VACCINES

Explaining alum: immunologists' dirty little secret

Aluminum hydroxide (alum) is a widely used adjuvant in human vaccines, but its mechanism of action remains ill-defined. In a new study, Lambrecht and colleagues find that a key factor in the adjuvant activity of alum is the activation of a population of inflammatory dendritic cells (DCs) through the induction of uric acid production, which leads to enhanced humoral and cell-mediated immunity.

Lambrecht and colleagues showed that immunization of DO11.10 mice — which have $CD4^+$ T cells specific for an ovalbumin (OVA)-derived peptide — with OVA alone led to transient T-cell proliferation followed by their deletion. By contrast, injection with alum-emulsified OVA (OVA–alum) resulted in an enhanced, persistent and recirculating T-helper-2-cell response. So, alum clearly enhances T-cell responses to an immunized antigen, but what triggers this effect? Emulsification of OVA in alum resulted in significantly increased recruitment of innate immune cells to the peritoneal cavity as early as 6 hours after intraperitoneal injection. $CD11c^+$ myeloid DCs isolated from the peritoneal cavity of OVA–alum-immunized mice were more efficient at antigen uptake and processing, and showed greater signs of functional maturation, compared with DCs from mice injected with OVA alone. In line with these observations, DCs isolated from OVA–alum-immunized mice were more potent stimulators of T-cell proliferation than control DCs.

Inflammatory monocytes, identified on the basis of a $CD11b^+Ly6C^+Ly6G^-F4/80^{mid}$ phenotype, were also found to take up significantly more antigen in the presence of alum and to traffic to mediastinal lymph nodes, the primary draining site of the peritoneum, within 24 hours of immunization. When isolated from mediastinal lymph nodes, $Ly6C^+$ inflammatory monocytes were even more potent T-cell stimulators than myeloid DCs from the same site and, most importantly, acquisition of CD11c expression by $Ly6C^+$ monocytes indicated their conversion to a DC phenotype. Depletion of recruited inflammatory monocytes and DCs from the peritoneum abolished both T-cell proliferation and IgG1 production following OVA–alum immunization, indicating that these antigen-presenting cells (APCs) were essential for alum to modulate the adaptive immune response.

Exposure to alum *in vitro* has no effect on the activation state of APCs, so by what mechanism does alum mediate its adjuvant activity *in vivo*? Examination of the peritoneal lavage fluid of immunized mice revealed that alum strongly induced the production of uric acid, an endogenous danger signal. Uricase, an enzyme that rapidly breaks down uric acid, abolished the recruitment of inflammatory monocytes and antigen-specific T-cell proliferation in OVA–alum-immunized mice, indicating that uric acid is crucial for alum's mechanism of action. Furthermore, mice lacking the adaptor molecule MyD88 (and therefore deficient in interleukin-1-receptor-dependent responses to uric acid) had severely impaired recruitment of inflammatory monocytes to the mediastinal lymph nodes compared with wild-type mice.

Unlike many mouse models, human vaccines are normally administered subcutaneously or intramuscularly. Importantly, the authors report that intramuscular administration of OVA–alum had similar effects to intraperitoneal immunization in mice. Therefore, these findings should further our understanding of how alum-containing vaccines trigger effective adaptive immune responses in humans.

Sarah Allan

ORIGINAL RESEARCH PAPER Kool, M. *et al.* Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J. Exp. Med.* 24 March 2008 (doi:10.1084/jem.20071087)
FURTHER READING Kono, H. & Rock, K. L. How dying cells alert the immune system to danger. *Nature Rev. Immunol.* **8**, 279–289 (2008)