VACCINES

Learning from our successes

The yellow-fever vaccine — YF-17D — is one of the oldest, most effective vaccines in use. But the secrets of its success have remained a mystery until now. Reporting in *The Journal of Experimental Medicine*, Troy Querec, Bali Pulendran and colleagues show that YF-17D — the main component of which is live attenuated virus — functions by activating dendritic cells (DCs) through multiple Toll-like receptors (TLRs), resulting in a diverse adaptive immune response.

The authors initially set out to examine how YF-17D initiates an adaptive immune response. They showed that YF-17D activates several subsets of human and mouse DCs *in vitro*. In response to YF-17D, monocyte-derived DCs showed increased expression of the p40 subunit of interleukin-12 (IL-12), as well as the pro-inflammatory cytokines IL-6, tumour-necrosis factor, CC-chemokine ligand 2 (CCL2) and CXC-chemokine ligand 10 (CXCL10), and the co-stimulatory molecules CD80 and CD86. Plasmacytoid DCs also produced large amounts of interferon-α (IFNα), which activates an antiviral programme in certain cells, in response to YF-17D.

Because DCs recognize micro-organisms through pattern-recognition receptors — such as the TLR family (each member of which binds distinct microbial components) — the authors then assessed how YF-17D activates DCs by examining DCs from mice that are deficient in various TLRs and TLR adaptor molecules. The absence of the adaptor molecules MyD88 (myeloid differentiation primary-response gene 88; which associates with all TLRs except TLR3) or TIRAP (Toll/IL-1-receptor domain-containing adaptor protein; which only associates with TLR2 and TLR4) resulted in reduced IL-6 and IL-12p40 expression, indicating that at least two adaptor molecules are involved in YF-17D-induced DC activation. The absence of TLR2, TLR7 or TLR9 (but not TLR4) also impaired IL-6 and IL-12p40 expression in response to YF-17D, and YF-17D activated cells transfected with TLR8, indicating that YF-17D can induce signalling through distinct TLRs.

The authors then examined how the activation of DCs through distinct TLRs influences the type of immune response, by examining the response to vaccination of MyD88- or TLR2-deficient mice with YF-17D. The absence of MyD88 decreased the production of T helper 1 (T_H1) cytokines (IFNγ) and increased the production of T_H2 cytokines (IL-4 and IL-5), whereas the absence of TLR2 increased the frequency

TRANSPLANTATION

Soluble CD154 initiates rejection

Blocking antibodies specific for CD154 (also known as CD40L) can prevent allograft rejection, and it is thought that this is a result of impaired interactions between T cells and antigen-presenting cells (APCs). However, compared with other antibodies that inhibit T-cell activation, very high levels of CD154-specific antibodies are required to prevent allograft rejection. Now, in a study published in *The Journal of Clinical Investigation*, it is shown that soluble CD154 derived from activated platelets can induce cardiac allograft rejection, providing a potential explanation for the requirement for very high levels of CD154-specific antibodies to prevent allograft rejection.

In addition to the very high levels of CD154-specific antibodies required to prevent allograft rejection, treatment with CD154-specific antibodies (unlike treatment with other antibodies that inhibit T-cell activation) has been associated with thromboembolic complications. Because human platelets release CD154 after activation, and because soluble platelet-derived CD154 activates APCs and endothelial cells *in vitro*, Xu *et al.* set out to investigate whether soluble platelet-derived CD154 has a role in allograft rejection. Initial experiments indicated that soluble human recombinant CD154 trimers induced the proliferation of mouse CD154-deficient splenocytes concomitantly stimulated with allogeneic splenocytes (isolated from BALB/c mice) and the production of interleukin-12 (IL-12) by mouse dendritic cells. Further analysis showed that whereas most untreated CD154-deficient mice transplanted with a cardiac allograft had not rejected their allograft 100 days after transplantation, most CD154-deficient mice infused with a single dose of soluble human recombinant CD154 trimers at the time of transplantation rejected their cardiac allograft within 34 days. Similarly, CD154-deficient mice infused with human platelets just before cardiac allograft transplantation rejected their allograft within 40 days unless they were treated with a blocking antibody specific for human CD154, in which case they maintained their allograft for the duration of the experiment (100 days post transplantation). A role for surgical trauma in the activation-induced release of CD154 by the human platelets was indicated by the observation that CD154-deficient mice infused with human platelets 30 days after cardiac allograft transplantation maintained their allograft for the duration of the experiment, whereas CD154-deficient mice infused with soluble human recombinant CD154 trimers at this time point rejected their allograft rapidly.

> “soluble CD154 derived from activated platelets can induce cardiac allograft rejection”

This study indicates that soluble human CD154 can induce cardiac allograft rejection and that during surgery, activation of human platelets induces the release of this molecule. As the authors point out, this has important implications for the clinical development of CD154-based therapies, as well as providing a potential explanation for the observations that very high levels of CD154-specific

of IFNγ-secreting T cells. These data indicate that distinct TLRs might differentially regulate the T_H1/T_H2-cell balance (which, in turn, influences the induction of humoral and cell-mediated immunity).

In addition to improving our understanding of YF-17D, unravelling the mechanism of action of such a successful vaccine might help in the design of vaccines against other microorganisms. The authors conclude that the efficacy of YF-17D is associated with the presence of multiple TLR ligands and therefore postulate that incorporating different combinations of TLR ligands into vaccine candidates might allow the stimulation of strong, appropriate immune responses.

Davina Dadley-Moore

ORIGINAL RESEARCH PAPER Querec, T. *et al.* Yellow fever vaccine YF-17D activates multiple dendritic cell subsets via TLR2, 7, 8, and 9 to stimulate polyvalent immunity. *J. Exp. Med.* **203**, 413–424 (2006)

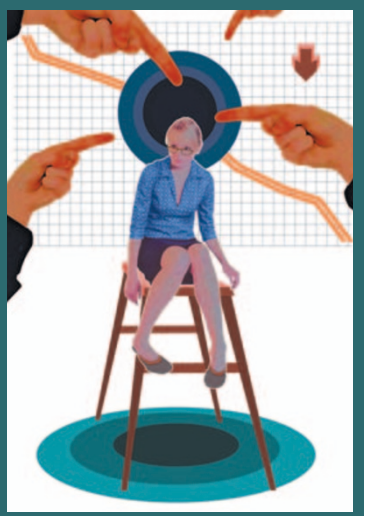
WEB SITE

Bali Pulendran's homepage: http://www.vaccines.emory.edu/scientists/pulendran.shtml

antibodies are required to prevent allograft rejection and that treatment with CD154-specific antibodies has been associated with thromboembolic complications.

Karen Honey

ORIGINAL RESEARCH PAPER Xu, H., Zhang, X., Mannon, R. B. & Kirk, A. D. Platelet-derived or soluble CD154 induces vascularized allograft rejection independent of cell-bound CD154. *J. Clin. Invest.* 23 Feb 2005 (doi:10.1172/JCI127155)



T-CELL MEMORY

Location, location, location

Can the tissue microenvironment influence memory CD8⁺ T-cell differentiation? Recent research published in *The Journal of Immunology* indicates that the anatomical location directly influences the differentiation of memory CD8⁺ T cells.

Two subsets of memory CD8⁺ T cells have previously been identified: CD8⁺ central memory T (T_{CM}) cells, which express CD62L and traffic through the blood, spleen and lymph nodes; and CD8⁺ effector memory T (T_{EM}) cells, which lack CD62L and circulate through the parenchyma of non-lymphoid tissue. It is unclear, however, whether pathogen-specific CD8⁺ T cells that persist in the intraepithelial compartment of the intestinal mucosa following clearance of infection fit neatly into the T_{CM}- or T_{EM}-cell subsets. This study compared the phenotype of these three CD8⁺ T-cell populations and showed that virus-specific CD8⁺ T cells from the gut do not resemble either the T_{CM}- or T_{EM}-cell populations found in the blood or spleen.

By transferring naive CD8⁺ T cells from the spleen of P14 transgenic mice — that is, T cells specific for an immunodominant epitope of lymphocytic choriomeningitis virus (LCMV) — to recipient mice that are then infected with LCMV, Masopust and colleagues could track a monoclonal homogeneous starting population of CD8⁺ T cells at all stages of the response.

A comparison of CD8⁺ T cells from the spleen and gut 85 days after infection showed a marked difference in phenotypic and functional properties. Gut CD8⁺ T cells expressed large amounts of granzyme B, CD69 and CD103 but small amounts of CD62L and Ly6C, whereas splenic CD8⁺ T cells showed the opposite phenotype. In addition, gut CD8⁺ T cells produced less interleukin-2 (IL-2) and lacked expression of the IL-15 receptor β-chain, and a smaller proportion of these cells produced the antiviral cytokines interferon-γ and tumour-necrosis factor than did splenic CD8⁺ T cells. Interestingly, although gut CD8⁺ T cells are $CD62L^{low}$, none of the phenotypic characteristics resembles those of T_{EM} cells (or T_{CM} cells), showing that a unique population of memory CD8⁺ T cells resides in the gut.

To examine the role of the microenvironment on the development of memory CD8⁺ T cells, memory T cells isolated from the spleen and gut of infected mice were transferred to naive mice, which were then challenged with LCMV. Examination of phenotypic characteristics following restimulation and migration of these transferred cells showed that both spleen and gut memory CD8⁺ T cells largely adopted the characteristics of their new environment. For example, gut memory CD8⁺ T cells remained $Ly6C^{low}$ within the gut but became $Ly6C^{hi}$ CD8⁺ T cells when localized in the spleen, similar to T_{CM} or T_{EM} cells, whereas spleen CD8⁺ T cells had reduced expression of Ly6C when they localized in the gut following secondary infection.

These data show that a unique population of memory CD8⁺ T cells, distinct from CD8⁺ T_{CM} or T_{EM} cells, is found in the gut. They also indicate that the microenvironmental location directly influences the differentiation of memory CD8⁺ T cells, but the mechanism involved has yet to be described.

Olive Leavy

ORIGINAL RESEARCH PAPER Masopust, D., Vezys, V., Wherry, E. J., Barber, D. L. & Ahmed, R. Gut microenvironment promotes differentiation of a unique memory CD8 T cell population. *J. Immunol.* **176**, 2079–2083 (2006)