structures characteristic of normal biofilms. Rather, it appears that the presence of the acyl-HSL normally initiates a process of differentiation that eventually leads to the maturation of the biofilm. Clearly, communication amongst cells by extracellular signaling molecules is a key step in the normal development of biofilms.

The work of Davies *et al.* has only scratched the surface of biofilm development, and these exciting results now open the way for much additional investigation.

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UPDATE: IMMUNOLOGY

# The Numbers Game for Virus-Specific CD8$^+$ T Cells


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A viral infection is a race. For the infected organism to survive, cell-mediated immunity has to develop faster than the spread of the pathogen. The outcome depends on how many essential cells are compromised by the time the protective immune cells [cytotoxic T lymphocyte effectors (eCTLs)] enter the site of infection. In the end, it doesn't matter whether it's the virus or the eCTLs that destroy the infected cells—too much damage leads to death or severe impairment. What are the numbers that underlie this precarious balancing act? Precise methods for virus titration have been available for more than 50 years. Measuring the other half of the equation, the clonal expansion of the virus-specific T cell response and the size of the eCTL population, has proven to be much more elusive.

The best estimates of virus-specific CD8$^+$ T cell numbers have been derived from limiting dilution analysis (LDA), a microculture technique in which lymphocytes undergo at least 10 cycles of replication before eCTL function is assayed. The LDA method is extremely tedious, technically demanding, and notoriously variable. Even worse, the assay clearly fails to measure the size of the eCTL population in sites of virus-induced pathology (*1*), probably because further stimulation of these highly activated lymphocytes induces apoptotic cell death in the LDA cultures. Nevertheless, LDA provides a reasonable measure of the size of the memory T cell (mCTL) pool, the greatly expanded virus-specific CD8$^+$ set that persists for the life of a laboratory mouse and is readily recalled to defensive eCTL activity after a secondary exposure to a pathogen.

The laboratories of Bevan (*2*) and of Altman and Ahmed (*3*) have triggered a revolution in our understanding of the virus-specific eCTL and mCTL responses by finally developing accurate methods for measuring eCTL responses. These researchers analyzed CD8$^+$ T cell–mediated immunity to murine lymphocytic choriomeningitis virus (LCMV) by using one or more of three recent technical developments. Two of the methods measure interferon-γ (IFN-γ) production after stimulation with viral peptide. T cell numbers are determined either by measuring secreted IFN-γ with a 24-hour ELISA spot assay or by staining cytoplasmic IFN-γ in fixed cells after stimulating for 6 hours in the presence of brefeldin A (which prevents secretion of the IFN-γ). The third method quantitates antigen-reactive T cells by direct staining of the virus-specific CD8$^+$ set with tetrameric complexes of major histocompatibility complex (MHC) class I glycoprotein plus peptide. This latter protocol to determine virus-specific CD8$^+$ numbers in the blood of people and monkeys infected with HIV and SIV (*4*). Tetramer staining looks set to be the gold standard for quantifying virus-specific CD8$^+$ T cells. The numbers are almost identical to those determined by the alternative flow cytometric technique involving short-term peptide stimulation and staining for IFN-γ.

The basic message from the LCMV experiments is that the size of the eCTL population is 10 to 50 times that suggested by previous LDA studies (*5*). As many as 70% of the activated CD8$^+$ T cells in the spleen of an LCMV-infected mouse (the virus grows in this site) are specific for one or another LCMV peptide presented by MHC class I glycoproteins; this represents an expansion of more than four orders of magnitude over a period of 7 days. Though the finding is dramatic, it was not totally unexpected, as the amount of eCTL activity is extremely high in this experimental system. More than 20 years after the discovery of MHC class I restriction with LCMV eCTLs (*6*), we finally know how many players are involved!

Even more surprising is that mCTL numbers are some 10 times those determined by LDA. Though a recent report indicates that elements of LCMV can be copied back into the mouse genome (*7*), most evidence contradicts the idea that mCTL survival depends on viral persistence (*5*). The tetramer experiments with HIV and SIV also detected very high numbers of peptide-specific CD8$^+$ T cells in blood (*4*). These viruses are never eliminated, raising the question of the relative balance between the eCTL and mCTL components in such ongoing confrontations.

Although the total numbers in the eCTL and mCTL compartments have been greatly underestimated, the kinetics and duration of the virus-specific CD8$^+$ T cell response derived from LDA are essentially correct. As exemplified by application of the tetramer technology to analyze immunity to an intracellular bacterium *Listeria monocytogenes* (*8*), a spectrum of secondary explosions is likely to occur in this field as these new approaches are applied to other pathogens and the tetramers are used to sort antigen-specific T cells for functional characterization.

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