Detergent-resistant membrane isolation

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Method Article

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Abstract

Introduction

Here, for the first time we have shown that a single receptor, CD40, by virtue of its re-localization within cholesterol-rich detergent-resistant membrane microdomains \(DRMs), can assemble a signalosome able to induce pro-inflammatory mediators and immunity to L. major, while the same receptor excluded from DRMs, as occurs in cholesterol depleted or L. major infected cells, signals IL-10 production and enhanced intracellular growth and lesion progression in BALB/c mice. There is good evidence that these microdomains are platforms for signal transduction. A further example of membrane microdomains is the lateral segregation of glycosphingolipids and cholesterol into liquid-ordered domains. Phase separation of cholesterol-enriched, liquid-ordered domains or lipid rafts has been clearly demonstrated in model membranes and also in biological membranes, although the length and time scales on which this phase separation occurs are a matter of debate. These observations inspired the thought that the lipid raft domain in the membrane is the domain in the liquid ordered phase, and that a strong correlation exists between the molecules recovered in the DRM fraction and those partitioned into raft domains in the membrane. Here we have isolated different signalosomes associated with CD40 in the DRM and non-DRM microdomains regulating the differential cellular responses.

Procedure

1.Plate 20-25x10^6 macrophages in culture flask. 2.Wash the cells and change the media next day. 3.Infect the macrophages with L. major for 6 h. 4. Wash the cells with plain RPMI \(Gibco) to remove parasites that are not internalized. 5. Where required treat the cells either with β-MCD\(10Mm) for 45 min and/or with cholesterol for 1h in the serum free medium at 37 ^{0}C. 6. After treatment or 72 h of infection stimulate the cells where required and then lyse the cells in cold 726 µl of TNE-buffer \(25 mM Tris-HCl pH 7.5, 50 mM NaCl, 5 mM EGTA) containing 1% Triton-X100 supplemented with protease and phosphatase inhibitors for 30 min on ice. 7. Mix the lysate with 1452 µl of 70% Nycodenz \(Sigma), dissolved in TNE-buffer. 8. Load this mix in the bottom of 4 ml of polyallomar ultracentrifuge tube. 9. Overlay this bottom loaded mix with 25, 21.5, 18, 15, and 8% Nycodenz, prepared in TNE-buffer. 10. Spin the tube at 200,000 × g for 4 h at 40C using SW 60 Ti rotor \(Beckman Coulter). 11. After centrifugation collect eleven fractions \(Fr.) each of 364 µl from top to bottom of the tube. 12. Analyse the fraction by SDS-PAGE and immunoblot. 13. Probe the blot with caveolin-1 antibody to confirm cav-1 positive \(Fr.2-Fr.5) or detergent resistant membrane fractions \(DRMs). 14. Probe the blot with anti-β-coat protein or anti-CD71 to confirm soluble \(Fr.7-Fr.11) fractions.