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From 'perfect mix' to '*potion magique*' — regulatory T cells and anti-inflammatory cytokines as adjuvant targets

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The efficacy of vaccines can be greatly improved by the addition of adjuvants, which enhance and modify immune responses. Historically, adjuvants have been discovered empirically by using experimental models. Unfortunately, many adjuvants are associated with side effects that make them unsuitable for use in humans. At present, few adjuvants are available, and of these the adjuvant that is most commonly used, alum, induces immune responses that are suboptimal in several respects. There is an obvious need for the development of improved adjuvants that can induce cell-mediated responses in addition to antibodies.

Recent advances in basic immunology have revealed the importance of innate pathogen-recognition receptors in shaping the adaptive immune response. The Review by Bruno Guy, in the July issue of *Nature Reviews Microbiology*[1], eloquently discusses how Toll-like receptor (TLR) and non-TLR innate-receptor signalling by antigen-presenting cells (APCs) can be used to enhance the immunogenicity of vaccines. Although the activation of APCs by innate receptors represents a promising approach to adjuvant development, we wish to draw attention to an alternative strategy, which could be used in conjunction with TLR agonists to optimize adjuvant activity. Specifically, we propose that selective interference with the activity of regulatory T (T_{Reg}) cells and suppressive cytokines could be used to boost responses to poorly immunogenic vaccines.

Naturally occurring $CD4^+CD25^+$ T_{Reg} cells are crucial for the induction and maintenance of self-tolerance and are present in peripheral tissues, such as the skin and gut, under normal, non-inflamed conditions[2]. T_{Reg} cells not only suppress self-antigen-specific immune responses, but also negatively regulate immune responses against foreign antigens, including those driven by TLR-stimulated APCs[3–6]. T_{Reg} cells can inhibit initial T-cell activation and downregulate ongoing immune responses, which suggests that they act both at the site of initiation of immune responses (secondary lymphoid organs) and at sites of inflammation; part of the regulatory activity of T_{Reg} cells is related to their ability to modify APCs such as dendritic cells (DCs)[3–6]. Notably, T_{Reg} cells themselves have been reported to express several TLRs, including TLR4, TLR5, TLR7 and TLR8, and stimulation by TLR ligands can enhance the suppressive functions of T_{Reg} cells[7–9]. These results suggest that although TLR stimuli can activate APCs, such signals might also enhance the suppressive functions of T_{Reg} cells. Although this mechanism is probably important in controlling inflammatory responses in the context of infection, such concomitant suppression might limit the generation of effective immune responses after administration of poorly immunogenic vaccines.

The idea that limiting T_{Reg} cell activity at the time of vaccination is an effective way of enhancing immune responses has been confirmed experimentally. Evidence for this has typically come from studies in which T_{Reg} cells are depleted using anti-CD25 monoclonal antibodies. Animals that are depleted of T_{Reg} cells show markedly superior primary and memory $CD8^+$ T-cell responses to both virus infection and vaccines[10,11]. Although such experiments provide a proof of principle, it is unlikely that this approach of T_{Reg} cell depletion would be of clinical use, as it has been associated with adverse consequences such as localized autoimmune disease[12].

Conversely, the transient inhibition of T_{Reg} cell function might be ideal for enhancing the immune response to vaccines. One strategy would be to block the trafficking of T_{Reg} cells to the site of vaccination. T_{Reg} cells express the chemokine receptors CCR4 and CCR8 and migrate in response to the chemokines CCL17 and CCL22. There is evidence to suggest that the secretion of these chemokines by DCs can attract T_{Reg} cells to the site of inflammation. Further, blocking CCL22 by monoclonal antibodies *in vivo* has been shown to significantly reduce T_{Reg} cell trafficking[13]. Similarly, the blockade of vascular endothelial growth factor has also been shown to reduce the migration of T_{Reg} cells[14]. Consequently, the inhibition of T_{Reg} cell migration at the time of vaccination — by blocking chemokines or growth factors through the use of antibodies or small-molecule antagonists — would alleviate the suppressive effects of T_{Reg} cells during the initiation of the immune response and allow for a greater response to the vaccine[15].

Cytokines are key regulators of the immune system that shape innate and adaptive immune responses. Because cytokines might have an adjuvant-like effect, researchers have attempted to use them to manipulate the immune response to vaccination[16]. Notably, the maturation-associated signalling of DCs (including that induced by TLR ligands) also leads to the production of anti-inflammatory cytokines such as interleukin-10 (IL-10). Importantly, suppression by IL-10 can have a significant effect on the outcome of the adaptive immune response to vaccination. Fms-like tyrosine-kinase-3-based immunoprophylaxis against infection has been shown to be improved by adjuvant treatment with anti-IL-10 antibody[17]. In this study, a single injection of anti-IL-10 antibody was associated with an increase in early IL-12 and interferon-γ production from innate and adaptive immune cells. This experimental model suggests that neutralizing monoclonal antibodies to IL-10 also have a potential application in vaccinology.

Together, these findings suggest that the use of combination adjuvants, which aim to both activate innate immune mechanisms and interfere with suppressive mechanisms, will achieve effective APC activation and generate robust responses to vaccines.

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1. Guy, B. The perfect mix: recent progress in adjuvant research. *Nature Rev. Microbiol.* **5**, 505–517 (2007).
2. Miyara, M. & Sakaguchi, S. Natural regulatory T cells: mechanisms of suppression. *Trends Mol. Med.* **13**, 108–116 (2007).
3. Oldenhove, G. *et al.* $CD4^+CD25^+$ regulatory T cells control T helper cell type 1 responses to foreign antigens induced by mature dendritic cells *in vivo*. *J. Exp. Med.* **198**, 259–266 (2003).

4. Tang, Q. *et al.* Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nature Immunol.* **7**, 83–92 (2006).
5. Houot, R., Perrot, I., Garcia, E., Durand, I. & Lebecque, S. Human $CD4^+CD25^{high}$ regulatory T cells modulate myeloid but not plasmacytoid dendritic cells activation. *J. Immunol.* **176**, 5293–5298 (2006).
6. Bayry, J., Triebel, F., Kaveri, S. V. & Tough, D. F. Human dendritic cells acquire a semimature phenotype and lymph node homing potential through interaction with $CD4^+CD25^+$ regulatory T cells. *J. Immunol.* **178**, 4184–4193 (2007).
7. Caramalho, I. *et al.* Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J. Exp. Med.* **197**, 403–411 (2003).
8. Crellin, N. K. *et al.* Human $CD4^+$ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in $CD4^+CD25^+$ T regulatory cells. *J. Immunol.* **175**, 8051–8059 (2005).
9. Lewkowicz, P., Lewkowicz, N., Sasiak, A. & Tchorzewski, H. Lipopolysaccharide-activated $CD4^+CD25^+$ T regulatory cells inhibit neutrophil function and promote their apoptosis and death. *J. Immunol.* **177**, 7155–7163 (2006).
10. Suvas, S., Kumaraguru, U., Pack, C. D., Lee, S. & Rouse, B. T. $CD4^+CD25^+$ T cells regulate virus-specific primary and memory $CD8^+$ T cell responses. *J. Exp. Med.* **198**, 889–901 (2003).
11. Toka, F. N., Suvas, S. & Rouse, B. T. $CD4^+$ $CD25^+$ T cells regulate vaccine-generated primary and memory $CD8^+$ T-cell responses against herpes simplex virus type 1. *J. Virol.* **78**, 13082–13089 (2004).
12. Sutmuller, R. P. *et al.* Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of $CD25^+$ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J. Exp. Med.* **194**, 823–832 (2001).
13. Curiel, T. J. *et al.* Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nature Med.* **10**, 942–949 (2004).
14. Li, B. *et al.* Vascular endothelial growth factor blockade reduces intratumoral regulatory T cells and enhances the efficacy of a GM-CSF-secreting cancer immunotherapy. *Clin. Cancer Res.* **12**, 6808–6816 (2006).
15. Johnson, Z., Schwarz, M., Power, C. A., Wells, T. N. & Proudfoot, A. E. Multi-faceted strategies to combat disease by interference with the chemokine system. *Trends Immunol.* **26**, 268–274 (2005).
16. Salerno-Goncalves, R. & Sztein, M. B. Cell-mediated immunity and the challenges for vaccine development. *Trends Microbiol.* **14**, 536–542 (2006).
17. Das, L., DeVecchio, J. & Heinzel, F. P. Fms-like tyrosine kinase 3-based immunoprophylaxis against infection is improved by adjuvant treatment with anti-interleukin-10 antibody. *J. Infect. Dis.* **192**, 693–702 (2005).