mechanisms of natural anti-oxLDL IgM monoclonal antibody on pathogenesis of atherosclerosis underlying the action of bacterial lipopolysaccharide (LPS) activation in the binding of oxLDL to macrophages.

Methods The Murine macrophage cell line RAW-264.7 was cultured in vitro. We prepared a strain of monoclonal antibody 3A6 specific for oxLDL with IgM isotype and purified it from ascites. Then, we utilised it to form complex with $Na^{125}I$-conjugated oxLDL. Influence of 3A6 on formation of foam cells was observed by Oil Red O staining and the affinity of $Na^{125}I$-conjugated oxLDL. After LPS stimulation on macrophages, anti-TLR4 neutralising mAb, p38MAPK specific inhibitor SB203580, NF-kB specific inhibitor PDTC or RNAi targeting Fcα/μ receptor were applied, respectively. The mRNA transcription and protein expression of Fcα/μ receptor in macrophages were studied by real-time RT-PCR and flow cytometry. Similarly, influence of 3A6 on formation of foam cells was observed by Oil Red O staining and the affinity of $Na^{125}I$-conjugated oxLDL.

Results Natural anti-oxLDL IgM monoclonal antibody 3A6 specifically inhibited the binding of CuoxLDL to naïve macrophages in vitro. 3A6 failed to inhibit the binding of CuoxLDL to LPS-activated macrophages and promoted the formation of CuoxLDL-mediated foam macrophages. Furthermore, 3A6 F $(ab')_2$ or pre-incubation with un-related IgM inhibited the binding of 3A6/CuoxLDL complex to LPS-activated macrophages, suggesting that the Fcα/μ receptor may be responsible for the binding of 3A6/CuoxLDL complex to LPS-activated macrophages. Indeed, LPS up-regulated the expression of Fcα/μ receptor in macrophages in a dose- and time-dependent manner, which was diminished by treatment with anti-TLR4 neutralising mAb. In addition, LPS induced the phosphorylation of p38MAPK and translocation of NF-kB p65, contributing to the up-regulated expression of Fcα/μ receptor in macrophages as treatment with specific inhibitor for p38MAPK (SB203580) or NF-kB (PDTC) attenuated the up-regulation of Fca/m receptor expression induced by LPS in macrophages.

Conclusions Natural anti-oxLDL IgM monoclonal antibody 3A6 specifically inhibited the binding of CuoxLDL to naïve macrophages and foam cell formation in vitro. LPS, through the TLR4 receptor, activated the p38MAPK and NF-kB pathways and up-regulated the expression of Fcα/μ receptor in macrophages, which promoted the binding of 3A6/CuoxLDL complex to macrophages through binding with Fc fragments and the formation of foam macrophages. Our findings provide a new explanation why increased LPS concentration deteriorates the pathogenesis of atherosclerosis.

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NATURAL IGM SPECIFIC FOR OXLDL PROTECTS MURINE MACROPHAGES FROM FOAM CELLS FORMATION BUT LIPOPOLYSACCHARIDE (LPS) DISRUPTS THE COURSE BY UP-REGULATING THE EXPRESSION OF Fcα/μ RECEPTOR

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Objective To explore the role and the exact molecular mechanisms of natural anti-oxLDL IgM monoclonal antibody played and involved in pathogenesis of atherosclerosis underlying the action of IgM antibody in the binding of oxLDL to macrophages. To explore the role and the exact molecular

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