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Membrane-Microfilament Interactions in the Cells of B-Chronic Lymphocytic Leukemia

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In the present study we have investigated the association between cell surface molecules and the cytoskeleton in malignant B-chronic lymphocytic leukaemia (B-CLL) cells. The rationale was the observation that close interactions between surface receptors and cytoskeletal proteins are involved in the regulation of several major lymphocyte functions, including activation and recirculation [1]. B-CLL cells were selected for this study for three reasons. First, B-CLL monoclonal B cells have a well-characterized phenotype [2]: they express on the membrane the monoclonal-antibody (MoAb)-defined cluster differentiation (CD) 19, 20, 21, 24 and 5 structures. Second, they have abnormalities that suggest defects of cytoskeleton function, such as the inability to cap surface immunoglobulins (sIg) and other ligand receptors [3]. Finally, these cells have a peculiar organization of F-actin that is predominantly associated with dot-shaped close-contact adhesion sites which have recently been characterized and described as podosomes [4, 5]. On those bases, our experimental approach was devoted to answering the following questions: (a) Can the different CDs present on the B-CLL cell surface be capped? (b) If such a phenomenon occurs, does it modify the cytoskeleton organization? (c) Can the perturbation of membrane-cytoskeleton interactions lead to any functional change in B-CLL cells?

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Monoclonal B cells from 12 patients with typical B-CLL were studied utilizing RFT1, B4 (Coulter), RFB7, RFB6 and BA1 (Menarini) to characterize CD5, 19, 20, 21 and 24, respectively. The analysis of cytoskeleton structures was performed as detailed in [5]; F-actin-containing microfilamentous structures were identified by means of rhodamine-isothiocyanate-labelled phalloidin (R-PHD). Short-term cultures were set up at 37 °C in air containing 5% CO_2 with RPMI medium and 10% fetal calf serum. The results indicate that CD5 can be capped on the surface of B-CLL cells by treating the cells with anti-CD5. The capping phenomenon becomes evident after 2 h of incubation, is maximal after 24 h and is more prominent when CD5 is cross-linked with an anti-mouse Ig Ab. In contrast, the CD5 molecules on the surface of normal and B-CLL T-lymphocytes cannot be capped. CD21 (C3d receptor) is the only other CD which can be capped on the surface of B-CLL cells. CD5 and CD21 co-cap on the surface of B-CLL cells and co-modulate. Finally, the incubation of B-CLL cells with anti-CD5 and/or anti-CD21 either abolishes or largely inhibits the organization of intracellular F-actin into podosomes.

The above data indicate that the lateral movements of CD5 and CD21 on the surface of B-CLL cells interfere with the organization of F-actin in the cytoplasm, and point to the existence of a strict spatial relationship on the membrane between CD5 and CD21. The possible functional significance of CD21 has been investigated in four cases. The in vitro stimulation of B-CLL cells with anti-CD21 does not modify their morphol-

ogy and phenotype. The picture changes drastically when anti-CD21 Sepharose-linked MoAb is used. After 72 h of stimulation, B-CLL cells transform into large blast-like elements with nucleoli, become $CD5^-$, $CD10^+$ and lose sIg. These activated cells are not proliferating, as they are unable to incorporate bromodeoxyuridine ($BUDR^-$). However, a very active wave of proliferation can be obtained by culturing B-CLL cells preactivated with Sepharose-linked anti-CD21 in the presence of 10% B-Cell Growth Factor (BCGF; CPI). The vast majority of cells enter the S phase of the cell cycle ($BUDR^+$) and reach a peak (>20%) after 72 h of culture. The same BCGF-induced proliferative activity can be observed in B-CLL cells preactivated with Sepharose-linked CB04 MoAb which detects the receptor for the C3b fraction of complement [6]. These data indicate that B-CLL cells may be a valuable model for investigating the interactions between surface receptors, i.e. complement receptors, and cytoskeleton. Their abnormalities may help in understanding the differentiation and proliferative properties of malignant B-lymphocytes.

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