

Microbubbles coated with anti-P-selectin antibody RB40.34

MB-RB40.34

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Chemical name:	Microbubbles coated with anti-P-selectin antibody RB40.34	
Abbreviated name:	MB-RB40.34, MBp	
Synonym:		
Agent category:	Antibody	
Target:	P-selectin	
Target category:	Antigen	
Method of detection:	Ultrasound	
Source of signal:	Microbubbles	
Activation:	No	
Studies:	<ul style="list-style-type: none">• <i>In vitro</i>• Rodents	Click on protein , nucleotide (RefSeq), and gene for more information about P-selectin.

Background

[PubMed]

Ultrasound is the most widely used imaging modality (1) and its role in noninvasive molecular imaging with ligand-carrying microbubbles is expanding (2). Microbubbles are spherical cavities filled by a gas encapsulated in a shell. The shells are made of phospholipids, surfactant, denatured human serum albumin, or synthetic polymer. Ligands and antibodies can be incorporated into the shell surface of microbubbles. Microbubbles are usually 2 to 8 μm in size. They provide a strongly reflective interface and

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resonate to ultrasound waves. They are used as ultrasound contrast agents in imaging of inflammation, angiogenesis, intravascular thrombus, and tumors (3-5). They may also potentially be used for drug and gene delivery (6).

Endothelial cells are important cells in inflammatory responses (7, 8). Bacterial lipopolysaccharide, virus, inflammation, and tissue injury increase tumor necrosis factor α (TNF α), interleukin-1 (IL-1), and secretion of other cytokines and chemokines. Leukocyte emigration from blood is dependent on rolling of leukocytes along endothelial cell surfaces and subsequent adherence to endothelial cell surfaces. Inflammatory mediators and cytokines induce chemokine secretion from endothelial cells and other vascular cells and increase their expression of cell surface adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), integrins, and selectins. Chemokines are chemotactic to leukocytes to sites of inflammation and tissue injury. The movement of leukocytes through endothelial junctions into the extravascular space is highly orchestrated through various interactions with different adhesion molecules on endothelial cells (9).

P-selectin is found on the cell surface of endothelial cells and platelets (8, 10). It binds to glycoprotein on the cell-surface of leukocytes. IL-1 and TNF α , released from inflammatory and ischemic stimuli, stimulate P-selectin and other adhesion molecule expression on the vascular endothelial cells leading to leukocyte adhesion to the activated endothelium. P-selectin and other selectins are involved in rolling and arresting leukocytes on the endothelium. Microbubbles targeted with antibodies against P-selectin are being developed as noninvasive agents for P-selectin expression in vascular endothelial cells of inflamed and damaged tissues (11).

Related Resource Links:

- Chapters in MICAD ([P-selectin](#))
- Gene information in NCBI ([P-selectin](#)).
- Articles in Online Mendelian Inheritance in Man (OMIM) ([P-selectin](#))

Synthesis

[PubMed]

For targeted microbubbles, biotinylated microbubbles were first prepared by sonication of an aqueous dispersion of decafluorobutane gas, distearoylphosphatidylcholine, polyethyleneglycol (PEG)- stearate, and distearoylphosphatidylethanolamine-PEG-biotin (11). Microbubbles were combined with streptavidin, washed, and conjugated with biotinylated monoclonal antibody (RB40.34) against P-selectin (MB_P) or isotype-control monoclonal antibody (R3-34) against α_v integrin (MB_{ISO}). Control lipid microbubbles (MB_C) were also prepared. The microbubbles are about $3.5 \pm 1.4 \mu\text{m}$ in diameter. An antibody to microbubble ratio was estimated to be $102 \pm 3 \times 10^3$ by flow cytometry.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Takalkar et al. (12) reported that MB_p (5×10^6 particles/ml) perfused through the flow chamber coated with P-selectin ($109 \text{ sites}/\mu\text{m}^2$) at a shear stress of $0.3 \text{ dyn}/\text{cm}^2$ accumulated at a rate of $565 \text{ mm}^{-2} \text{ min}^{-1}$. Attachment rates increased at higher plate surface densities of P-selectin and MB_p detachment was reduced. Accumulation rate first increased with shear, reached a maximum stress force at $\sim 0.6 \text{ dyn}/\text{cm}^2$, and then decreased. Minimal microbubble attachment was observed on a plate that lacked P-selectin or was blocked with RB40.34. Microbubble detachment was tested by stepping up shear stress at 30-s intervals. Half-maximal detachment was reached at $34 \text{ dyn}/\text{cm}^2$. Therefore, accumulation and retention of MB_p is possible under physiologic flow conditions and is strongly influenced by shear stress and surface density of the target receptor. Furthermore, Rychak et al. (13) demonstrated that deformed MB_p exhibited significantly greater attachment efficiency than spherical MB_p at stress forces of $0.4\text{--}1.35 \text{ dyn}/\text{cm}^2$.

Animal Studies

Rodents

[PubMed]

Lindner et al. (11) performed ultrasound assessment of inflammation with MB_p by intravital microscopy of the cremasteric venules of control ($n = 5$) and TNF α -stimulated wild-type mice ($n = 5$). Retention of MB_p, MB_{iso}, and MB_c increased ($P < 0.05$) with TNF α treatment because of increased attachment to activated leukocytes. Extensive attachment of MB_p directly to the endothelium was observed in TNF α -stimulated mice, resulting in retention of MB_p (16 microbubbles/measurement) >3 -fold ($P < 0.01$) greater than either MB_{iso} (4 microbubbles/measurement) or MB_c (2 microbubbles/measurement). Enhanced retention of MB_p was completely abolished in TNF α -stimulated P-selectin-deficient mice. Renal ultrasound imaging of the kidneys of wild-type mice ($n = 5$) undergoing ischemia-reperfusion injury showed that video intensity signal was significantly higher ($P < 0.05$) for MB_p ($12 \pm 2 \text{ U}$) than either MB_{iso} ($6 \pm 3 \text{ U}$) or MB_c ($5 \pm 3 \text{ U}$). In P-selectin-deficient mice ($n = 3$), the signal for MB_p was equivalent to that from control microbubbles.

Rychak et al. (13) demonstrated that deformed MB_p exhibited significantly greater attachment than spherical MB_p in the cremasteric venules of TNF α -treated wild-type mice ($P < 0.05$, $n = 4$). There was no difference in adhesion of deformed MB_p and spherical MB_p in the cremasteric venules of TNF α -treated P-selectin-deficient mice ($n = 3$); rather, the adhesion measurements were 6- and 2-fold lower, respectively.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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