T15 has a striking ability to protect mice against infection by virulent pneumococci. Perhaps because of its ability to recognize this and other pathogens, T15 has been selected by evolution to represent a large proportion of the natural autoantibody repertoire in mice and humans.

Hyperlipidemia has long been recognized as a major risk factor for atherosclerosis. OxLDL is taken up by macrophages, which populate early atherosclerotic lesions, converting the macrophages into lipid-laden foam cells. Natural autoantibodies (such as T15) can block this uptake[6].

Macrophages seem to recognize oxLDL through the same receptors that allow them to respond to microbial invaders by initiating innate immune responses, thereby increasing natural autoantibody production by B-1 cells. Indeed, titers of these natural antibodies rise with the development of atherosclerosis in a mouse model.

Binder *et al.* tested whether raising the natural levels of T15 might prevent atherosclerosis in a mouse model deficient in LDL receptors. Because they have high levels of LDL, the mice develop atherosclerosis spontaneously. The investigators immunized these mice with a pneumococcal vaccine to raise T15 levels.

Immunization greatly increased naturally occurring phosphorylcholine-specific IgM antibodies, which reacted with both the components of pneumococcal cell wall and with oxLDL in the atherosclerotic lesions themselves. Notably, pneumococcal immunization reduced atherogenesis in LDL receptor–deficient mice. Plasma from these mice blocked oxLDL uptake by macrophages. Finally, the investigators found that IgM antibodies in sera from humans with pneumococcal pneumonia reacted significantly with both pneumococcal polysaccharides and oxLDL (**Fig. 1**).

These experiments establish for the first time a surprising connection between naturally occurring autoantibodies, microbial antigens and atherogenesis.

Before seriously considering whether we can prevent atherosclerosis with a pneumococcal vaccine, the function of antibodies to oxLDL must be defined in humans. The type and specificity of these oxLDL-recognizing antibodies vary, and so might their roles in disease progression. Some oxLDL-specific antibodies do exactly the opposite of what was described by Binder *et al.*; that is, they enhance the uptake of oxLDL by macrophages and accelerate atherosclerosis in a mouse model[7].

The issue may be further complicated by the presence of autoantibodies directed against T15 itself, described during the early studies of the T15 antibody[8,9]. Autoantibodies against oxLDL antibodies may block the protective effect of oxLDL-specific antibodies. Autoimmunity can be "good" or "bad", physiogenic or pathogenic, depending on the circumstances. It is important to understand the rules before we intervene.

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Prime boost vaccines power up in people

Harriet L Robinson

Vaccines using DNA for priming and recombinant modified vaccinia Ankara virus (MVA) for boosting have shown great promise in preclinical models. Now, this novel protocol appears effective in humans (pages 729–735).

65 British volunteers have rolled up their sleeves to test a new approach to vaccination, for the first time in people. In this issue, McConkey *et al.* report success in the initial safety and immunogenicity studies of a malaria vaccine consisting of a DNA 'prime' followed by a 'boost' with a replication-defective poxvirus, modified vaccinia Ankara (MVA)[1].

New approaches to immunization have sprung from the understanding of DNA and the ability to construct expression plasmids, recombinant viruses and recombinant bacteria. The first genetically engineered vaccine, a hepatitis B virus vaccine, used recombinant protein to raise protective antibodies. More recently, genetically engineered vaccines have been developed to elicit cell-mediated as well as humoral immunity. The ability to raise cell-mediated immunity has been necessary to combat such scourges as HIV/AIDS, malaria and tuberculosis.

Particularly powerful among these T-cell vaccines have been combinations of DNA and live viral vectors, in which a DNA vaccine is used to prime a T-cell response and a recombinant viral vaccine is used to boost the response, or in which one recombinant viral vector is used for priming and a second viral vector for boosting[2].

These heterologous prime-boost immunizations elicit immune responses of greater height and breadth than can be achieved by priming and boosting with the same vector. The first immunogen initiates memory cells; the second immunogen expands the memory response. Outside of the immune response to the common vaccine insert, which undergoes a tremendous boost, the two agents do not raise responses against each other and thus do not interfere with each other's activity. In preclinical models, this strategy has reinvigorated efforts to construct vaccines for AIDS[3,4], malaria[5], tuberculosis[6] and cancer[7].

The malaria vaccine described by Hill and colleagues is the first human evaluation of a heterologous prime-boost vaccine. The DNA-

The author is at the Emory Vaccine Center and Yerkes National Primate Research Center, 954 Gatewood Drive, Emory University, Atlanta, Georgia, 30329 USA.
e-mail: hrobins@rmy.emory.edu

MVA combination not only proved safe, but also elicited large numbers of T cells and provided partial protection against a malaria challenge, extending to humans the promise that such vaccines have shown in monkeys and mice.

To get at just the right vaccine combination, the investigators evaluated 20 different conditions of priming and boosting by rolling volunteers from priming studies into boosting studies. The scope and breadth of this initial trial staked boundaries for future trials, conducting dose escalations and testing different numbers of immunizations. Under the most favorable conditions, single modality immunizations elicited fewer than 100 responding T cells per million white blood cells. In contrast, immunizations combining DNA priming with MVA boosting resulted in frequencies of responding cells that were ten times higher.

Impressively, T cells were elicited against all regions of the 557-amino acid malaria protein expressed in the immunogen. This contrasted with the low response against a 232-amino acid multiepitope string that had been fused to the C terminus of the expressed protein. The more robust response to the natural protein than the multiepitope string is good news. Despite the power of genetic engineering, expressing proteins is far easier than defining the histocompatibility types in a target population, identifying immunodominant epitopes for a pathogen and constructing a string of epitopes.

For those of us developing vaccines for which T cells, not antibody, will be the correlate for protection, the study adroitly pioneers the screening of volunteers for vaccine-elicited T cells. White blood cells were harvested and placed directly in enzyme-linked immunospot analyses to allow an *ex vivo* count of responding T cells. Assays were conducted on fresh, rather than frozen, cells to facilitate the scoring of proapoptotic T cells in peak effector responses. Consistent with the temporal pattern of T-cell responses, points were taken at one, rather than at two, weeks after boosting, when antibody responses would have been at their peak. The ability to detect enzyme-linked immunospot analysis responses was optimized by accepting, rather than attempting to zero, the nonspecific chatter of peptide stimulations. And finally, data were presented as geometric means as well as medians, to better represent patient-to-patient variability in the numbers of elicited T cells.

This heterologous prime-boost protocol is but one of several currently in human trials. McMichael and Hanke, supported by the International AIDS Vaccine Initiative, have obtained promising results in DNA-MVA trials for HIV and AIDS, being conducted in England and Kenya[8]. In these trials, as in the current trial, the protein portion of the immunogen seems more effective than the multiepitope string at eliciting T cells. Approximately 800 volunteers are participating in trials for an AIDS vaccine developed by Merck that uses DNA priming and replication-defective adenovirus boosting and, more recently, replication-defective adenovirus priming followed by boosting with an avian poxvirus developed by Aventis. Many more modalities of priming and boosting are being tested in preclinical models. Provocatively, different protocols are raising different patterns of $CD4^+$ and $CD8^+$ T-cell responses[9] and potentially different levels of T cells to dominant and subdominant epitopes. Thus, the rules of heterologous prime-boost immunizations are just beginning to be understood. Interesting science, as well as the potential to control major human pathogens, awaits those of us pursuing this powerful new approach to vaccination.

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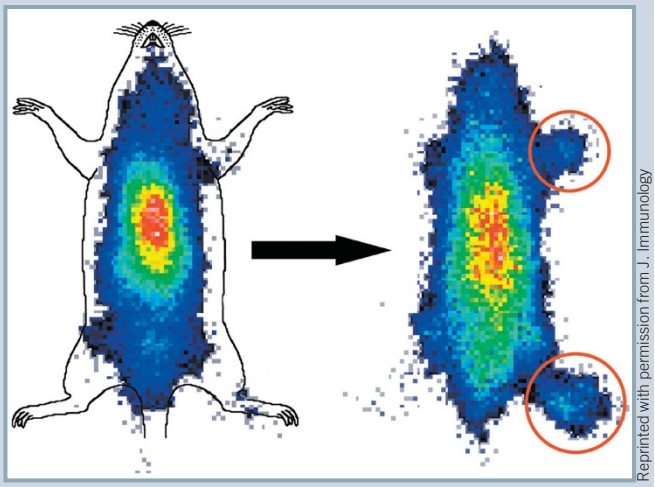
Pausing paw proliferation

Methotrexate, perhaps the most widely prescribed drug for rheumatoid arthritis, slows the rapid proliferation of cells in the joints of patients with the disease. But its efficacy is limited by side effects that can include low sperm count and liver damage. In the May issue of the *Journal of Immunology*, Andreas Wunder *et al.* efficiently deliver methotrexate to the joints, increasing the dose where it is most needed. The investigators conjugated methotrexate to serum albumin, which enters fast-metabolizing cells such as those in inflamed joints. On the left is a mouse 5 minutes after injection with radiolabeled methotrexate-albumin conjugate, treated so that it has arthritic right paws. On the right is the same mouse after 13 hours, with accumulation of the drug in these paws. Mice treated with the conjugate fended off arthritis better than mice treated with methotrexate alone, and they accumulated less of the drug in their livers and kidneys. Similar uptake occurred in cells cultured from the joints of patients with rheumatoid arthritis. The combination, which has shown promise in human cancer trials, could minimize toxicity while boosting the effectiveness of methotrexate.



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Charlotte Schubert