UNDERSTANDING BINDING PROPERTIES OF MONOCLONAL ANTI-CHOLESTEROL ANTIBODIES

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Monoclonal anti-cholesterol antibodies (ACHA, IgG clones AC1 and AC8 and IgM clone AC9), made in our laboratory, reacted with clustered cholesterol or structurally closely related sterols, but not with other lipids, assayed by ELISA. They bound to lipoproteins, but only IgG clones bound to locally clustered cholesterol (in lipid rafts and caveolas) in the cell membrane of various intact immunocytes (1,2). Based on these results we aimed to test the reactivity of our antibodies using large scale multiplex analysis, microarray. Cholesterol, cholesterol analogues and lipoproteins were printed onto nitrocellulose membrane and binding of AC1, AC8 and AC9 was visualized by fluorescent secondary antibody. Furthermore, sequences of variable regions of ACHA clones' heavy and light chain were determined to support interpretation of microarray data. We found that all ACHA clones, with different affinities, were capable of binding to cholesterol and lipoproteins printed onto membranes. They also showed different extent of cross-reactivity to DNA, reflecting that cholesterol and DNA share structurally common epitopes for anti-cholesterol antibodies. Sequence analysis showed that AC1, AC8 (IgG3) and AC9 (IgM) mAbs differ from each other and use gene segments in germ-line configuration for their antigen-binding portion. In the future, we plan to develop microarray-based high throughput raft-analytical method for the detection of total cellular or membrane cholesterol that might be both perspectivic in direction of rapid and reliable diagnostics and therapy of lipid raft-related diseases.

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