production of angiotensin II which binds its cell surface receptor, found throughout the myocardium including cells of the conduction system, and initiates a cascade of intracellular events which culminates in cell growth. For the nonmyocyte population, several peptides including local and circulating ANG II, bradykinins and other growth peptides are proposed to stimulate proliferation of vascular smooth muscle cells, fibroblasts and endothelial cell within the ischemic tissue. Alternatively, the response of the cardiac myocyte population to hypertrophic stimuli (i.e volume overload by expansion) is to increase myocyte size without an increase in cell number. Thus, antagonism of the renin-angiotenin system may serve not only to regulate proliferation of the non-myocyte population but potentially may influence the anatomical composition of the interstitial compartment in relationship to the remaining cardiac myocytes during or subsequent to ischemic injury.

SUMMARY

As shown previously, evolving concepts on the pathogenesis of acute thrombosis and now left ventricular dysfunction have had dramatic benefits for the care of patients after an acute myocardial infarction. The topics outlined in the foregoing discussion represent several promising areas for future therapeutic intervention. The use of highly specific monoclonal antibodies, for example, may emerge from current studies on the cellular and molecular biology of leukocytes-adhesion interaction to combat leukocyte-mediated injury. The manipulation of endogenous host defenses may open novel approaches for the use of stress proteins as cytoprotective agents in first line defense for myocardial preservation. The results of the peptide growth factor, bFGF, in stimulation of angiogenesis and myocardial salvage are encouraging but much more work remains before it reaches the clinical arena. Finally, the advances made to modulate the effects of infarct expansion and ventricular remodeling might spurn new fields of investigations to identify the anatomical and molecular substrates for ventricular arrhythmogenesis, which along with the residual myocardial reserve remain the major causes of death from coronary heart disease.

Figure 22



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