Erythrocyte membrane abnormalities in myeloproliferative disorders

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Chronic myelogenous leukaemia is a haematologic malignancy characterized by excessive growth of myeloid cells and their progenitors. The role of the cytoskeleton in perturbations of phospholipid distribution has been investigated in chronic myelogenous leukaemia (CML) erythrocytes. The effect of defective spectrin tetramer formation and reorganization of cytoskeletal components on the topographic distribution of band 3 protein was studied by determining the agglutination of CML erythrocytes by concanavalin A (con A). CML erythrocytes were agglutinated by con A, whereas normal erythrocytes were agglutinated only after trypsin digestion. Our studies have shown comparable rate constants of $^{35}SO_4^{2-}$ efflux in normal and CML erythrocytes and decreased ankyrin binding. Increased binding of autologous IgG to CML erythrocytes compared to normal ones suggests clustering of band 3 protein and early removal of CML erythrocytes from circulation, accounting in part for the anaemia associated with the disease.

MYELOPROLIFERATIVE disorders result from the development of an abnormal haemopoietic stem cell (Figure 1). Since these stem cells are multipotent, abnormalities may reside in each of the lineages (red cells, granulocytes, platelets) derived from that clone. The erythrocyte membrane structure is well characterized. Identification of erythrocyte membrane abnormalities in myeloproliferative disorders will help in identifying mechanisms responsible for altered mechanical properties and biological recognition (by macrophages and reticuloendothelial cells) of these erythrocytes.

Structure of the erythrocyte membrane

The erythrocyte membrane skeleton is composed of a lipid bilayer underlying which is a meshwork of peripheral proteins located in the cytoplasmic surface. This flexible and elastic meshwork of proteins comprises the cytoskeleton. It is responsible for maintaining the biconcave shape of the erythrocyte, for its reversible deformability and for membrane structural integrity[1-3]. Spectrin, a long, rod-like molecule of two non-identical subunits, is the major component of the membrane skeleton. Spectrin dimers associate into tetramers and higher oligomers. The tetramer is the predominant form of spectrin in the normal erythrocyte membrane[4]. Spectrin tetramers interact with actin, protein 4.1 and adducin to form the cytoskeletal network[5-7].

This network is linked to the membrane by two proteins. Protein 4.1 binds to the end of spectrin distal to its self-association site and attaches the spectrin network to the membrane through glycophorin[8].

Ankyrin binds to the beta subunit of spectrin and attaches it to the membrane via band 3 protein[9]. A schematic diagram showing the organization of the erythrocyte membrane cytoskeleton is given in Figure 2.

The membrane skeleton restricts the lateral mobility of integral membrane proteins in the plane of the membrane[10], and also stabilizes the slow transbilayer movement of the phospholipids of the membrane[11,12]. Defects and deficiencies in the skeletal components have been described in several hereditary haemolytic anaemias[13-18], where they give rise to mechanically and/or thermally unstable erythrocytes[19] with shortened life spans.

Band 3 is the major integral membrane protein of the mammalian erythrocyte membrane[20,21]. The band 3 gene has been sequenced from human[22], murine[23] and chicken[24] erythrocytes. This 95-kDa polypeptide possesses two distinct structural domains. The membrane-associated domain is responsible for the anion exchange activity of band 3, which mediates the physiological exchange of chloride and bicarbonate across the erythrocyte membrane. The amino-terminal cytoplasmic domain serves as an anchor for the membrane

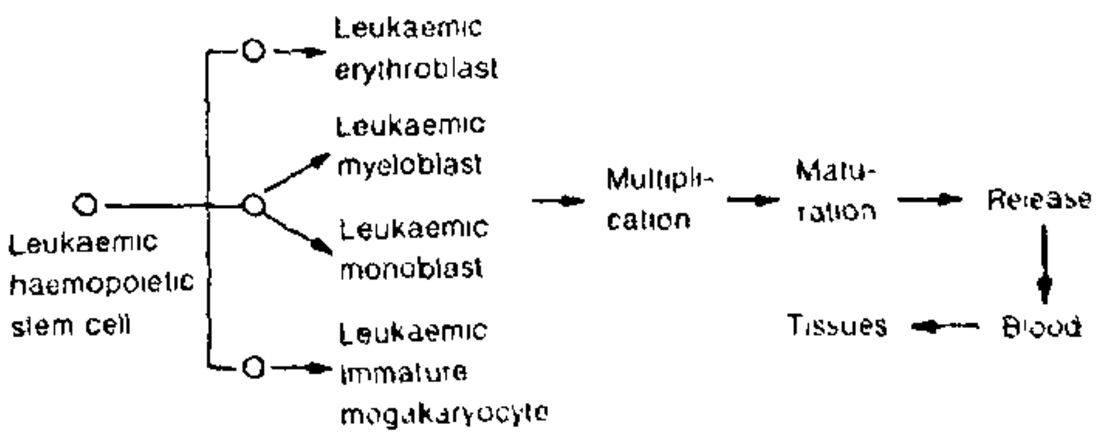

Figure 1. The malignant process in myelogenous leukaemia is believed to reside in a heamopoietic stem cell. At least four major steps in haemotopoiesis are regulated stem cell proliferation, commitment to one of the three major haemopoietic cell lines (red cells, granulocytes, platelets), multiplication and maturation of early committed cells, and release of mature cells into the blood. These control points are partially or totally defective in myelogenous leukaemia [adapted from *Hematology* (eds Williams, W. J., Beutler, E., Erslev, A. J. and Lichtman, M. A.), McGraw-Hill Book Company, Singapore, 1986]

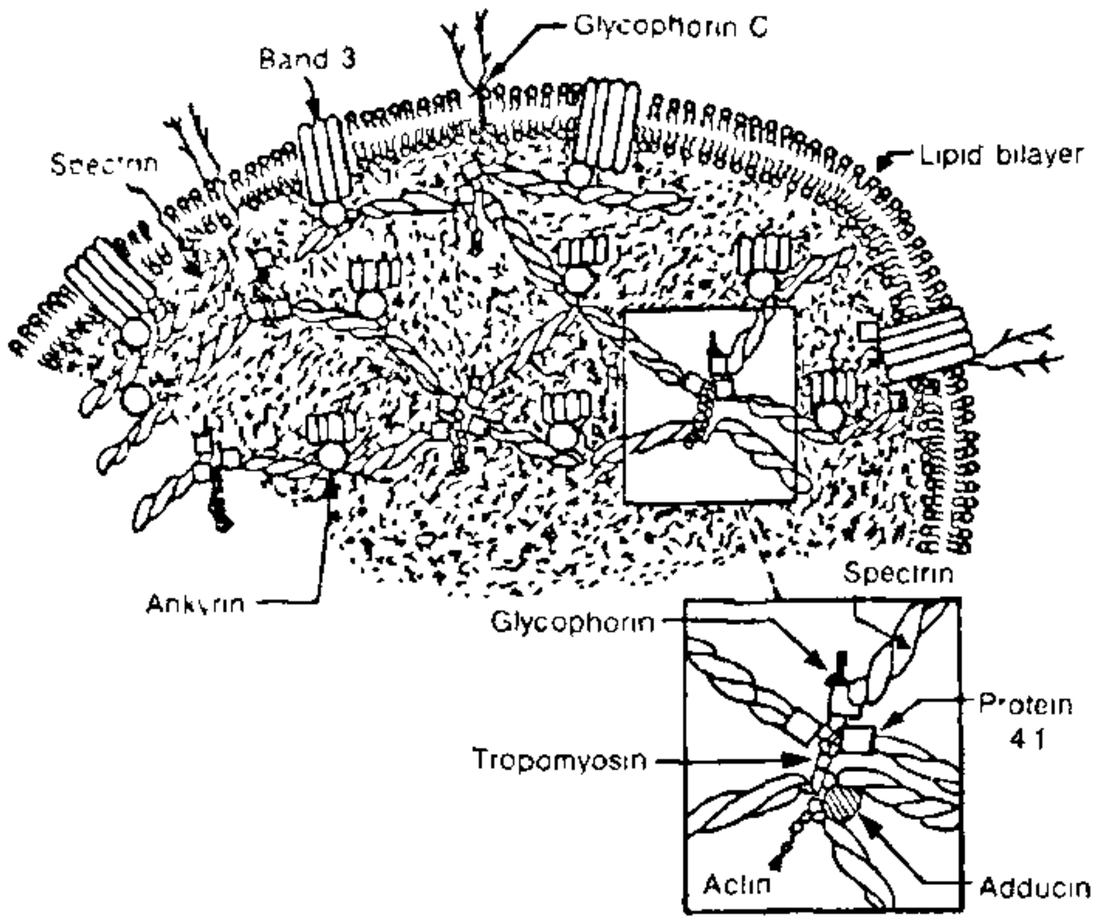

Figure 2. Schematic diagram of the erythrocyte membrane (from ref. 3)

cytoskeleton and glycolytic enzymes[25,26]. Initial efforts at identifying the ankyrin-binding site of the human erythrocyte anion exchanger suggest that this site is likely to involve a fairly extended sequence in the midregion of the cytoplasmic domain and possibly requires several contacts mediated by each of the subdomains of the cytoplasmic domain[27].

Using monoclonal antibodies, another view has been projected suggesting that ankyrin binds to band 3 in a region near a cysteine cluster of residues 202 and 317 which is in close proximity to a proposed regulatable hinge[28].

The phospholipids in the human erythrocyte membrane are asymmetrically distributed over the two halves of the bilayer. The outer monolayer contains neutral phospholipids, phosphatidylcholine (PC) and sphingomyelin, whereas phosphatidylethanolamine (PE) and phosphatidylserine (PS) are mainly restricted to the inner leaflet of the bilayer[29].

Among the mechanisms suggested to be responsible for maintaining this asymmetry are (i) interactions between membrane skeletal proteins and the aminophospholipids PE and PS[30–32], and (ii) an ATP-dependent translocation of both aminophospholipids from the outer towards the inner monolayer[33].

Studies with erythrocytes from patients suffering from chronic myelogenous leukaemia

Chronic myelogenous leukaemia is a haematologic malignancy characterized by excessive growth of myeloid cells and their progenitors[34,35]. Kumar and Gupta[36] first showed the altered distribution of membrane phospholipids in erythrocytes from patients suffering from chronic myelogenous leukaemia (CML). Reed *et al.*[37] have shown increased binding of merocyanine 540 to the erythrocytes from patients suffering from myeloproliferative disorders, compared to normal erythrocytes. Schlegel *et al.*[38] have reported altered recognition of CML erythrocytes (like experimentally produced lipid-symmetric erythrocytes) by endothelial cells as well as monocyte-derived macrophages as a consequence of the loss of phospholipid asymmetry. CML erythrocytes have also been reported to display high levels of calcium-binding proteins which may possibly be responsible for perturbation of membrane structure[39].

In our laboratory, the role of the cytoskeleton in the altered recognition of CML erythrocytes has been investigated. The treatment of erythrocyte ghosts with low ionic strength buffer at 4°C for a prolonged period (24–48 h) and subsequent fractionation on Sepharose 4B leads to three major peaks attributable to the spectrin–actin–band 4.1 complex, tetrameric spectrin and dimeric spectrin[40]. In normal human erythrocytes, spectrin is predominantly in the tetrameric form. CML ghosts showed considerable amounts of spectrin dimer and reduced tetramer[41]. CML erythrocytes were also found to be thermally more sensitive than normal erythrocytes becoming poikilocytes at 46°C.

Favourable arrangements of α- and β-chains at the head-end allow the formation of spectrin tetramers or oligomers, which are the functional units in the membrane skeleton. Similarly, actin, ankyrin, band 4.1 and the tail end of spectrin where it interacts with band 4.1 should be in a favourable position to build up the protein meshwork which is believed to be responsible for the shape and stability of the red blood cell. The arrangement of skeletal proteins in CML erythrocytes was studied using the bifunctional crosslinking agent dimethyladipimidate (DMA) with a span of 8.6 Å. Skeletal proteins in CML ghosts were found to undergo a spatial redistribution so that they become crosslinkable by DMA.

The effect of defective spectrin tetramer formation and reorganization of cytoskeletal components on the topographic distribution of functionally important membrane proteins was studied by determining the agglutinability of CML erythrocytes using concanavalin A (con A). CML erythrocytes were found to be agglutinable by con A, whereas normal ghosts are agglutinable only after trypsinization. However no increase in the number of con A receptors was found. Since band 3 is the con A receptor, possible alteration in functions of band 3 in CML erythrocytes was investigated. The comparable rate constants of sulphate self-exchange in normal and CML erythrocytes suggest that there is probably no significant structural alteration of band 3 in the anion transport domain[42].

Ankyrin is the key protein involved in the interaction of the cytoskeletal network with band 3. Scatchard analysis of the binding of labelled ankyrin to vesicles prepared from normal and CML erythrocyte ghosts revealed decreased ankyrin-binding sites (9–15 $\mu g\ mg^{-1}$ membrane protein) in CML patients compared to normal volunteers (39–45 $\mu g\ mg^{-1}$ membrane protein). It is possible that this may lead to partial loss of anchorage of the cytoskeleton to the membrane[42]. The decreased proportion of spectrin tetramers and the decreased number of ankyrin-binding sites in CML erythrocytes compared to normal erythrocytes, may both contribute to an increased lateral mobility of band 3. The rearrangement of band 3 into clusters provides the recognition site for autologous antibodies against senescent cells. These bind to remove aged cells from the circulation[43]. It may, therefore, be speculated that clustering of band 3 in CML erythrocytes leads to their removal from the circulation, accounting in part for the anaemia associated with the disease. We have indeed found increased binding of autologous IgG to CML erythrocytes compared to normal erythrocytes.

The mechanisms underlying altered band 3 structure/function in CML erythrocytes await further investigation.

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