

β1 INTEGRIN MEDIATES PRESSURE-STIMULATED PHAGOCYTOSIS

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Background: Changes in extracellular pressure that occur in infection, inflammation, or positive pressure ventilation may influence monocyte and macrophage phagocytosis. Phagocytosis requires engagement of transmembrane integrin receptors, but whether pressure-derived signals influence phagocytosis by affecting integrins is unknown.

Methods: We examined the role of β1-integrin heterodimers in pressure-induced phagocytosis. We assayed phagocytosis by adding opsonized fluorescent latex beads to cells at ambient or 20mmHg increased pressure for 2 hours. After washing away extracellular beads, we counted intracellular beads. Integrin phosphorylation was assessed by Western blot before phagocytosis.

Results: Pressure did not alter phagocytosis in β1-integrin-null GD25 fibroblasts, but stimulated phagocytosis in GD25 fibroblasts expressing wild type β1-integrin (18.67 ± 1.97 vs. $31.66 \pm 3.54\%$, $n=6$, $p<0.01$). In PMA-differentiated THP-1 macrophages, pressure stimulated β1-integrin T788 phosphorylation by $30.5 \pm 7.8\%$ ($n=6$, $p<0.05$), but did not alter S785 phosphorylation. We therefore further compared the effects of pressure in GD25 cells expressing wild type β1-integrin or β1-integrin with point mutations at three potentially important phosphorylation sites. Pressure stimulated phagocytosis in cells expressing an inactivating S785A point mutation or a T788D substitution that should mimic a constitutively phosphorylated threonine (26.15 ± 3.30 vs. $39.66 \pm 2.01\%$, $n=6$, $p<0.01$, and 19.44 ± 0.22 vs. $34.80 \pm 2.98\%$, $n=3$, $p<0.01$ respectively). In contrast, pressure did not alter phagocytosis in cells expressing a TT788/9AA mutation, in which both T788 and T789 phosphorylation sites were rendered inactive.

Conclusions: These results suggest that pressure stimulates phagocytosis via increasing β1-integrin T788 phosphorylation. These results also suggest that the effects of pressure on phagocytosis are not limited to macrophages but may be generalized to other phagocytic cells. Interventions that target β1-integrin threonine 788 phosphorylation may modulate phagocytic function.