Vaccine wakes from the dead

Fred R Frankel

A new immunization approach could result in safer, more effective vaccines for intracellular infectious agents and cancer (pages 853–860).

For intracellular pathogens, such as tuberculosis or HIV, vaccines made with dead pathogens tend to be less effective than live vaccines. But live vaccines also carry risks. Brockstedt *et al.*[1] address this quandary by venturing into a 'gray zone' between life and death. They introduce a promising new vaccine protocol that consists of bacteria that are unable to reproduce but are still metabolically viable—sufficiently viable, at least, to generate cellular immune responses.

Traditional vaccines, often based on killed pathogens or molecules isolated from them, usually elicit a humoral, antibody-based response. Such approaches have successfully controlled or eliminated major diseases such as polio, smallpox, pertussis and diphtheria. But pathogens causing other diseases, such as tuberculosis, malaria and AIDS, have resisted such easy strategies, as they infect and reside within cells, generally avoiding the effects of a humoral response. These agents require vaccines that can elicit potent cellular immunity—by mimicking the infection and inducing an army of pathogen-specific T cells that can kill infected cells.

Cellular immunity originates when antigen-specific T cells recognize infected host cells bearing pathogen-derived peptides on their surface. Recognition leads to activation of the T cells, which expand clonally, acquire useful effector functions and home to sites of inflammation and infection, where they can attack the festering cause of the response (**Fig. 1**). A subset of effector cells, CD8 cells, is important for eradicating intracellular pathogens, as these acquire killer functions that can destroy the infected cell. Generation of CD8 T cells is most efficient when pathogens replicate and express their genes inside the host cells. Killed agents cannot do that, and therefore illicit a poorer cellular response.

After the pathogen is eliminated, the vast expansion of antigen-specific T cells collapses, leaving behind a small but important fraction to become memory T cells. Vaccines, designed to combat future infections, depend on the establishment of potent, long-lived memory T cells, ever ready to re-expand rapidly to become killers.

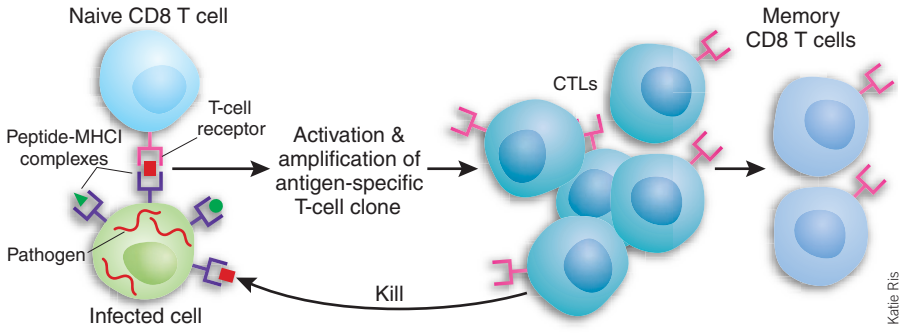

Figure 1 Vaccination mimics natural infection with a pathogen. A pathogen-infected cell expresses pathogen-derived peptides on its surface in association with major histocompatibility complex (MHC) class I molecules. Circulating naive antigen-specific CD8 T cells may recognize these pathogen peptide–MHC class I complexes through their T-cell receptors. This encounter, if accompanied by other stabilizing interactions, leads to T-cell activation, further differentiation and expansion of the antigen-specific cells to form a large clone. The resulting progeny T cells, now with lytic activity, circulate in search of cells expressing the initiating peptide.. After the offending peptide-labeled cells are killed, the army of T cells collapses, leaving behind a smaller population of memory T cells able to quickly respond to a subsequent attack by the pathogen.

Mackaness[2] was the first to focus on the cellular nature of protection generated after infection with *Listeria monocytogenes*, finding that antibodies had no role. Subsequently, Portnoy and colleagues leapt at the implications of this work and proposed the use of recombinant *Listeria* to produce vaccines against other intracellular pathogens[3–6]. But the problem of vaccine safety raised its head.

Brockstedt *et al.*[1] have now come up with a clever solution. If the genetic material of a bacterial cell is damaged by even a single cross-linking of the two strands of DNA, for example by chemical agents or radiation, transcription of the damaged gene and chromosome replication are blocked. Bacteria, born billions of years ago in an ultraviolet light–intense environment, have evolved mechanisms to remove such damage. One, excision repair, is mediated by three genes (*uvrA*, *uvrB* and *uvrC*) that recognize and excise the damaged nucleotides. This action allows the chromosome and the bacterial cell to carry on, free of damage. Inactivation of any of these *uvr* genes leaves an almost insurmountable block for the cell, and most DNA-damaged cells die.

The authors calculate and show that 20–30 random cross-links, introduced into the *Listeria* chromosome by photochemical treatment with a synthetic psoralen, assure that as few as one in 10^{10} Δ*uvrAB* bacteria is able to survive. Thus, these bacteria really are dead; the rare cell that survives such treatment can easily be handled by a host's innate defenses.

Such a culture, however, contains bacteria in which most of the thousands of genes on their chromosomes are unaltered. The culture—as a whole—therefore expresses all of the functions of a normal, undamaged population of bacteria, and should be able to express normal pathogen-related molecules that are necessary to induce a normal cellular immune response (**Fig. 2**).

The authors show that this is indeed the case. Although the psoralen-treated cells could not divide and generate progeny, they continue to synthesize proteins and elongate morphologically for up to 6 hours without septating, and escaped the phagolysozome after infection of cultured cells (unlike heat-killed *Listeria* or *Listeria* mutants lacking the virulence factor hemolysin). Because they retain full metabolic activity, the cells expressed the cellular functions, except replication, necessary to induce protective immunity. In fact, the authors found that cul-

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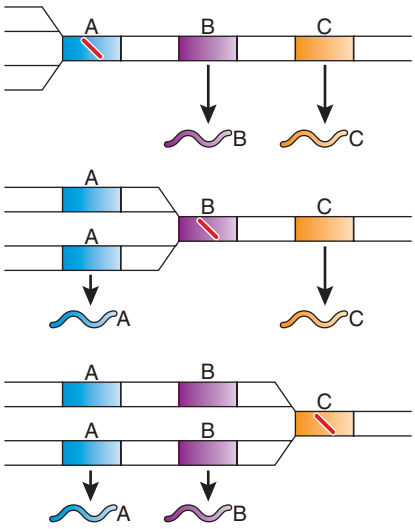

Figure 2 The walking dead. Psoralen-treated Δ*uvrAB Listeria* are unable to replicate their chromosomes beyond the point of a random cross-link introduced by the treatment. Because of the replication block, septation and cell division are also blocked. Nevertheless, undamaged genes continue to be expressed properly. The culture as a whole expresses all genes, including any necessary to induce an immune response.

tured cells infected with the damaged Δ*uvrAB* bacteria express *Listeria*-coded peptides on their surface and could activate CD8 T cells *in vitro*, whereas equally attenuated nonmutant bacteria were ineffective.

But could these psoralen-treated Δ*uvrAB* bacteria elicit an immune response *in vivo*? The authors show that recombinant vaccine carrying the gene encoding ovalbumin could protect mice from viral infection by vaccinia virus that carried the same gene. They also found that immunization with a single booster injection was as effective as the live vaccine at protecting mice from infection with wild-type virulent *Listeria*. To test their approach in a tumor-vaccine model, the authors generated *Listeria* expressing a tumor antigen. Mice implanted with CT26 tumor cells develop lung nodules 20 days after implant and usually die. But, impressively, mice vaccinated on three consecutive days, starting shortly after tumor cell infusion, were protected against nodule formation and death. Protection was accompanied by the appearance *in vivo* of epitope-specific cytolytic CD8 T cells.

Will the approach be of general use? Brockstedt *et al.*[1] have created a protocol that may be applicable to a wide range of organisms—indeed, they show that they can 'kill' *Bacillus anthracis* using the same approach. Their organisms really are dead, yet they retain full metabolic activity and express the cellular functions, except replication, to induce protective immunity. The strategy seems promising, but a crucial question will be whether the efficacy seen in these mouse studies can translate to primates. It is known that at least 24 hours of antigen presentation is necessary to achieve a full immune response[7]. But, unable to replicate, these organisms probably are destroyed within hours by polymorphonuclear leukocytes of the innate immune system. This may explain why after immunization with the ovalbumin-expressing vaccine the authors found somewhat weaker CD8 responses compared to the live vaccine.

The recognition of *Listeria* as a valuable vaccine vector has resulted in a plethora of attenuation models, beginning with Δ*actA* bacteria (blocked in intercellular spread)[6,8,9], conditionally lethal Δ*dal*Δ*dat Listeria* (blocked in cell wall synthesis) that are unable to multiply unless supplied transiently with D-alanine[10–12] and metabolic mutants[13]. As vaccinology is unfortunately still based primarily on empiric observation, these various approaches can only be compared in head-to-head studies. But this new protocol may have a leg up on the others as a cancer vaccine in an arena which *Listeria* has already shown considerable promise[14] and in which safety is of utmost importance.

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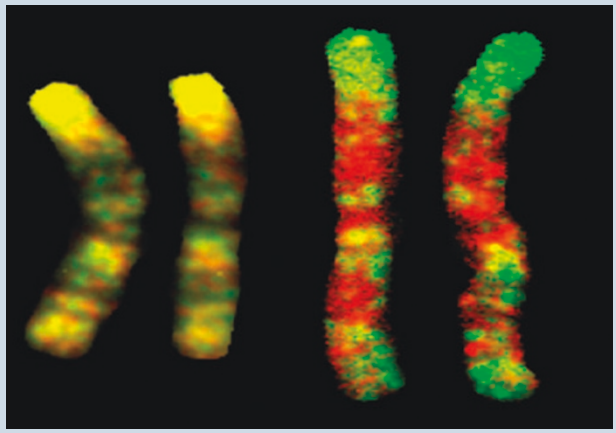
Growing older, growing apart



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Monozygotic twins originate from a single fertilized egg, but they are not truly 'identical'—for instance, they can develop different chronic diseases. But are the differences due to nature—subtle sequence changes in the DNA—or nurture? In a recent issue of the *Proceedings of the National Academy of Sciences*, Mario Fraga *et al.* take an alternate approach to this debate, implicating epigenetic alterations in the differences (doi:10.1073/pnas.0500398102).

Fraga *et al.* analyzed DNA methylation and histone acetylation in lymphocytes of 80 twins. They found significant differences in these epigenetic modifications between siblings in 35% of the twin pairs—and the differences increased with age. To show this, they generated labeled PCR probes for each individual that reflected the distribution of methyl groups along their DNA, and hybridized these to chromosomes. Shown are the DNA methylation patterns of chromosome 1 from 3-year-old twins (left pair) and 50-year-old twins (right pair). The younger twins showed similar patterns of methylation, visualized as an overlap of green and red probes (yellow). The chromosomes from the older twins show substantial disparity in methylation patterns (distinct red and green pattern).

Do such epigenetic differences lead to variations in gene expression that might explain phenotypic differences between monozygotic twins? In this study, the answer was yes—gene expression patterns in the 3-year-old twins were virtually identical, but varied widely in the 50-year-old twins. So fear not, identical twin, you are unique.

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