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GRIP AND STICK AND THE LYMPHATICS

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ABSTRACT

Kaposi sarcoma is a common, though not inevitable consequence of AIDS. There is a body of opinion that believes that this sarcoma is derived from lymphatic endothelium, or at least from a failure of vascular endothelium to distinguish between whether it is attempting to be a blood vessel or a lymphatic. While immunodeficiency and its consequences have proved to be the most significant area of research, the general biology of endothelium, and especially angiogenesis, has perhaps been neglected. I predict that the most important new concept in the biology of endothelium is the recognition of mechanico-receptors as a determinant of its behavior. The concept is illustrated by articles from Oxford (Ryan 1989), from Boston, Massachusetts (Ingber & Folkman 1989), and from Moscow (Shirinsky et al 1989). Most authors studying endothelium have concentrated on blood vascular endothelium and ignored the rich lymphatic bed. Since the lymphatic is par excellence a mechanical receptor, this is perhaps surprising. The lymphatic functions by its responsiveness to mechanical forces, it is a fine control for hydrostatic pressure within the interstitium, and morphologically, its flat and attenuated endothelium linked to strong anchoring fibers is biologically exactly the kind of behavior required of a cell that is responsive to mechanical factors. Perhaps the best known mechanical receptor is the stretch receptor in the muscle fiber. The linkage of this receptor to the enzyme protein kinase C has been described. Ryan has also pointed out that protein kinase C may be an important mechanico-receptor in the fibroblast and possibly also universally in all cells, including lymphatic endothelium. This enzyme is linked to the phosphorylation of vinculin which binds cytoskeleton to the cell membranes. Distortions of the membrane which displace cholesterol, bring protein kinase C into the membrane, where it is able to phosphorylate the cell glue vinculin. Associated with this capability is the capacity to induce inhibitors of proteolysis. Such inhibition is essential if firm adhesion or anchorage is to occur. It is suggested that the anchoring fiber linkage to the lymphatic endothelium contains such biological mechanico-receptors. Since activation of proteases is a feature of transformed cells and loss of anchorage is a feature of malignant cells, and since also shape change seems to be a feature of the endothelium in Kaposi sarcoma, it is suggested that alterations in the biology of the mechanico-receptor be taken into account when attempting to explain Kaposi sarcoma.

Although lymphatics are composed of endothelium, it is perhaps surprising that investigators specializing in the field of blood vessel endothelium almost never mention the lymphatic system. On the other hand, it is also a feature of current interest in the lymphatic systems relating to the pathogenesis of AIDS that lymphatics are thought of in immunological rather than biological terms.

Perhaps the most important concept relating to the biology of endothelium to be developed since our last symposium (1) is the transduction of biochemical

signals by mechanical forces. The concept that there are mechanico-receptors on the endothelial cell has been discussed by Boston investigators (2) and by a group in Moscow (3). Neither group have referred to lymphatic endothelium, whereas Ryan (4-7) and Ryan and Barnhill (8) have consistently proposed that this concept is especially relevant to lymphatic endothelium. Essentially the concept states that mechanical forces tending to distort cells produce shape changes that affect the composition of cell membranes which are reflected ultimately in the biochemical behavior of the cell. It is pointed out that flat cells behave differently from round cells (2,9,10) and in this respect it may be significant that no endothelial cell is more flat than the lymphatic. The concept requires that cells should feel the effects of mechanical forces and again it should be emphasized that the lymphatic senses changes in the forces of hydrostatic and oncotic pressure in its environment and responds to this alteration by aiding the clearance of fluid and macromolecules. It is probable that in Kaposi sarcoma the lymphatic is neither flat nor sensing its environment. Recent studies of fibronectin receptor suggest that anchorage and migration can be differentiated (11). Both require adherence but it is conceivable that a change in receptor function could change a cell from stable anchorage to the transient adherence needed for migration.

The Mechanico-Receptor

It has been suggested that the mechanico-receptor may be protein kinase C (5,6). This enzyme is responsible for phosphorylation of cell adhesive materials, such as vinculin and adducin (12). It is an enzyme that spends some of its time in the cytoplasm but can be moved into the cell membrane to phosphorylate materials therein. Such linkage to the cell membrane occurs when the cell membrane is distorted and cholesterol is displaced, and it also occurs as a result of a number of cytokines and agents controlling the use of calcium. One of the interesting tools for studying protein kinase C is phorbol ester and the role of this agent in modulating cell adhesion has been described (13). One of the best understood mechanico-receptors is the stretch receptor in muscle. Recent studies of the development of the muscle fiber or the myofibroblast by Adamo et al (14) have shown that protein kinase C is predominantly cytosolic in mouse cultured muscle cells, but with stretch response development there is a requirement that protein kinase C should become membrane attached. In the course of the development of the muscle cell, there is an inverse relationship between the stretch response and the capacity to undergo mitosis (14). Protein kinase C is such a universal enzyme that serious consideration should be given to the possibility that all cells use it as a mechanico-receptor.

Protease Inhibition for Stick and Grip

Pollack and Rifkin (15) were one of the first to publicize the role of proteases in the prevention of anchorage. It is an important part of the concept of the mechanico-receptor that mechanical forces should be felt by cells as a result of anchorage. Any protease activity which disrupts adhesion or anchorage is likely to interfere with the mechanico-receptor response. Ryan (5-7) and his group have consistently pointed out that inhibitors of urokinase may be the most significant element in the protection of adhesion plaques from lysis. The observation that urokinase is co-localized with vinculin, a major constituent of the cell membrane's adhesive material (16,17) brings animal cells in line with more primitive unicellular organisms which habitually hide away a protease somewhere in their periplasm and which can be activated when lysis of the cell membrane is required for the manipulation of adherence and division for sexual activity (18). Many cells also use activators and inhibitors of proteases for remolding their environment, either to reshape it or to clump together within it. Stick and grip require that either such proteases are inhibited (15) or that some

substitutes such as a glycoprotein resistant to proteases can be introduced as a locking device (19). The center point for both stick and grip may well be the phosphorylation of vinculin. This phenomenon requires protein kinase C to be moved from the cytoplasm and incorporated in the cell membrane (16).

Transformed Cells and Protease Activation

With respect to the development of Kaposi sarcoma in the pathogenesis of AIDS, one should be aware that transformation of cells by viruses is a well-known cause of activation of proteolytic behavior, loss of adhesion and change in cell shape. It is also now well understood that when cells develop a more malignant phenotype, they frequently lose their capacity to adhere. Were one to explore this concept, the spindle-shape of the supporting cells in Kaposi sarcoma and the shape variability of the endothelium itself would need to be studied. The accompanying edema which upsets the transmission of mechanical forces or the frequent association of increased proteolysis with edema (20) would all need to be taken into account. McNeill et al (21) postulate that certain cytokines such as basic fibroblast growth factor are released as a result of mechanically-induced disruptions of the endothelial cell. It also now seems likely that what matters for cytokine activity is not so much cell shape as change in shape. The observation mentioned above relating to fibronectin receptor function also distinguishes between fixed attachment and the unstable attachment of migration. The role of shape change in determining the response to cytokines has been discussed by Ingber and Folkman (2), and the observations of Krishnamurti et al (22) that phorbol ester is a more potent producer of inhibitor when added to cells that are firmly attached rather than rounded and dis-attached, is also of interest, since the focus of the phorbol ester is known to be protein kinase C--the proposed mechanico-receptor.

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