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Bad bugs: good for cancer therapy?

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Components of *Listeria* and *Escherichia coli* (Fig. 1) have been used to prime the immune system in a novel approach to fighting cancer [1]. Recombinant *E. coli* expressing listeriolysin and ovalbumin were used to induce dendritic cells to efficiently present ovalbumin to cytotoxic T lymphocytes in mice. The activated cytotoxic T lymphocytes were able to prevent the growth of ovalbumin-labelled melanoma cells in mice.

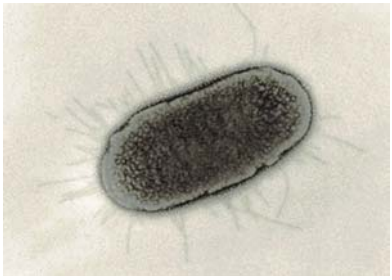

Figure 1. Electron micrograph of *Escherichia coli.* Used with permission of the Wadsworth Center, New York Department of Health (http://wadsworth.org).

Cell-mediated immunity

The key to using the immune system to overcome cancer uses the cell-mediated arm of the immune system. Cancers progress by evading cell-based immune attack; therefore, this research offers a way to efficiently present tumour antigen and activate cytotoxic T lymphocytes to kill tumour cells.

Antigens presented to dendritic cells (DCs) must trigger maturation of the DC or the result will be tolerance rather than immune attack on the antigen. Darren Higgins, a microbiologist at Harvard Medical School (http://www.hms.harvard.edu) and colleagues at London's Hammersmith Hospital used *E. coli*, which – as with all bacteria – drives DCs to mature. The matured DCs then present the *E. coli* antigens to cytotoxic T lymphocytes, which are subsequently activated and, in turn, attack and lyse cells with those antigens.

Higgins and colleagues selected a mouse melanoma strain labelled with an ovalbumin marker antigen as their target cell and created a recombinant *E. coli* expressing ovalbumin protein. Upon exposure to the recombinant *E. coli*, the mouse DCs phagocytosed the bacteria and were driven into maturation. The matured DCs then presented the ovalbumin protein to cytotoxic T lymphocytes, which were only slightly more toxic to the ovalbumin-labelled melanoma than naïve cytotoxic T lymphocytes.

However, when the researchers engineered recombinant *E. coli* with ovalbumin plus listeriolysin (a pore-forming cytolysin from *Listeria monocytogenes*) the outcome changed. When *E. coli* expressing both proteins were phagocytosed by DCs, the phagosome was perforated by the listeriolysin, and ovalbumin flooded the cytosol of the DC. The resulting presentation of antigen strongly activated cytotoxic T lymphocytes against the ovalbumin. Listeriolysin thus appears to augment the efficient presentation of ovalbumin antigen to cytotoxic T lymphocytes.

Mice vaccinated with DCs that have been exposed to *E. coli* expressing ovalbumin and listeriolysin mounted a strong immune response when challenged with ovalbumin-labeled melanoma cells. However, mice vaccinated with DCs exposed to a *E. coli*–ovalbumin recombinant without listeriolysin mounted only a minimal immune response to melanoma cells expressing ovalbumin. The researchers then vaccinated the mice with live recombinant *E. coli*–ovalbumin–listeriolysin without the DCs and saw a stronger immune response. When they vaccinated mice with *E. coli*–ovalbumin–listeriolysin that had been killed by paraformal-dehydefixation, they saw an even stronger cell-mediated response against the ovalbumin-labelled melanoma.

Future work

William Robinson, Professor of Medicine and a cutaneous oncologist at the University of Colorado Health Sciences Center (http://www.uchsc.edu), finds the use of listeriolysin to stimulate cell-mediated toxicity an interesting concept. He notes, however, that human melanoma cells rapidly mutate and have only minimally antigenic surface markers. He thinks it is a long step from attacking the very antigenic ovalbumin to the slightly antigenic cells of human cancer, which are so heterogeneous and unstable.

Ralph Reisfeld, a microbiologist at The Scripps Institute (http://www.scripps.edu), says that using listeriolysin to enhance presentation of antigen to cytotoxic T lymphocytes is a welcome advance. But he warns that there are many complications between this work in mice and the creation of a vaccine effective against any human cancer. The use of bacteria as the vector is one of his concerns as the risk of pathogenicity, either acute or late, exists with any bacterium. The bacteria chosen for a vaccine must be maximally innocuous. He too, points out that ovalbumin, the marker antigen used by Higgins is, in mice, a xeno-antigen.

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Any human tumour antigen chosen for a vaccine in humans will not be a xeno-antigen and additionally must not provoke immune attack on normal cells.

Ronald Levy, Professor of Medicine at Stanford School of Medicine (http://www.stanford.edu) who has reviewed DC vaccines [2], agrees that that this paper offers some interesting possibilities for creating human cancer vaccines. He expressed optimism that tumour markers for at least some cancers would prove to be useful vaccine targets.

Higgins, whose primary interest is bacterial and viral infection, not oncology, noted in earlier work that listeriolysin forced macrophages into a much stronger presentation of antigen to cytotoxic T lymphocytes [3]. When discussing this finding with his co-authors in the UK they resolved to use the approach against cancer. Although gratified by this proof-of-concept work, Higgins says his group is planning significant follow-up studies using listeriolysin-based vaccines against bacteria, viruses and cancers. Choosing the bacterial vector for human vaccines is a major issue, which must be determined not by its ease of use but rather by safety of the resultant bacterium, he says. He notes that the choice of the appropriate target antigen for each variety of cancer is another area that will require much work.

References

1 Radford, K.J. *et al.* (2002) A recombinant *E. coli* vaccine to promote MHC class 1-dependent antigen presentation; application to cancer immunotherapy. *Gene Ther.* 9, 1455–1462

2 Timmerman, J.M. and Levy, R. (1999) Dendritic cell vaccines for cancer immunotherapy. *Ann. Rev. Med.* 50, 507–529

3 Higgins, D.E. *et al.* (1999) Delivery of protein to the cytosol of macrophages using *Escherichia coli* K-12. *Mol. Microbiol.* 31, 1631–1641

News in brief

Targets and mechanisms

Friendly stomach bugs

A specific type of microbe promotes the development of blood vessels in the intestinal lining, according to researchers at the Washington University School of Medicine (http://medicine.wustl.edu/). Jeffrey Gordon and co-workers have discovered that *Bacteroides thetaiotaomicron*, a naturally occurring gut bacterium, interacts with Paneth cells in the intestine and encourage the growth of capillaries [1].

The gut naturally contains a complex collection of bacterial species, some of which are known to be beneficial to the host. This study provides a new example of such symbiosis. The researchers used confocal microscopy to provide 3D views of intestinal tissue sections. This enabled the comparison of capillary density between groups of six-week-old mice with and without gut bacteria. The development of blood vessels stopped early in the group with no gut bacteria, but growth could be reinitiated upon colonization with bacteria from normal mice. Further tests showed that implantation of *B. thetaiotaomicron* was as effective as introducing the whole microbial society, thus implicating the bacterium as the causative agent.

To investigate the pathway of bacterially mediated blood-vessel growth, Gordon and his team engineered mice that lacked Paneth cells, a constituent of the intestinal lining that defends against bacterial attack. Without these cells, blood vessels could not completely develop, even when *B. thetaiotaomicron* was introduced. The team concluded that Paneth cells and *B. thetaiotaomicron* co-operate to stimulate postnatal capillary formation. 'Our findings illustrate the importance of co-evolution of animals and their microbial partners,' said Gordon. 'Unravelling the molecular foundations of these relationships may provide new ways of preventing or treating a variety of diseases.'

1 Stappenback, T.S. *et al.* (2002) Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.202604299 (http://www.pnas.org)

Staph's secret weapon revealed

The secret of *Staphylococcus aureus'* success might be its ability to interfere with T cells, according to research carried out at Texas A&M University System Health Science Center (http://tamushsc.tamu.edu/com/com.html) [2].

Eric Brown and co-workers suspected that *S. aureus* promotes its own survival by expressing MHC class II analog protein (Map), which works by affecting the host animal's immune system. Using mice as hosts, the team compared the incidence of disease in animals infected with two types of *S. aureus*: one group with functioning Map and the other group that was deficient in the protein.

They found that mice infected with Map-deficient *S. aureus* suffered less arthritis, osteomyelitis and abscess formation than control animals. The team went a step further by showing how Map weakens the immune system. 'T cells or mice treated with recombinant Map had reduced T-cell proliferative responses,' they explained. Their data suggests that Map is 'an immunomodulatory protein that may play a role in persistent *S.aureus* infections by affecting protective cellular immunity.'

New treatments stemming from these new findings might provide the body blow needed against *S. aureus,* a pathogen that is becoming increasingly resistant to current drugs and thought to be carried by over 20% of healthy humans.

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