# MACROPHAGE DEPLETION REDUCES ANGIOGENESIS IN THE EX OVO CHICK CHORIOALLANTOIC MEMBRANE ASSAY

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Introduction: Macrophages play a role in various biological processes including angiogenesis. Models such as the chick chorioallantoic membrane (CAM) assay are frequently used to study blood vessel development, but few data are available on the role of chicken embryonic macrophages in angiogenesis. We therefore aimed to investigate the angiogenic role of these macrophages by means of their depletion in the CAM model. Methods: After 2.5 days of incubation, chicken eggs were opened into a weigh boat and the embryos were further incubated at 37.8°C and 80% humidity. To deplete macrophages, 25µl of clodronate liposomes (CL) were injected intravenously in the CAM after 8.5 days of total incubation. PBS liposomes were used as control. Macrophage depletion up to 3 days after CL treatment was assessed with immunohistochemistry and flow cytometry. The chicken macrophage specific antibody KUL01 was used for staining. To monitor angiogenesis, both plain collagen type I plugs and plugs containing angiogenic cells were placed on the CAM immediately after injection of liposomes. Plugs were placed on a mesh to allow quantification of angiogenesis. After 3 additional days of incubation, images of the plugs were taken using a stereomicroscope. Angiogenesis was quantified as the percentage of squares in the mesh which contained blood vessels. Results and conclusion: A significant 3.4-fold reduction of the macrophage population was observed 3 days after injection of CL compared to injection with PBS liposomes. Furthermore, the vascular ingrowth in both plain and angiogenic plugs was significantly lower in macrophage-depleted embryos compared to control embryos. These results suggest that endogenous chicken macrophages have a direct role in the vascularization of collagen plugs.