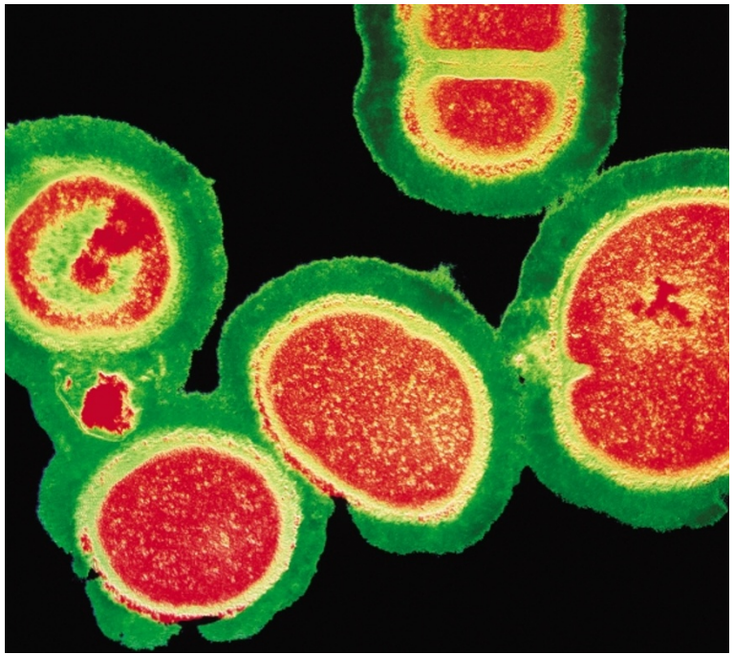
VACCINES

Dead or alive



Live vaccines usually give much better protection from infection than subunit vaccines or those based on preparations of dead or replication-defective microbes — but what is the immunological basis of this phenomenon? Increased antigen dose, altered antigen presentation and the nonspecific effects of inflammation are all possible mechanisms. Now, Gregoire Lauvau and co-workers, reporting in *Science*, have compared how live and killed vaccines interface with the immune system, using *Listeria monocytogenes* infection of mice as a model.

Although immunization with live *L. monocytogenes* induces long-lasting, $CD8^+$ T-cell-dependent protective immunity, vaccination with heat-killed *L. monocytogenes* (HKLM) is ineffective. To find out why, the authors tracked $CD8^+$ *L. monocytogenes*-specific T cells using MHC tetramers. They found that mice primed with live bacteria and HKLM-primed mice form recall $CD8^+$ T-cell responses of equivalent magnitude.

Why, then, does this $CD8^+$ T-cell memory response fail to protect HKLM-primed mice? One possible explanation is that $CD4^+$ T-cell memory, which is not elicited by HKLM vaccination, is crucial for protection. To test this, $CIITA^{-/-}$ mice (which are $CD4^+$ T-cell deficient) were immunized and challenged with live *L. monocytogenes*. These mice were immune to infection and their $CD8^+$ T-cell recall responses were indistinguishable from those of wild-type mice, indicating that protective immunity does not depend on $CD4^+$ T-cell memory.

The authors then took a closer look at the effects of live and HKLM vaccination on $CD8^+$ T-cell priming. Naive $CD8^+$ T cells from *L. monocytogenes*-specific TCR transgenic mice were transferred into an adoptive host which was then immunized. After immunization with live bacteria, the transgenic $CD8^+$ T cells were found to undergo expansion, develop cytotoxic activity, and downregulate the resting-cell marker CD62L. Conversely, following immunization

MUCOSAL IMMUNOLOGY

On guard!

The major histocompatibility complex (MHC) class I and II molecules present peptide antigen to $CD8^+$ and $CD4^+$ T cells, respectively. As well as classical MHC molecules, the mouse MHC encodes several non-classical MHC class I-like molecules, such as the thymus leukaemia (TL) antigen, whose functions remain incompletely understood. TL is expressed almost exclusively on intestinal epithelial cells and has been proposed to have a role in presenting antigen to intestinal epithelial lymphocytes (IELs). Reporting in *Science*, Hilde Cheroutre and colleagues now show that TL interacts with CD8αα homodimers on IELs, with important consequences for the mucosal environment.

Cheroutre *et al.* used TL tetramers to identify cells that bind to TL. Most IELs were stained by the tetramers, but not splenocytes, and only a minority of thymocytes were stained. Tetramers bound equally well to $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ IELs, and binding was irrespective of TCR specificity. Production of the TL tetramers in insect cells ensured that peptide–TL interactions were not possible, so peptide binding to TL molecules was not a requirement for the interaction with IELs.

As TL-tetramer binding is virtually specific for IELs, IELs express the CD8αα homodimer, and TL molecules contain a CD8α-binding motif, Cheroutre and colleagues reasoned that TL might bind CD8αα. This seemed to be the case — TL tetramers showed no staining on IELs from CD8α-deficient mice, and thymocytes from CD8β knockout mice, which express CD8αα homodimers, showed elevated TL-tetramer binding in comparison to wild-type mice. Studies using surface plasmon resonance, in which the binding of TL to CD8αα molecules immobilized on a chip was assessed, confirmed a preferential and high-affinity binding of TL to CD8αα.

So, what are the immunological consequences of TL–CD8αα interactions? This was tested by stimulating CD8α-deficient and CD8α-transfected T cells (expressing the same antigen-specific TCR) with TL^+ and TL^- forms of peptide-pulsed presenting cells. Increased interleukin-2 (IL-2) production by the CD8αα-expressing T cells was observed when they were stimulated with the TL-positive presenting cells. This enhanced cytokine production was confirmed using antigen-stimulated transgenic IELs, and intracellular cytokine staining revealed enhanced interferon-γ (IFN-γ) production. Polyclonally-stimulated wild-type IELs also showed increased IL-2 and IFN-γ production in the presence of TL. In contrast to the cytokine results, TL–CD8αα interactions decreased the proliferative and cytotoxic responses of IELs.

The results of this study indicate that CD8αα is not a TCR co-receptor and it affects T-cell function independently of TCR specificity. The authors speculate that TL–CD8αα interactions have a role in the maintenance of barrier function and homeostasis in the gut epithelium — inhibition of proliferation would ensure that cellular expansion would not disrupt the epithelial layer, and inhibition of cytotoxicity would ensure that the integrity of the epithelial layer is maintained. Further work will be required to elucidate the precise mechanisms by which TL–CD8αα interactions exert their regulatory effects in the intestine.

Elaine Bell

References and links

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with HKLM, specific CD8$^+$ T cells undergo fewer divisions, do not become cytotoxic, and remain CD62L^{hi}. This indicates that although HKLM vaccination leads to CD8$^+$ T-cell memory, effector T cells — which are crucial for protective immunity — do not develop.

It is likely that this defect in effector cell generation is due to suboptimal antigen presentation, but at what level? When mice were primed with live bacteria and HKLM at the same time, two subsets of CD8$^+$ T cells appeared — CD62L^{hi} and CD62L^{lo}. This shows that nonspecific inflammatory signals, released by infection with live bacteria, cannot rescue the defective antigen presentation of HKLM. Furthermore, live infection and HKLM might target distinct subsets of antigen-presenting cells.

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ENCYCLOPEDIA OF LIFE SCIENCES Vaccines

WEB SITE

Eric Pamer's lab: http://www.ski.edu/lab_homepage.cfm?lab=214

VIRAL IMMUNITY

Winning moves

Chess is usually won and lost by key moves made in the early stages of the game. So too is the strategic battle between a virus and the host immune system. Host–virus interactions in the acute phase of infection are thought to be crucial in determining the outcome — ranging from rapid viral clearance to persistent infection. Hepatitis C virus (HCV) can establish a chronic infection that leads to liver disease, but the acute phase of infection is most commonly asymptomatic. By the time liver damage alerts clinicians and immunologists to a case of HCV infection, it is already checkmate.

But now, a study published in the *Journal of Experimental Medicine* provides the first insight into immune interactions in the acute phase of human HCV infection. Robert Thimme and co-workers measured virus levels, liver pathology and immune responses in five individuals who developed acute infection after accidental exposure to HCV through needlestick injuries. Within this group, three patterns of infection were observed: symptomatic acute infection leading to chronic infection (two subjects); asymptomatic acute infection leading to chronic infection (two subjects); and asymptomatic acute infection that was cleared (one subject).

The presence of alanine aminotransferase in the blood was used as an indicator of liver damage. In all subjects, viraemia quickly rose to high levels within the first 2–3 weeks after exposure — but the onset of liver damage was delayed by several weeks. This indicates that the virus itself is not directly cytopathic. Later, the virus levels decline, but there was no simultaneous increase in liver damage, showing that acute-phase HCV infection is partially controlled by non-cytopathic mechanisms.

Studies in chimpanzees (the only animal model of HCV infection) have implicated early CD8$^+$ T-cell responses in viral clearance. Is this true in humans? Several HLA-A2-restricted HCV epitopes have been characterized, so HLA-A2 tetramers loaded with specific HCV peptides can be used to track specific CD8$^+$ T-cell responses. The one individual who cleared infection was HLA-A2 positive. HCV-specific CD8$^+$ T cells with an activated (CD38$^+$) phenotype were readily detectable throughout the course of infection, but, in general, these cells did not produce the effector cytokine interferon-γ (IFN-γ). Only at 12 weeks post-infection did a subset of IFN-γ$^+$, HCV-specific T cells appear, coinciding with viral clearance and resolution of liver disease. Intriguingly, these cells were CD38$^-$. A vigorous CD4$^+$ T-cell proliferative response to HCV was also observed, and this also correlated with viral clearance.

By contrast, the two subjects with asymptomatic infection leading to chronic infection had very weak CD4$^+$ T-cell responses. One of these individuals was also HLA-A2 positive, but no HCV-specific CD8$^+$ T-cell responses were detected using the tetramers. In the two patients that developed chronic infection after symptomatic acute infection, only the CD4$^+$ T-cell responses could be measured. Both individuals had strong HCV-specific CD4$^+$ T-cell responses that developed later. The function of this response is not clear, but it did correlate with a partial control of viraemia.

Clearly, there are different patterns of early host–virus interaction that lead to chronic HCV infection. These results indicate that HCV persistence might be due to a fundamental failure in both CD4$^+$ and CD8$^+$ T-cell responses early in infection. In particular, the emergence of an IFN-γ$^+$, CD38$^-$, non-cytopathic CD8$^+$ T-cell response might be key to the host's victory over HCV.

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ORIGINAL RESEARCH PAPER Thimme, R. *et al*. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J. Exp. Med.* **194**, 1395–1406 (2001)

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