Investigating the Contribution of Neutrophils to Alum Adjuvant Function *in vivo*

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The aluminium salts (alum) have been widely applied as adjuvants in a variety of human and veterinary vaccines for over 70 years. Despite this, their use in vaccines has been largely empirical and no consensus exists on the mechanisms by which they potentiate the immune response. Recently the role of the innate immune system in shaping the development of adaptive (T and B cell) immune responses has been highlighted for microbial stimuli during infection or as adjuvants. However less is known about how non-microbial agents such as alum perform this role. The potential role of neutrophils in the mechanism of action of alum adjuvant is highlighted by their very early appearance at the injection site. Neutrophils have a well-recognised role in inducing inflammation, furthermore recent studies suggest they play an immunoregulatory role, by aiding the recruitment of other immune cells through the secretion of cytokines, chemokines and other mediators. To directly investigate the role played by neutrophils in the alum-induced response to the model antigen Ovalbumin (OVA), we employed an *in vivo* depletion strategy using a specific monoclonal (NIMP-R14). We then read out the resulting T cell response using mice expressing transgenic, OVA-specific T cell receptors and a Green Fluorescent Protein (GFP) reporter of IL-4 production. Our results revealed that neutrophil depletion not only decreased the number of OVA-specific T cells found in the draining lymph nodes but also reduced the number of IL-4 producing OVA-specific T cells, suggesting a diminished Th2 response. Neutrophil depletion did not appear to significantly reduce the activation or division of the OVA-specific T cells present in the draining lymph nodes. Analysis of the subcutaneous injection site demonstrated a transient absence of cellular infiltration in depleted animals at day one, returning by day five or later. We conclude that neutrophils play an essential role in potentiating and shaping the adaptive immune response induced by alum adjuvant *in vivo*.