Synopses of Research Articles

Malaria Vaccine Trial Results Are Negative, but Important

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A malaria vaccine called ME-TRAP, which targets the pre-erythrocytic stage of the disease, was not effective at reducing natural infection rates in semi-immune African adults, according to the report of a randomized controlled trial published this month in *PLoS Medicine*. "This first field efficacy trial was an important milestone in the progression of new recombinant vectored vaccines to deployable products," says Adrian Hill (University of Oxford, United Kingdom), the lead investigator of the study. "The safety profile was excellent and the efficacy data provide a first indication of the levels of cellular immunogenicity that will be required for preventing infection," he says.

Hill and his co-workers used a heterologous prime–boost vaccination technique. They gave the volunteers two vaccines—a DNA priming vaccine followed by a modified vaccinia virus Ankara (MVA) that acted as a booster. The DNA and MVA vaccines both had the same insert coding for thrombospondin-related adhesion protein (TRAP; a pre-erythrocytic antigen) and a string of T cell epitopes (called ME for "multiple epitopes").

Hill's team had previously shown that ME-TRAP vaccines given in prime–boost sequence could induce large T cell responses in healthy volunteers from the UK and could delay parasitemia in a sporozoite challenge test (Nat Med 9: 729–735). The next step was to do a randomized controlled trial in Gambia to determine whether this vaccination strategy could provide protection against natural *Plasmodium falciparum* infection.

The researchers recruited volunteers from 13 Gambian villages that were close to the alluvial flood plain and so were at high risk of developing malaria. They randomly assigned the 372 volunteers to receive either two doses of the DNA ME-TRAP vaccine followed by a single dose of MVA ME-TRAP, or three doses of rabies vaccine. This three-dose schedule is similar to the one used by the World Health Organization/United Nations Children's Fund Expanded Program on Immunization. Two weeks before the third dose was given, all the volunteers received antimalarial drugs to clear blood-stage *P. falciparum* infections.

The time to first infection, the primary end point of the study, was similar in the two groups, with an estimated vaccine efficacy of only 10%. However, the effector T cell response to the TRAP antigen T9/96, measured one week after the third vaccination, was 80 times higher in the DNA/MVA vaccine group than in the rabies vaccine group.

"It is absolutely crucial that results like these are published, since the failures, as well as the successes, need to be documented if we are to move towards rational strategies for optimizing malaria vaccines," says Tom Smith from the Swiss Tropical Institute, who was not involved in the study. "At the same time, it makes sense to move on quickly without shedding too many tears, in a field that is moving much faster than it was before the recent injections of money from the Gates Foundation, but where it is still impossible to second-guess the results of field trials. This is partly because we do not have any good proxy measures of effective immunity in *P. falciparum,* and partly because this is a fertile area for trying out new techniques, such as DNA vaccines, where there is still a lot to learn."

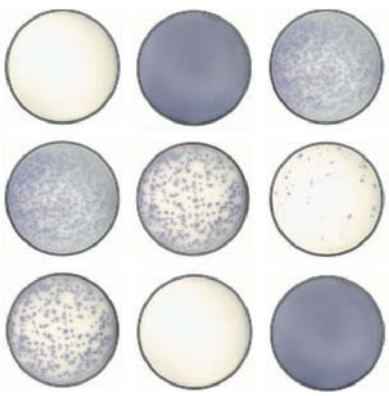
Hill is planning to do further trials that address the important question of whether this type of vaccine can prevent the symptoms of malaria. "The next step," says Hill, "is to assess newer vaccine regimes that employ two viral vectors rather than DNA and to study prevention of malaria rather than infection."

Moorthy VS, Imoukhuede EB, Milligan P, Bojang K, Keating S, et al. (2004) A randomised, controlled, double-blind efficacy trial of DNA/MVA ME-TRAP prime-boost immunisation against malaria infection in Gambian adults. DOI: 10.1371/journal.pmed.0010033

Supervised Treatment Interruptions Fail to Control HIV-1 Viremia

DOI: 10.1371/journal.pmed.0010048

Highly active antiretroviral therapy (HAART) for the treatment of individuals infected by HIV-1 is limited by high costs, drug resistance, and drug-related toxicities. This has led researchers to investigate new treatment options, including ways to boost immune responses to better control HIV. One such approach has been termed supervised treatment interruption (STI)—in which HAART is intermittently stopped once viral load has been reduced to a low level, in order to boost natural immunity by brief exposure to virus. The goal is to allow for the eventual discontinuation of drug treatment.



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ELISPOT assays detect HIV-specific cytotoxic T lymphocyte responses

Preliminary evidence, published by Bruce Walker and his colleagues from Harvard Medical School in *Nature* in 2000, suggested that this approach worked in persons treated in the earliest stages of acute HIV infection. HIV-1 viral loads in newly infected patients remained suppressed for a median of six months after therapy had been stopped. However, a follow up paper, published this month in *PLoS Medicine* by the same research group, shows that the viral load rebounded in eight of the 14 patients by one year.

"The findings are very straightforward and very important," comments Danny Douek from the Vaccine Research Center, National Institutes of Health, United States, who was not involved in the study. "In almost every case, virus rebounded and no clinical benefit from the interruption could be determined."

Walker's team first considered the possibility of STI in 1997 after they demonstrated that HAART given to patients recently infected with HIV

could protect T helper cells, which are normally destroyed in the earliest stages of infection. They hypothesized that early treatment of acute HIV-1 infection with HAART might boost the immune response, allowing it to control the HIV-1 infection without the need for continuous therapy. "We did not know at that time whether the T helper cells would be functional," explains Walker. "The only way to tell this was to stop medications and see if the immune response could control the virus."

To test this hypothesis the researchers did an open-label trial of STIs; they published data from six months follow-up in the *Nature* paper. "The key finding was that we were able to get at least transient control of virus in all eight persons studied, and in five of eight the viral load was less than 500 copies (very low!) at the time of publication," explains Walker. However, at that point they did not know how long the protective effects would last.

The first evidence that protection was not complete came two years later when Walker's team reported a case of superinfection; one of the patients in the original experiment was infected with a second strain of HIV, even though the first virus was still well controlled. "This paper was important because it indicated that the amount of immunity might be enough for the person's own virus, but might not protect against closely related viruses circulating in the population," says Walker.

The *PLoS Medicine* study adds more concern since it shows that although most persons can indeed transiently control their own virus, they do so for only a limited amount of time. "We expanded the study to 14 persons, and now have about five years of follow-up on some of the patients," says Walker. "Although we were able to use early treatment and structured treatment interruption to boost immunity and have 11 of 14 patients control their virus, most of the persons ultimately 'broke through,' meaning that they had a recurrence of viremia." At the present time the researchers do not know what causes the loss of viral control.

Walker and colleagues conclude that treatment interruptions should probably be avoided outside the setting of controlled clinical trials, whereas Douek goes a step further: "The study shows that even early short-term treatment and structured treatment interruptions, using current strategies, impart only transient benefit and are unlikely to serve as a reasonable therapeutic option in the future."

Kaufmann DE, Lichterfeld M, Altfeld M, Addo MM, Johnston MN, et al. (2004) Limited durability of viral control following treated acute HIV infection. DOI: 10.1371/journal.pmed.0010036

The Quest for a Vaccine That Yields Tumor-Killing T cells

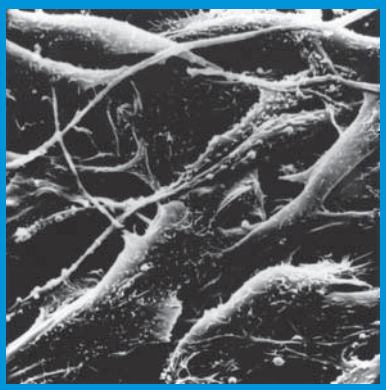
DOI: 10.1371/journal.pmed.0010050

The immune system has a remarkable capacity for fending off infectious diseases, and it has become clear that these same defenses can recognize and destroy cancer cells. In fact, they do so on an ongoing basis, and cancer develops only when immune surveillance breaks down. Many patients with established tumors also mount an immune response against some antigens that are specific to, or enriched in, the tumor. This response, however, is rarely effective against the disease.

The idea of enlisting the immune system to fight cancer has been around for a long time, and has led to the development of various cancer vaccines designed to alert the immune system to the presence of a tumor and to induce a response that, selectively and potently, will eliminate tumor cells. Vaccines include whole tumor extracts or specific proteins and peptides that are selectively expressed or enriched in tumors, by themselves or with a variety of adjuvants.

There have been some spectacular successes, in particular with immune therapy to malignant melanoma, a tumor type that seems naturally to be more immunogenic than others. However, even in melanoma, success is usually restricted to a fraction of the patients, with no obvious explanation of why the strategy works for a particular patient and fails in most others. The emphasis has consequently shifted from clinical outcomes to monitoring a patient's immune response. What type of response is necessary and sufficient to eliminate tumor cells is still unclear, but the hope is that understanding the immune response in patients that show clinical benefit will answer that question.

Peter Lee and colleagues used state-of-the art technology to dissect the endogenous immune response to vaccination with heteroclitic melanoma peptides, i.e., melanoma-associated peptides that have been engineered to elicit a stronger immune response. They focused on cytotoxic T lymphocytes (CTLs), and compared CTL clones from four melanoma patients who had vaccine-induced T cell responses and two melanoma patients with spontaneous anti-tumor T cell responses. The researchers analyzed several hundred CTL clones (to get a sense for the complexity of the responses in individual patients) for T cell receptor variable chain beta expression, recognition efficiency, and ability to lyse target melanoma cells. Most T cells isolated from vaccinated patients were poor at tumor cell lysis compared with T cells from endogenous responses to cancer.



DOI: 10.1371/journal.pmed.0010050.g001

Melanoma—a prime target of cancer vaccines (Photo: Timothy Triche, National Cancer Institute)

The authors suggest that the high doses of peptides administered in vaccinations and the increased binding capacity of heteroclitic peptides to major histocompatibility complex molecules—the very quality that makes them more immunogenic—induce many T cells with low recognition efficiency for the native peptides they encounter on the tumor cells. Their findings also bring into question the ability to deduce the recognition efficiency and tumor reactivity of T cell responses from ELISPOT and tetramer staining assays—the two standard measures of T cell responses to vaccines—which has implications for rational vaccine design in general.

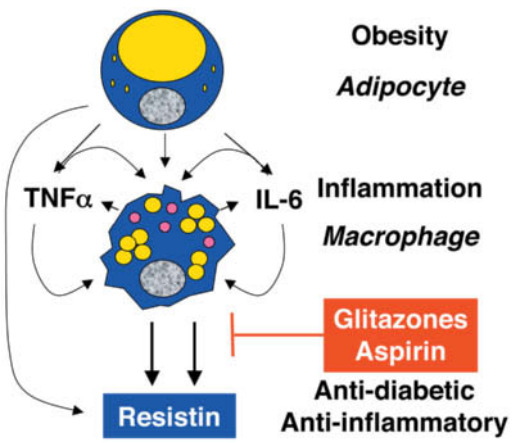
Stuge TB, Holmes SP, Saharan S, Tuettenberg A, Roederer M, et al. (2004) Diversity and recognition efficiency of T cell responses to cancer. DOI: 10.1371/journal.pmed.0010028

Resistin Response to Inflammation

DOI: 10.1371/journal.pmed.0010049

Obesity, in particular visceral adiposity, is positively correlated with insulin resistance and type 2 diabetes. Although the link is well established in humans and in rodent models, the mechanisms involved in obesity-related insulin resistance are not clear. One possibility is that hormones secreted by adipocytes compromise peripheral insulin sensitivity, and a number of candidates for such adipocyte signals have been identified. One of them, resistin, was discovered a few years ago by Mitchell Lazar and colleagues, who showed that the protein is expressed by mouse adipocytes and regulated by a group of anti-diabetic drugs called thiazolidinediones. Several lines of evidence from functional studies in rodents suggested that resistin could be the missing mechanistic link between obesity and diabetes.

The human homolog of resistin has subsequently been under intense investigation, but initial studies revealed more differences than similarities between the human and rodent proteins: human resistin is mostly expressed in macrophages, not in adipocytes, and its serum levels do not correlate as clearly with obesity, insulin resistance, or diabetes. Similarly, genetic association studies between allelic variants of the resistin gene and metabolic abnormalities have so far been inconclusive. These results prompted some of the scientists in the field who had jumped on the resistin bandwagon after the initial results in rodents to jump off again. Others, including the resistin discoverers, continue their quest to uncover resistin's role in humans, and have started to think outside the framework defined by the mouse data.



DOI: 10.1371/journal.pmed.0010049.g001

Connections between obesity and inflammation

Starting with the role of macrophages in inflammation and encouraged by the fact that obesity and insulin resistance are associated with markers of systemic inflammation, Lazar and colleagues examined the resistin response to inflammatory stimulators. As they report in this issue, resistin production in macrophages and serum levels in patients are significantly increased by these stimulators. This response can be blocked by the thiazolidinedione rosiglitazone and by aspirin, two drugs that have dual anti-inflammatory and insulin-sensitizing actions and antagonize the immune regulator NF-kappaB. The researchers go on to show that activation of NF-kappaB is sufficient to induce resistin expression. And NF-kappaB is necessary for the resistin response to inflammatory stimuli.

Lazar and colleagues now view obesity as a state of chronic inflammation and speculate that in obese individuals inflammatory cytokines lead to elevated production of resistin by macrophages and elevated serum resistin levels, which in turn contribute to insulin resistance and diabetes. This is consistent with some studies that have found higher resistin levels in obese individuals and patients with insulin resistance and/or diabetes, but not all studies have found such differences.

Jeffrey Flier, an obesity researcher who was not involved in the study, calls the article "an excellent and timely paper that demonstrates the fact that inflammatory pathways induce resistin expression and levels in human monocytes ex vivo, and in intact humans. The work appears to provide a novel link between inflammation and insulin resistance, through monocyte derived resistin." He points out, however, that "several other factors also appear to contribute directly to insulin resistance in inflammation (e.g., cytokines themselves, without invoking resistin) so the full biologic implications of the high resistin levels for insulin resistance in humans cannot be determined from this study." Resistin, it seems, continues to resist easy interpretations.

Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, et al. (2004) An inflammatory cascade leading to hyperresistinemia in humans. DOI: 10.1371/journal.pmed.0010045