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Decay-accelerating factor in the cardiomyocytes of normal individuals and patients with myocardial infarction*

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Summary. The presence of decay-accelerating factor (DAF) was clearly demonstrated on the surface of normal cardiomyocytes. In patients who had died of myocardial infarction (MI) cardiomyocytes displayed different appearances: outside the ischaemically damaged region the myocytes showed no significant variations in DAF expression when compared with controls without MI. Within myocardial zones damaged by ischaemia, however, apparently normal myocytes showed large gaps in surface staining of DAF or formed clusters which were entirely devoid of reactivity with anti-DAF antibodies. The number of DAF-deficient myocytes increased with the extent of necrosis and also with the number of days between onset of MI and death. Even though injury to myocytes is to a large extent related to anoxia and to the presence of free oxygen radicals, the complement system also appears to be involved; DAF may have protective functions against complement-mediated injury. We speculate that phospholipase may be involved in the removal of DAF from the cardiomyocyte surface.

Key words: Decay-accelerating factor – Cardiomyocytes – Myocardial infarction

Introduction

Decay-accelerating factor (DAF) is a glycoprotein present in the membranes of many mammalian cells, capable of regulating the complement cascade at the level of the C3 convertases of the classical, as well as the alternative pathway (Nicholson-Weller et al. 1982). During complement activation fragments such as C3b and C4b can be deposited on surfaces of host cells other than the target cells to be destroyed (Medof et al. 1984). This may lead to damage of host bystander cells, either by phagocytosis and/or, perhaps, by insertion of membrane attack complexes. Other membrane proteins capable of protecting host cells against complement-induced damage are complement receptor 1, which also acts at the level of C3 convertase (Fearon 1980; Kinoshita et al. 1986; Iida and Nussenzweig 1981) and C8-binding protein, a membrane glycoprotein which inhibits complement functions at the level of the late components (Zalman et al. 1986).

DAF has a molecular weight of 70 kDa and is inserted into membranes by a glycosylphosphatidylinositol anchor, as described for several other surface proteins, for example Thy-1 antigen and acetylcholinesterase (Davitz et al. 1986; Tse et al. 1985; Low and Saltiel 1988). This mode of attachment has the advantage of facilitating lateral mobility of the protein in the lipid bilayer (Ishihara et al. 1987; Thomas et al. 1987). By moving freely in the membrane DAF may thus counteract the action of randomly deposited C3b and C4b and inhibit damage to host cells (Davitz 1986). In paroxysmal nocturnal haemoglobinuria, DAF is deficient in erythrocyte membranes, which renders them more susceptible to lysis (Nicholson-Weller et al. 1983; Pangburn et al. 1983). However, erythrocytes are not the only cells bearing DAF. It was shown to be present on the surface of other blood cells, such as platelets, granulocytes, monocytes and lymphocytes (Kinoshita et al. 1985; Nicholson-Weller et al. 1985). Affected paroxysmal nocturnal haemoglobinuria leucocytes show synthesis of aberrant DAF proteins (Carothers et al. 1990) and DAF is partially internalized and partially released from human polymorphonuclear cell membrane to the fluid phase, which may contribute to the presence of DAF in body fluids (Tausk et al. 1989). Recently, DAF has also been demonstrated on vascular endothelial cells (Asch et al. 1986), on vascular smooth muscle cells in human atherosclerotic lesions (Seifert and Hansson 1989), in the epithelial surface of the cornea, conjunctiva, gastrointesti-

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nal mucosa, exocrine glands, renal tubules, synovial membrane (Medof et al. 1987) and on the microfibrils found at the periphery of elastic fibres (Werth et al. 1988).

The purpose of this study was to determine if DAF can also be detected in human cardiomyocytes. Recent reports have suggested that complement may be involved in the pathogenesis of lesions observed in myocardial infarction (Schäfer et al. 1986; Maroko et al. 1978). These authors point to the presence of membrane attack complexes in the infarcted area of the myocardium of those who die, as well as the increased survival time of dogs suffering from experimentally induced myocardial infarction, when these were treated with cobra venom factor. Even though a deleterious role of complement during myocardial infarction has not yet been established, it seemed timely to verify whether cardiomyocytes contain DAF in their membranes, which might exert a protective effect against complement-mediated damage during ischaemia. Furthermore, a modification of DAF expression on the cardiomyocytes of patients with acute myocardial infarction would be of particular interest.

Materials and methods

Hearts obtained at the autopsy of five patients (3 males, 2 females, aged 58–81 years) were used for this study. All five patients exhibited left ventricular myocardial infarction. The time period between onset of signs of myocardial infarction and death varied between 1 day in patient 1; 1–2 days in patient 2; 2–3 days in patient 3; 4–5 days in patient 4; and at least 6 days in patient 5. Post-mortem intervals for patients 1–5 were 3 h, 7.5 h, 17 h, 4.5 h, and 3.5 h, respectively. Fully established myocardial infarction was documented histologically for patients 2–5, whereas patient 1 showed early ischaemic myocardial damage with incipient myocyte necrosis and neutrophil granulocyte margination in blood vessels, but not cohesive coagulation necrosis.

As controls, the myocardial tissue of patients who had died from diseases other than myocardial infarction were used.

After dissection of the hearts, myocardial areas displaying typical infarction or ischaemic discoloration of tissue at gross examination were selected. By ischaemic discoloration we mean the peculiar pale aspect of myocardium seen in early phases of ischaemic damage, but without increase of tissue friability and haemorrhagic demarcation. Starting at the normal-looking myocardium and advancing towards the infarcted area, a tissue strip of 10 mm width was then excised, thus representing normal muscle, the border zone, and the ischaemic or necrotic tissue region itself. This tissue strip was divided into 3–4 consecutive rectangular blocks of equal size, and each of these samples containing the different zones to be analysed were split into halves to obtain corresponding samples suitable for both light microscopy and immunohistochemistry. For light microscopy tissue was dehydrated in graded alcohols and xylene, and embedded in paraffin. Sections were stained with haematoxylin and eosin.

The preparation of anti-DAF monoclonal antibody IA 10 has been described (Kinoshita et al. 1985). Biotinylated antibody and ABC reagents were purchased from Dakopatts (Copenhagen, Denmark). For immunohistochemical detection of DAF unfixed myocardial tissue (see above) was cut into samples of up to 7 mm size and quickly frozen. Sections of 5 µm thickness were cut in a cryostat at −20° C. Sections were air-dried for several hours, fixed for 10 min in acetone at room temperature and sequentially incubated for 30 min each with antibody IA 10 directed against DAF, biotinylated antibody directed against mouse immunoglobulins, and avidin-biotin-peroxidase complex (Hsu et al. 1981). Bound peroxidase was visualized by incubation for 8 min in substrate solution containing 10 mg 3,3′-diaminobenzidine tetrahydrochloride and 0.1 ml 3% hydrogen peroxide in 50 ml of 0.1 M imidazole buffer, pH 7.0. The sections were lightly counterstained with haematoxylin and mounted in glycerol gelatin.

Incubation with diluent buffer (TRIS-buffered saline containing 0.4% gelatin, pH 7.5) without primary antibody served as negative controls.

Results

Strong anti-DAF staining of cardiomyocyte surface areas was observed in normal myocardium and in non-ischaemic myocardium of the five patients. Corresponding control sections tested without primary antibody exhibited no staining (Fig. 1A, B). Intensity, localization and amount of staining were similar, irrespective of the different post-mortem intervals ranging from 3 to 17 h. Anti-DAF staining was present in the form of a thin band at the cell surface facing the extracellular matrix and was also detectable as a thin, linear structure in the junctional area of the cells. No staining was observed within the cytoplasm of normal cardiomyocytes. In addition to myocytes, all tissue samples revealed variable degrees of fibrillary staining in the space between muscle cells, containing fibroblasts. In blood vessel walls, staining was found mainly in the adventitia and sometimes in the endothelial lining, but not in the tunica media.

The ischaemically damaged myocardial zone seen in patient 1 (estimated age of the lesion: 1 day) showed a majority of myocytes with anti-DAF staining not different from normal tissue. However, there also were cells with large gaps in the surface staining (Fig. 2C), and perivascular clusters of myocytes devoid of staining, resulting in pale areas in the preparation. Patient 2 with a myocardial infarction of 1–2 days of age exhibited intact DAF staining of cells in the zone adjacent to the lesion, whereas myocytes within the damaged area, which was infiltrated with neutrophil granulocytes, frequently lacked staining or showed gaps in the surface stain. In patient 3, myocytes forming the border zone expressed surface DAF in a manner similar to normal control cells, but the infarction zone itself was not sufficiently represented in the immunohistochemical preparations to allow for reliable analysis. Patients 4 and 5 (myocardial infarctions of more than 4 days of age) showed extensive necrosis with formation of granulation tissue and with mixed cellular infiltration. Numerous cardiomyocytes within this area lacked DAF staining, whereas morphologically preserved cells exhibited transitions from no staining, staining with gaps, and continuous staining (Fig. 2D).

Interestingly, the infarcted area of patient 5 contained perivascular groups of morphologically non-necrotic and enlarged myocytes with a pale and slightly granular or vesiculated cytoplasm. In some of these cells granular reaction product was found within the cytoplasm, and of about the same staining intensity as the surface DAF stain (Fig. 3A). This population of cells

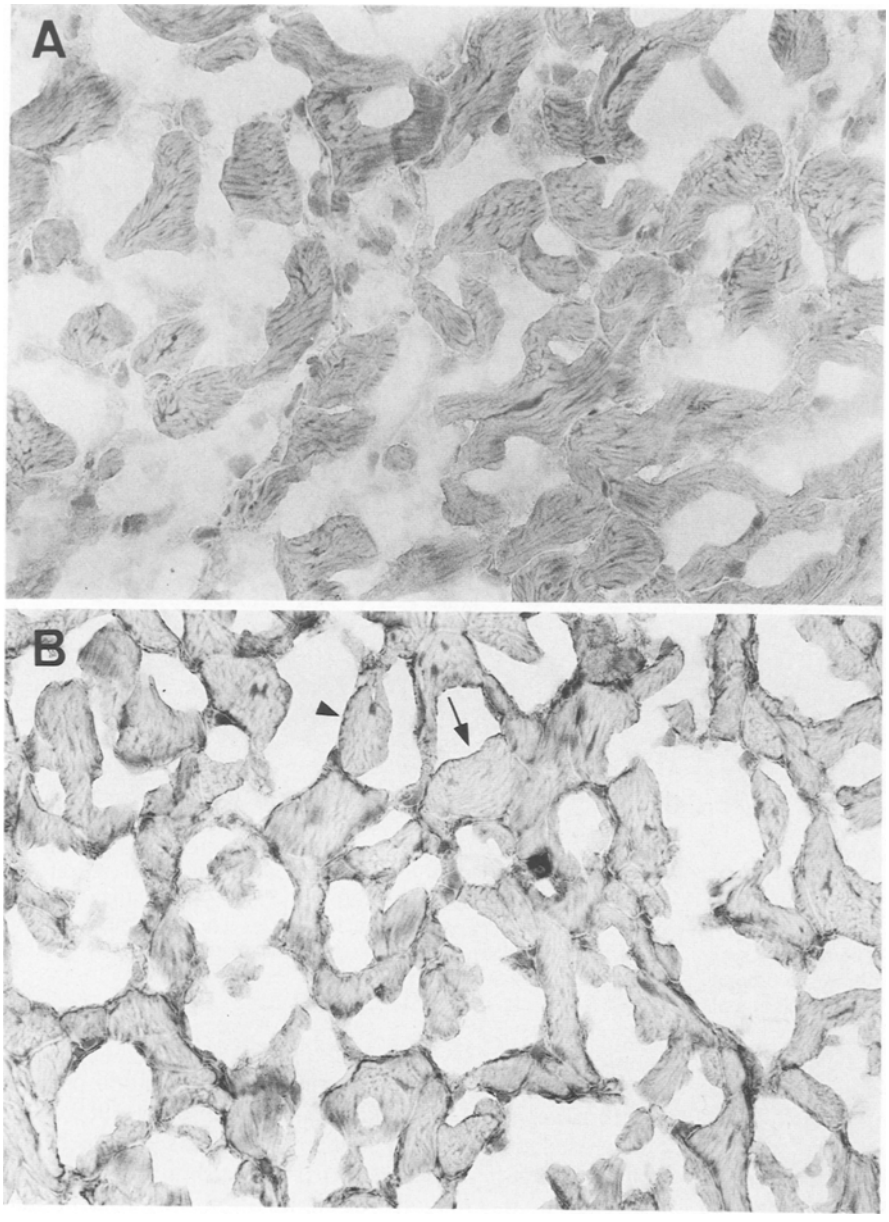

Fig. 1 A, B. Surface expression of decay accelerating factor in cardiomyocytes, using IA 10 anti-decay-accelerating factor (DAF) monoclonal antibody and the ABC method. **A** Negative control, haematoxylin counterstain, ×320. **B** Anti-DAF staining of normal ventricular myocardium. The peroxidation product is visible as a dark band along the myocyte contours, sometimes in the form of a thin line (*arrow*), sometimes as a structure slightly indenting the periphery of the cell (*arrowhead*). ABC preparation, ×320

was surrounded by damaged myocytes partly lacking DAF expression (Fig. 3B).

Discussion

We have shown that DAF is present on the surface of normal cardiomyocytes, but further studies are necessary to determine its origin and more precise cellular localization. Our findings may have implications for the pathogenesis of myocardial infarction, since several studies suggest that complement is involved in cellular damage associated with ischaemia. Complement membrane attack complexes (Morgan 1989) may contribute to tissue injury either primarily, by disrupting membranes and causing cell death, or secondarily by stimulating cells to produce toxic pro-inflammatory mediators. If C4b and C3b fragments are deposited on cardiomyocytes the presence of DAF could inhibit the assembly of C3-convertase and, consequently, C5-convertase, thus preventing injury to heart muscle cells (Medof et al. 1984). It appears that complement is not the primary pathogenic factor in the cascade of events leading to ischaemic cell injury, and damage to myocytes by oxygen radicals can be reduced by superoxide dismutase (Jolly et al. 1984; Zweier et al. 1987). Our group has proposed that the necrosis of a few cardiomyocytes leading to the release of mitochondria into the extracellular space may induce complement activation (Kovcsovics et al. 1985). Indeed, human heart mitochondria have been shown to activate C1 in vitro (Storrs et al. 1981). Activation is even stronger when the outer mitochondrial membrane has become leaky (Peitsch et al. 1987), a process which is known to occur frequently in acute myocardial infarction. When exposed to plasma, the inner membranes, due to their cardiolipin content, activate the first component of com-

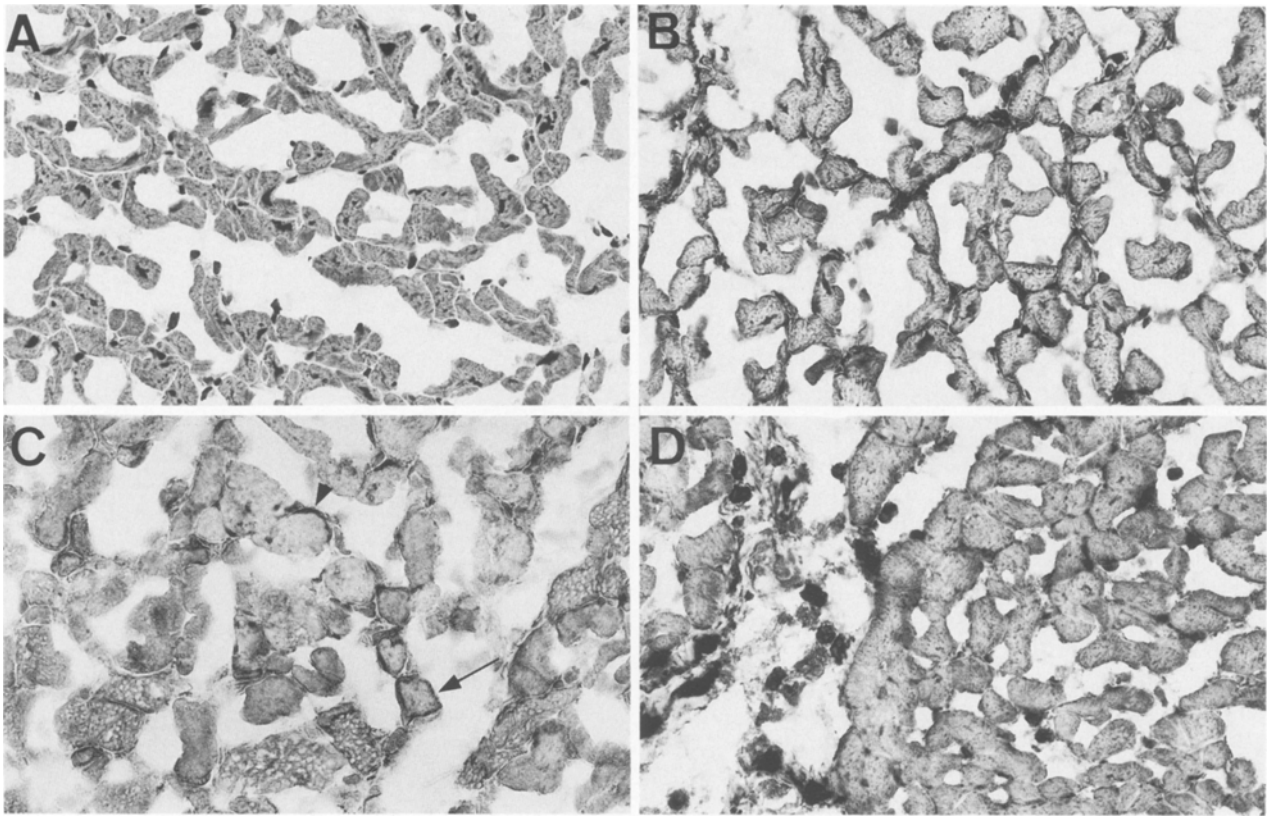

Fig. 2A–D. **A** Negative control, haematoxylin counterstain, ×270. **B** Anti-DAF staining of normal ventricular myocardium. ABC preparation, ×270. **C** Anti-DAF staining of ischaemically damaged myocardium (patient 1; estimated age of the lesion: 1 day). Surface staining is heterogeneous, with cells still encircled by reaction product (*arrow*) and with other cells showing an incomplete surface stain (*arrowhead*). Anti-DAF staining is lacking in part of the cells. ABC preparation, ×270. **D** Ischaemically damaged area (patient 4), infiltrated by leucocytes which are stained due to activity of endogeneous peroxidase (left side of figure). Note that the anti-DAF staining of myocytes is reduced, peroxidation product being visible mainly at contact sites of the cells. ABC preparation, ×270

plement and thus trigger the complement cascade (Peitsch et al. 1988). This will lead, among other factors, to the formation of C5a and to insertion of membrane attack complexes (Schäfer et al. 1986; Rossen et al. 1988). C5a is a potent chemotactic factor for neutrophil granulocytes, and their infiltration of the lesion site contributes to the tissue damage in myocardial infarction (Yancey 1988). The oxidative burst resulting from the adhesion of neutrophils to cardiomyocytes may produce additional oxygen radicals (Engler and Covell 1987) which lead to further cellular injury and complement activation. Thus, the expression of DAF on heart muscle cells may again protect them by interrupting this vicious cycle, and may counteract terminal C5b-9 complex-mediated damage, down-regulate the C3 amplification loop, and promote decay or dissociation of complement complexes. These considerations are speculative, but a protective role of DAF has been proposed for other cell systems. Thus, DAF levels correlate inversely with tumour cell sensitivity to complement-mediated cell killing in a series of melanoma cell lines (Cheung et al. 1988). It has been reported that lymphocytes (CD4+ as well as CD8+ cells) from persons with AIDS or AIDS-related complex show enhanced susceptibility to complement-mediated damage that correlates with diminished expression of lymphocyte DAF in comparison with other surface markers (Lederman et al. 1989). The increased complement susceptibility was decreased by incorporation of DAF into patients' lymphocytes (Lederman et al. 1989). It was claimed that this defect could contribute to the lymphocyte depletion of AIDS, and that this provides additional evidence for the importance of DAF in regulating complement activation. Anti-DAF antibodies render erythrocytes (Pangburn et al. 1983), glomerular epithelial cells (Quigg et al. 1989), Raji lymphoblasts (Fujita et al. 1988) and melanoma cells sensitive to complement-mediated killing. The demonstration of DAF on cardiomyocytes may thus explain their relative resistance against complement-mediated injury. The apparent decrease of DAF expression by heart muscle cells from ischaemic lesions is intriguing. It does not appear to be related to different post-mortem intervals, as shown by the similar intensity and the localization of the staining in the non-ischaemic tissue of the five cases with post-mortem intervals ranging from 3 to 17 h. Whereas these normal tissue sections displayed a strong homogeneous staining of myocytes, those located in the ischaemic area showed considerable heterogeneity with gaps in the staining of surface DAF, and areas of apparently intact myocytes expressing no staining at all. The

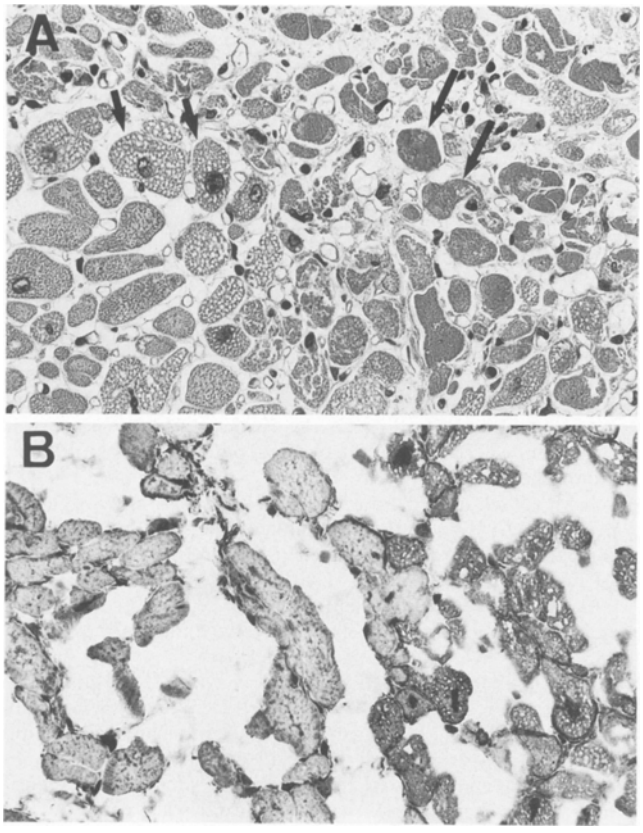

Fig. 3A, B. Myocardium of patient 5: myocardial infarction of at least 6 days of age. **A** Haematoxylin and eosin-stained paraffin section. Necrotic myocytes are visualized as ovoid cells lacking a nucleus and showing a dark cytoplasm (*large arrows*). The larger cells with nuclei and granular cytoplasm are preserved myocytes (*small arrows*); ×270. **B** Anti-DAF staining of a frozen section representing a corresponding tissue area. Necrotic myocytes (left half of figure) express a discontinuous staining or lack surface stain. In contrast, preserved cells (right half of figure) corresponding to the granular cells in **A** show a stronger surface staining, and some cells display stain in the cytoplasm as well. ×270

observation that lack of staining was seen with myocytes that showed no morphological signs of necrosis suggests that cell damage alone may not be the only mechanism involved. Loss of DAF expression could be related to decreased biosynthesis, decreased intracellular transport, increased degradation of the factor, or combinations of these. It is known that the numbers of DAF molecules on the surface of neutrophils and of T-lymphocytes can be modulated by inflammatory mediators and by mitogens (Berger and Medof 1987; Davis et al. 1988). Modulation of DAF expression is supported by the findings that DAF synthesis by endothelial cells appears to involve protein kinase C activation (Bryant et al. 1990) and that DAF, which is not found in smooth muscle cells of normal human arterial walls, can be induced on vascular myocytes during atherogenesis and in culture (Seifert and Hansson 1989). Differential expression of DAF and its modulation may thus play a regulatory role in pathological conditions involving complement activation. For the situation where DAF expression is reduced in cardiomyocytes located near an ischaemic lesion, an attractive explanation would be the activation of glycosylphosphatidyl-inositol-dependent phospholipases which could remove the polypeptide of DAF from the phospholipid anchor in the cell membrane. Recently, a glycosylphosphatidylinositol-specific phospholipase D has been detected in plasma (Davitz et al. 1987). This enzyme has a pH optimum at 5.4 and is much less active at pH 7.4 (Cardoso de Almeida et al. 1988). It is conceivable that the lactic acid accumulation occurring in the ischaemic areas during myocardial infarction would facilitate removal of DAF from the cardiomyocytes. Myocyte death may cause the release of intracellular enzymes, such as phospholipases, and cleavage of DAF through the action of phospholipase C has been demonstrated, variations in the sensitivity of human DAF cleavage apparently being related to structural heterogeneity of the DAF anchor (Walter et al. 1990). However, it appears that gaps in the staining of surface DAF also occurs in areas with morphologically intact cells. At this stage it is clear that DAF staining is decreased in ischaemically damaged heart muscle cells, and in myocytes directly surrounding ischaemic lesions, but a cause and effect relationship to cell damage by a complement-dependent mechanism is speculative. Immunohistochemical analysis of myocardium in patient 5, who had the oldest lesion, displayed perivascular clusters of non-necrotic, large myocytes with distinct DAF staining also in the cytoplasm. This finding, which will have to be confirmed in other patients with myocardial infarction dating from more than 4 days, could signify the beginning of repair (DAF synthesis) after acute myocardial infarction.

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