In sensory ganglia, the authors observed decreased expression of CGRP, in contrast with increased release of this peptide in the spinal cord. Further alterations in sensory ganglia include a decreased expression of substance P, increased expression of galanin and neuropeptide Y (all three peptides are involved in pain transmission). Decreased expression of the artemin receptor GDNF family receptor-α3 (GFR-α3) also occurred. The expression of markers selective for pain-sensing nerves (purinergic $P2X_3$ receptors and isolectin B_4) was decreased, and one of the sensory neuron–specific sodium channels ($Na_v1.8$, an ion channel implicated in spontaneous nerve firing) had been translocated to the sciatic nerve, although the exact location relative to the ligature site was unclear.

All these alterations, except for the decrease in GFR-α3, were partially reversed by artemin (**Fig. 1**). This suggests that the antihyperalgesic effect of artemin might result from the normalization of CGRP release and of the expression of CGRP, substance P or $P2X_3$ receptors, and that artemin's antiallodynic effect might be related to the reversal of changes in neuropeptide Y. However, it is not clear why artemin only partially restored most of these changes while fully abolishing pain behavior. It remains to be seen whether these biochemical changes represent nonspecific actions of artemin or mediate its pain-suppressing effects.

The challenge now is to determine how artemin might be working. One common feature of neuropathy is continuous pain resulting from spontaneously generated neuronal activity in sensory fibers—so-called ectopic firing. Boucher *et al.*[10] found that GDNF reduced ectopic firing, probably by reversing injury-induced changes in sodium channel expression. Does artemin work in a similar way? If so, do both of these molecules use a common signaling pathway that could be targeted to treat neuropathic pain?

Because artemin changed the expression of dynorphin, perhaps it could affect also other opioids such as enkephalins, analgesic peptides in the peripheral and central nervous systems. Similar effects are observed with opioid peptides in the brain, which contribute to the pain relief produced by another trophic protein, brain-derived nerve growth factor[11]. The exact receptor selectivity of artemin also is unclear, but the identification of specific antagonists should aid in receptor identification.

In the study of Gardell *et al.*, once artemin was discontinued, the analgesia and neurochemical changes vanished. In clinical practice, therefore, artemin would require lifelong application. A detailed evaluation of artemin-induced side effects during long-term application in rats will therefore be essential before this compound can enter clinical studies. Nevertheless, Gardell *et al.* move us one step closer to a treatment for painful neuropathies.

1. Gardell, L.R. *et al. Nat. Med.* **9**, 1383–1389 (2003).
2. Ralston, D.D. *Pain Rev.* **5**, 83–100 (1998).
3. Suzuki, R. & Dickenson, A.H. *Neuroreport* **21**, R17–R21 (2000).
4. Woolf, C.J. & Salter, M.W. *Science* **288**, 1765–1769 (2000).
5. Sindrup, S.H. & Jensen, T.S. *Pain* **83**, 389–400 (1999).
6. Dellemijn, P. *Pain* **80**, 453–462 (1999).
7. Honma, Y. *et al. Neuron* **35**, 267–282 (2002).
8. Baloh, R.H. *et al. Neuron* **21**, 1291–1302 (1998).
9. Bennett, D.L. *et al. J. Neurosci.* **20**, 427–437 (2000).
10. Boucher, T.J. *et al. Science* **290**, 124–127 (2000).
11. Siuciak, J.A., Wong, V., Pearsall, D., Wiegand, S.J. & Lindsay, R.M. *Eur. J. Neurosci.* **7**, 663–670 (1995).

Cancer vaccine targets leukemia

John Donnelly

Effective cancer vaccines targeted against specific antigens have eluded researchers for decades. When combined with a drug, one such vaccine now shrinks tumors in a mouse model of promyelocytic leukemia (pages 1413–1417).

Scientists have sought to use immunization as a method of treating cancer since the beginning of immunobiology. Over many decades, various approaches to eliciting both innate and acquired immune responses against tumors have been tried, some with a degree of success. However, immunotherapy has yet to be incorporated into first-line therapies for more than a very few types of cancers, such as the use of IL-2 immunotherapy for metastatic renal cell carcinoma[1].

Treating cancer with something that looks more like a modern-day vaccine, with a defined antigen and an optimized adjuvant and delivery platform, is still in the future. In this issue, Padua *et al.* edge us closer to this goal by testing a vaccine against acute promyelocytic leukemia (PML) in a particularly informative mouse model[2]. They found that the vaccine prolonged survival in combination with all-*trans* retinoic acid (ATRA), a drug already used to treat patients with this form of leukemia. This finding is potentially relevant for patients because ATRA alone tends to provide only short-lived remission, unless combined with other cytotoxic therapies. And even when combined with other therapies, ATRA results in a cure rate of approximately 50%, so there is room for further improvement.

There are three basic challenges in attempting to treat cancer by using the adaptive immune system: breaking peripheral tolerance to generate adaptive immune responses (T cells or antibodies) against self or slightly modified self antigens; causing the effectors to home to the site where tumor cells are present; and maintaining effector function in the tumor environment, which may contain inhibitory agents such as transforming growth factor-β.

Obtaining proof of concept for candidate immunotherapies presents additional challenges. Animal models that use human tumors implanted into mice require immune-deficient hosts, as hosts with functioning immune systems will reject exogenous tumors. Immune-deficient models can be suitable for the study of innate immunity, but clearly not for the study of acquired immunity.

Putting human oncogenes into mouse tumors allows for experiments showing that an immune response can target a particular tumor. However, the oncogene may still essentially be a foreign protein, and thus may not accurately mimic the induction of an immune response to self. The most relevant tumor immunotherapy

John Donnelly is at Chiron Vaccines, 4560 Horton Street, Emeryville, California 94608, USA.
e-mail: John_Donnelly@chiron.com

models use endogenous mouse tumors that also express the antigens of interest. Even in these models, however, it may not be possible to exactly test the intended product, but rather a model product that incorporates the mouse homolog of the target antigen.

In both animal studies and clinical trials, the candidate tumor vaccine must generate effective responses to self or modified self antigens as rapidly as possible, and do so in a tumor-bearing host, in order to be effective. These are formidable challenges to vaccine-platform technologies that were initially developed with infectious disease in mind, where the antigens are foreign and immunization occurs before exposure to the pathogen.

Despite these challenges, significant progress is being made, reflected in a number of recent successful phase 1 and 2 clinical trials. For the most part, these approaches use cells as sources of antigen, delivered either directly as tumor cells, as heat shock proteins extracted from tumor cells or as dendritic cells loaded with tumor antigens. The vaccines often are combined with administration of cytokines, most frequently granulocyte-macrophage colony-stimulating factor (GM-CSF)[3–8]. The vaccines themselves are challenging, although not impossible, to mass-produce.

The clinical results are very important because they help define the attributes of a successful cancer vaccine platform: colocalization of antigens and costimulatory molecules, efficient antigen presentation, local or systemic delivery of cytokines as adjuvants and inclusion of multiple antigens if possible. Building on this success, a vaccine based on plasmid DNA formulated in polylactide coglycolide microparticles has shown promise against premalignant lesions induced by human papilloma virus[9].

The study by Padua *et al.* represents an advance on several fronts[2]. Human acute PML is associated with chromosomal translocations involving retinoic acid receptor-α (RAR-α)[10], activating the PML-RAR-α oncoprotein. PML accounts for about 10% of all acute myelogenous leukemias, which strike about 14,000 individuals per year in the US.

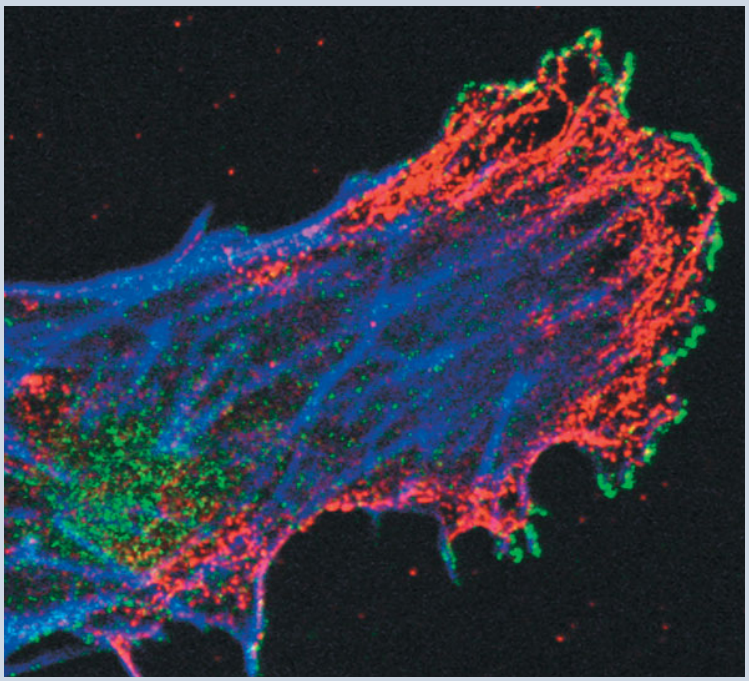
Padua *et al.* studied spontaneous leukemias arising in transgenic mice expressing the rearranged human receptor gene. These tumors have the advantages of a well-characterized transforming mutation that also provides a cell-surface target for an immune response, and are transplantable into the parental mouse strain. In addition, the leukemias retain the ability to signal through the transgenic receptor and are susceptible to ATRA. This compound is used to treat leukemias because it induces granulocytic differentiation and eventual death of the leukemia cells. ATRA is more effective in immune-competent mice than in immune deficient ones, suggesting a role for acquired immune responses induced during chemotherapy[11].

Padua *et al.* began their studies with the observation that mice immunized with the transgenic leukemia cells survived best if they had formed antibodies to the PML-RAR-α receptor. The authors reasoned that a vaccine might further promote survival, so they generated an effective DNA vaccine by fusing PML-RAR-α to fragment C of tetanus toxin. The latter is a potent stimulus of $CD4^+$ T-cell responses in mice, and in this case increased the potency of the DNA vaccine. ATRA or the DNA vaccine alone both improved the survival of mice that had been injected with the leukemia cells. But given together, the combination

Tinged migration

Cell migration enables essential processes such as wound healing, but also gives legs to cells during metastasis. In the October *Developmental Cell*, Shiro Suetsugu *et al.* take a close look at how cells move. Shown is a migrating mouse fibroblast induced to migrate with platelet-derived growth factor. The cell is stained for actin (blue), matrix metalloproteinase-2 (MMP-2; red) and WASP family verprolin-homologous protein-2 (WAVE-2; green). During migration, MMP-2 and other proteases degrade the extracellular matrix, breaking the way for the leading edge of the cell. At this leading edge, WAVE proteins activate the Arp-2/3 complex, which nucleates actin and causes rapid polymerization. These processes precede lamellipodium extension and attachment to the substratum, which create a scaffold for the next step of leading-edge extension. The investigators found that WAVE-2 is essential for leading-edge extension and directed migration. Another protein, WAVE-1, colocalizes with MMP-2 and is essential for MMP-dependent migration in the extracellular matrix. The researchers are now investigating the role of WAVE proteins in metastasis.

Charlotte Schubert



prolonged the survival of half the mice significantly, to 120–300 days. In contrast, ATRA treatment alone did not result in any survivors at all beyond 80–100 days.

This study meets the key requirements for validity of a mouse tumor challenge model: the tumor is syngeneic, the treatment can be given after tumor implantation, and the antigen is closely related to self (the mouse and human RAR-α amino acid sequences are very similar). Furthermore, this study shows synergy between the immunotherapy and conventional chemotherapy, which reflects the clinical setting in which the vaccine is most likely to be used. Given the medical need for effective leukemia therapies, and given that DNA vaccines have been generally safe and well tolerated in clinical trials thus far, this approach should be tested clinically in the near future.

The successful clinical application of a cancer immunotherapy that is based on an infectious disease–type immunization platform is still in the future. Improvements to animal models to make them more representative of human disease will facilitate the selection of antigen targets and the design of platforms to be used. This type of incremental progress may well enable the next round of clinical achievements.

1. Fisher, R.I., Rosenberg, S.A., Sznol, M., Parkinson, D.R. & Fyfe, G. *Cancer J. Sci. Am.* **3**, 568–569 (1997).
2. Padua, R.A. *et al. Nat. Med.* **9**, 1413–1417 (2003).
3. Small, E.J. *et al. J. Clin. Oncol.* **18**, 3894–3903 (2000).
4. Dummer, R. *Curr. Opin. Invest. Drugs* **2**, 844–888 (2001).
5. Belli, F. *et al. J. Clin. Oncol.* **20**, 4169–4180 (2002).
6. Su, Z. *et al. Cancer Res.* **63**, 2127–2133 (2003).
7. O'Rourke, M.G. *et al. Cancer Immunol. Immunother.* **52**, 387–395 (2003).
8. Peterson, A.C., Harlin, H. & Gajewski, T.F. *J. Clin. Oncol.* **21**, 2342–2348 (2003).9.
   Sheets, E.E. *et al. Am. J. Obstet. Gynecol.* **188**, 916–926 (2003).
10. Park, D.J., Vuong, P.T., DeVos, S., Douer, D. & Koeffler, H.P. *Blood,* published online 31 July 2003 (doi:10.1182/blood-2003-02-0412).
11. Westervelt, P. *et al. Proc. Natl. Acad. Sci. USA* **99**, 9468–9473 (2002).

Life on the inside for *Mycobacterium tuberculosis*

John D McKinney & James E Gomez

M. tuberculosis **persists in the body, sequestered inside macrophages and subverting the phagocytic machinery to create a membrane-bound home. Microarray profiling studies reveal how the bacterium settles into its new environment.**

Resident tissue macrophages provide one of the first lines of host defense against infection. As a rule, microorganisms that encounter the macrophage's phagocytic embrace meet a swift and 'degrading' end within lysosomes, the cell's "garbage disposal" organelle. Evolution delights in providing the exception to the rule, and a few professional killers have learned to exploit the macrophage as a base of operation. Some intracellular bacteria, such as *Shigella* and *Listeria*, escape from the phagosome and replicate in the cytoplasm of the host cell; others, such as *Salmonella* and *Mycobacterium*[1], replicate and persist within an altered phagosomal compartment. The environmental conditions that *M. tuberculosis* encounters within the phagosome—and the adaptive responses that allow the organism to replicate and persist therein—have largely remained elusive.

No longer. In the 1 September issue of the *Journal of Experimental Medicine*, Schnappinger *et al.*[2] provide evidence that the phagosome is an inhospitable habitat, poor in conventional nutrients and damaging to bacterial components (**Fig. 1**). Activation of macrophages with the cytokine interferon-γ (IFN-γ) makes life in the phagosome even harsher by lowering the pH, fusing the phagosome with lysosomes and stimulating the production of nitric oxide (NO), a potent antimicrobial

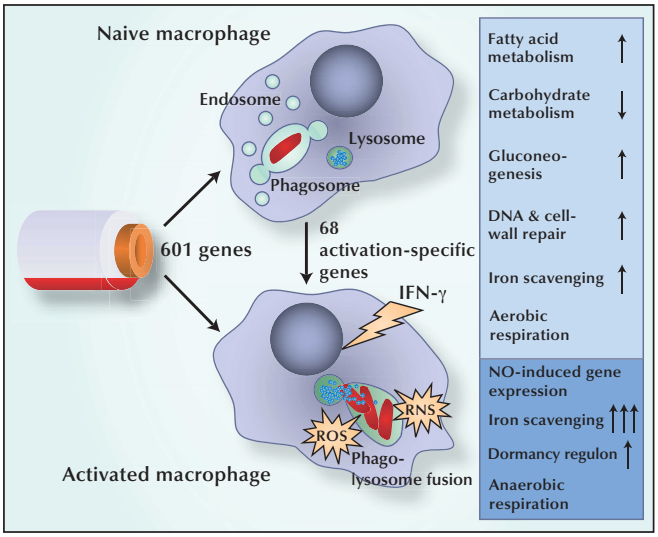


Figure 1 *M. tuberculosis* moves in. *M. tuberculosis* gene expression in naive and activated macrophages reflects environmental stresses that can be mimicked *in vitro*. In naive macrophages, *M. tuberculosis* resides in a modified phagosome that intersects the recycling endosome network and does not undergo acidification, maturation or fusion with lysosomes. The macrophage-activating cytokine IFN-γ promotes phagosome acidification and phagolysosome fusion. Antimicrobial mechanisms induced by IFN-γ include the generation of reactive nitrogen (RNS) and oxygen (ROS) species. A total of 601 *M. tuberculosis* genes are differentially expressed within macrophage phagosomes relative to log-phase axenic cultures. Regulation of a set of 68 activation-specific genes is restricted to activated macrophages and is dependent on the macrophage's ability to produce NO.

John D. McKinney and James E. Gomez are in the Laboratory of Infection Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA.
e-mail: mckinney@rockefeller.edu or gomezj@rockefeller.edu