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Eosinophils and Lung Mucosal Antibody Production
Is Location the Key?

Since their discovery in 1874 (1), eosinophils have presented an enigma to immunologists. What is their role in the immune response? Much work has been done to investigate their function, focusing on immune responses where there is an increase in eosinophils. These include Th2 conditions resulting from parasitic infections, first described for nematodes (2) and later for a number of other infections, and under conditions of allergic responses (3–6). Eosinophils tend to be increased under the influence of IL-5 produced by Th2 cells but also by cells such as type 2 innate lymphoid cells and mast cells. Studies exploring eosinophils have provided evidence for their role in clearance of infection but also in contributing to infection, depending on the agent. In addition, a pronounced presence of eosinophils has been reported in allergic responses, particularly at mucous surfaces, such as in allergic airway inflammation, eosinophilic esophagitis, and eosinophilic gastritis.

More recently, attention has focused on a potential role for eosinophils in regulating the antibody response. This was first observed in alum-adjuvanted immunization in mice (7, 8), alum being one of the most common adjuvants approved for use in humans. Alum administration led to an increase in an IL-4–positive population that was subsequently identified as eosinophils, whose numbers increased in bone marrow (and spleen). Furthermore, the absence of these cells impaired the early development of the IgM response (8), and eosinophils have been shown to regulate the number of B cells (9).

Of note, a role for eosinophils in the survival of plasma cells in the bone marrow has been previously suggested, at steady state and after immunization (10). In these experiments, alum-adjuvanted protein vaccination in mice lacking eosinophils led to reduced IgM and also to reduced IgA levels (8, 11). This happened despite normal formation of germinal centers, and despite no apparent effect on affinity maturation of the antibody response. However, these findings have not been observed by others and remain somewhat controversial (12). Furthermore, most studies seeking to understand a role for eosinophils in the antibody response have largely examined systemic immunization, with less information on mucosal immunization.

In this issue of the *Journal* (pp. 186–200), Prince and colleagues describe their use of silver nanoparticle (AgNP)–adjuvanted immunization, delivered intratracheally, to explore the role of eosinophils in lung and systemic antibody production (13). Although the use of AgNPs as an adjuvant is less common than the use of alum in experimental studies and in humans, it is increasingly being explored as a potent adjuvant in inducing robust immune responses after intraperitoneal immunization. Indeed, AgNP-adjuvanted immunization has been shown to induce protection against influenza infection in mouse models (14). When delivered intratracheally, AgNPs have been shown to be picked up by macrophages (15) and to induce Th2 responses at seemingly lower concentrations than used by the current authors (16), although this response may be dose-dependent (17). It is, therefore, of interest that intratracheal delivery of AgNP-protein antigen (Keyhole Limpet Hemocyanin [KLH], in this case), induced strong IgM and IgG antibodies in BAL, as well as in serum (Figure 1A). The authors also looked at antibody-secreting cells (ASCs) and found increases in the number of ASCs secreting IgM, IgG, and IgA in the lung-draining lymph node (Figure 1A). The noteworthy advance of this work is that, in the absence of eosinophils (examined using the ΔGATA mice on the C57Bl/6 background), AgNP-protein antigen immunization through the lung led to reduced IgM as well as IgG in BAL and in serum (Figure 1A). Numbers of IgM- and IgG-secreting ASCs were partially rescued by the delivery of eosinophils isolated from the BAL of vaccinated wild-type mice into eosinophil-deficient mice, even though no effect was observed on antibody titers (Figure 1B). Furthermore, *in vitro* experiments demonstrated that eosinophils were able to produce IL-6 and could modulate the secretion of antibodies by lymph node cells from vaccinated eosinophil-deficient mice (Figure 1C).

Surprisingly, there was no effect of the absence of eosinophils on numbers of IgA-secreting ASCs at 10 days postimmunization. However, after intratracheal prime immunization and boosting, there was a reduction in IgA production at day 13 in the absence of eosinophils. However, there was also a surprising rebound at day 27 postimmunization, and higher numbers of IgA-secreting ASCs were observed in the mice lacking eosinophils (Figure 1A). Together, these findings suggest that class switching is not dependent on eosinophils but that, early in the response, IgM- and IgG-secreting ASCs require support from eosinophils. However, IgA-secreting ASCs have a more complex relationship with eosinophils.

Of interest is the observation that intratracheally delivered AgNP-adjuvanted antigen led to an increase in $CD4^+$ T cells to the lung, and this was significantly increased in the absence of eosinophils. This finding suggests a role for eosinophils in modulating the recruitment of $CD4^+$ T cells to the lung after immunization. Indeed, a role for eosinophils in recruiting $CD4^+$ T cells to the lung in response to alum-adjuvanted protein delivered intraperitoneally and protein delivery to the lung has previously been reported (18, 19). The authors' finding that $CD4^+$ T cell recruitment to the lung may be negatively regulated by eosinophils goes in the opposite direction to what was previously reported. It is likely that the nature of the

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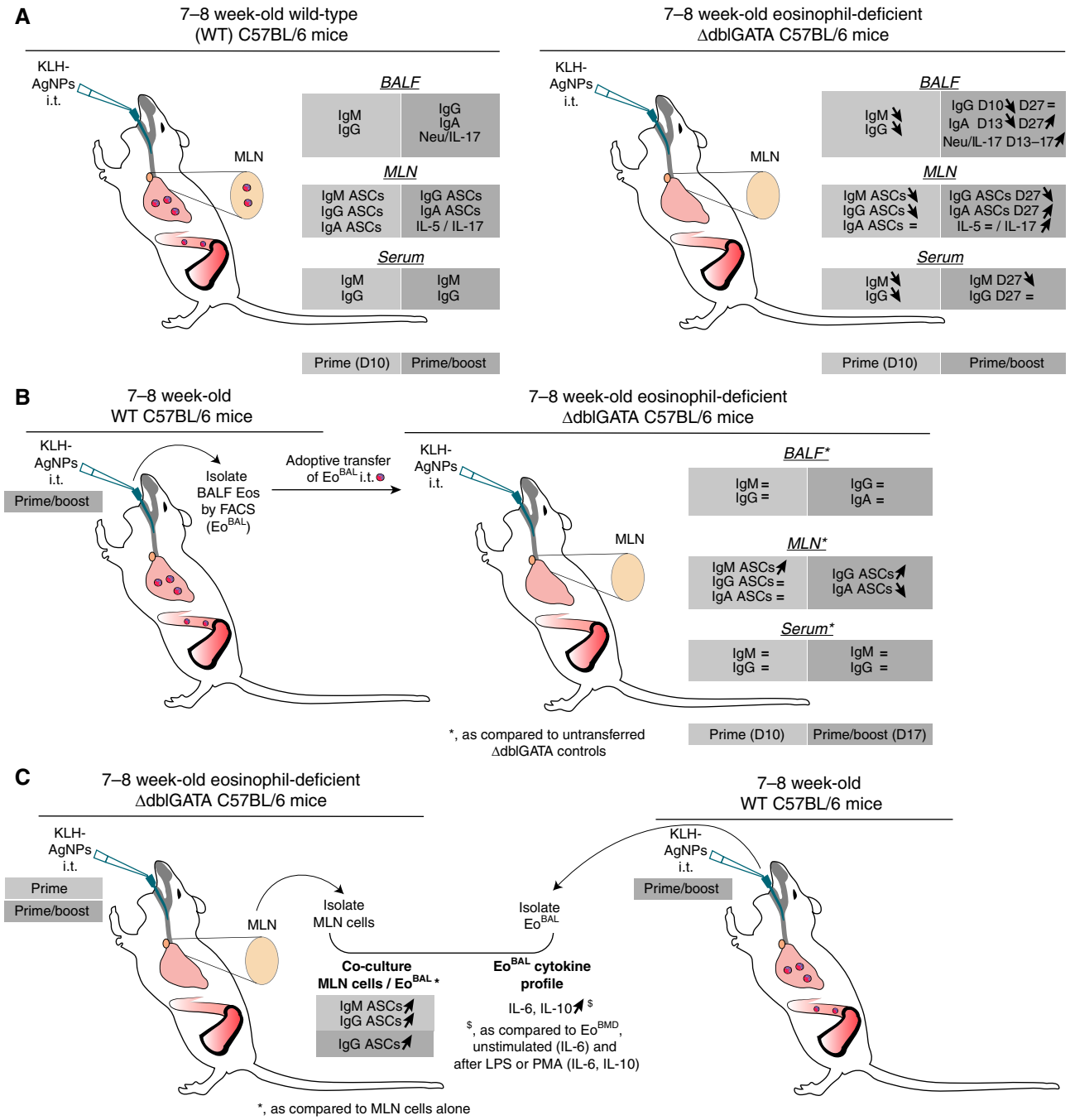

Figure 1. Eosinophils can regulate antibody responses during pulmonary vaccination with KLH and silver nanoparticles (AgNPs). IgM, IgG, and IgA represent KLH-specific antibody titers; IgM, IgG, and IgA antibody-secreting cells (ASCs) are ASCs secreting KLH-specific IgM, IgG, and IgA, respectively. (*A*) Seven- to 8-week-old wild-type (WT) and eosinophil-deficient ΔDBLGATA C57BL/6 mice were injected intratracheally (i.t.) with KLH combined with AgNPs as an adjuvant. BAL fluid (BALF), mediastinal lymph node (MLN), and serum readouts were assessed 4, 7, and 10 days after priming, as well as 13, 17, 22, and 27 days after priming (day 0) and boost (day 10). (*B*) Eosinophils isolated from the BAL (Eo^{BAL}) of KLH/AgNP-vaccinated WT mice (prime+boost) were adoptively transferred into eosinophil-deficient ΔDBLGATA C57BL/6 mice to assess the extent to which Eo^{BAL} can modulate the immune responses to KLH/AgNPs. (*C*) *In vitro* (co-)culture experiments were performed to evaluate the cytokine profile of Eo^{BAL} and its ability to modulate the secretion of antibodies by MLN cells from vaccinated eosinophil-deficient ΔDBLGATA C57BL/6 mice. BMD = bone marrow–derived; Eo = eosinophil; KLH = keyhole limpet hemocyanin; LPS = lipopolysaccharide; PMA = phorbol 12-myristate 13-acetate.

inflammation induced by the AgNPs controls how eosinophils are engaged, so this may be contextual. Indeed, it would be of interest to determine whether eosinophils interact with these AgNPs to produce cytokines and chemokines that subsequently modulate the recruitment of various cells to the lung.

Prince and colleagues also report that on prime and boosting, an apparent Th17-mediated pathway kicks in to be able to support IgA production in the absence of eosinophils, and although this was less well explored, elevated IL-17A/F and high neutrophil numbers were observed in BAL in the absence of eosinophils. Hence, eosinophils may suppress IL-17A/F responses, and in their absence, these IL-17A/F responses can be unleashed.

Given these interesting findings, it would be of some interest to further explore whether and which type of lung eosinophils (20) affect affinity maturation of the resulting antibody, given the proposed supporting role of eosinophils for ASCs. In addition, AgNPs have been suggested to induce some lung injury (21) and, at least when delivered to the gut, may also affect the local microbiota as well (22). Could this also play a role in the ability of this adjuvant to act by means of eosinophils in generating robust lung mucosal antibody responses? ■

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