
Choosing the right memory T cell for HIV

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An experimental simian immunodeficiency virus vaccine boosts production of memory T cells at the site where the virus first contacts the body—in the mucosa (293–299). The approach has the potential to result in more effective HIV vaccines than those currently under development.

In this issue of *Nature Medicine*, Hansen *et al.*¹ present a new type of approach to

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vaccination against HIV. The aim of their experimental vaccine is to harness immune cells residing in the mucosa of the vagina and rectum—in contrast with more established approaches that coax a response from memory cells further removed from the site of infection, in the lymph nodes. Although more needs to be done to demonstrate how

effective this approach could be, their vaccine shows promise, protecting four out of twelve macaques from simian immunodeficiency virus (SIV) chronic infection¹. To date, the only vaccine that has protected from chronic SIV infection is attenuated SIV, an approach that poses safety concerns^{2,3}.

The researchers based their vaccine on

an infectious replication-competent rhesus macaque cytomegalovirus that expresses the SIV structural Gag, Pol and Env proteins and a chimeric Rev-Tat-Nef protein (RhCMV-SIV)¹. They observed that the vaccine protected the macaques from mucosal acquisition of SIV, which they suggest is mediated by preexisting SIV-specific CD8⁺ effector memory T cells¹ (T_{EM} cells). These cells are generated and maintained by the continuous expression of SIV antigens by the RhCMV-SIV vaccine. The authors further speculate that other vaccines based on viral vectors that transiently express SIV/HIV antigens have failed to protect nonhuman primates from SIV acquisition because they elicit mainly CD8⁺ T cells with a central memory phenotype and function (T_{CM} cells).

Mammals have evolved a highly sophisticated immune system to limit the damage induced by pathogens, including viruses. Some viruses, such as influenza, cause acute infection, kill their target cells and are eliminated mainly by antibodies produced by B cells. Antibodies represent an effector arm of the immune response, because these proteins distribute from the blood to tissues and mucosal fluids, where they bind and destroy viruses, inhibit their entry into cells, and mediate the killing of infected cells by natural killer cells.

Other viruses, such as HIV, Epstein-Barr virus and cytomegalovirus, remain within the cells of the host and cause persistent infection. To protect from such viruses, the immune system has evolved CD4⁺ and CD8⁺ T cells. Both T cell subtypes are recruited in tissues by dendritic cells sensitized through the Toll-like receptors to the presence of pathogens. CD4⁺ T cells provide help to naive CD8⁺ T cells so they can proliferate and acquire the ability to recognize foreign antigens presented on the surface of the infected cells and kill them.

Naive CD8⁺ and CD4⁺ T cells become memory T cells once they have encountered the antigen. There are two main subtypes of memory CD8⁺ T cells that can be differentiated by their surface expression of receptors, cytokine production and ability to proliferate⁴: T_{EM} cells and T_{CM} cells.

CD8⁺ T_{EM} cells are differentiated T cells that constitute the frontline defense within the epithelial layer and the lamina propria of mucosal sites, such as the urogenital, gastrointestinal and respiratory tracts, which are the portal of entry of most pathogens. T_{EM} cells have a limited ability to proliferate but promptly recognize and kill infected cells; it is believed that T_{EM} cells are maintained by continuous antigen exposure.

CD8⁺ T_{CM} cells reside mainly in lymph nodes and are believed to undergo homeostatic proliferation in the absence of antigen, have higher proliferative capacity, and require more time to differentiate and become competent at killing cells than CD8⁺ T_{EM} cells. Thus, CD8⁺ T_{CM} cells can be thought as a long-lasting immunological reserve that requires time to be fully deployed upon the reencounter with the pathogen.

Hansen *et al.*¹ propose that because CD8⁺ T_{CM} cells reside in lymph nodes and must expand, differentiate and migrate to the mucosa to kill cells infected by SIV/HIV, they may not be sufficiently prompt to limit mucosal and distal dissemination of the virus. In contrast, CD8⁺ T_{EM} cells that reside at mucosal sites and have an immediate killing function can quickly eliminate the cells infected by the incoming virus and halt systemic dissemination of SIV.

Most HIV infections worldwide are acquired through heterosexual or homosexual transmission. In humans, as well as in macaques⁵, the memory CD8⁺ T_{CM} cell 'reserve' is mainly located in the lymph nodes that drain from the female and male genital tracts, whereas frontline memory CD8⁺ T_{EM} cells are located within and underneath the epithelium layer that lines the female and male urogenital tracts and the rectum.

The hypothesis of Hansen *et al.*¹, that CD8⁺ T_{EM} cells can protect from SIV acquisition, suggests the need for continuous expression of HIV antigens at mucosal sites. Such continuous expression would require vaccines that target mucosal sites and either a vaccine vehicle that persists or repeated administration of a vaccine vehicle that wanes. Both scenarios raise important issues about feasibility, safety and cost for the development of an HIV vaccine.

Fortunately, macaques offer a unique opportunity to address some of the questions raised by this study. The authors suggest that the protection in four of the twelve RhCMV-SIV-vaccinated macaques is due to the presence of SIV-specific CD8⁺ T_{EM} cells at mucosal sites, using the frequency of these cells in the bronchialveolar lavage as a surrogate for their frequency in the rectal mucosa. The direct analysis of the quantity and specificity of SIV-specific CD8⁺ T_{EM} cells in rectal mucosa, though technically challenging, will provide definitive support to this hypothesis. Determining whether protection is lost after experimental depletion of total CD8⁺ T cells immediately before challenge exposure would also be very informative, even though caveats, such as incomplete CD8⁺ T cell depletion at mucosal sites and depletion of natural killer

cells, would need to be accounted for.

RhCMV-SIV vaccine also induced a high frequency of SIV-specific CD4⁺ T_{EM} cells, a kind of T cell that provides help to CD8⁺ T cells and that at the same time is a target for SIV infection. Fortunately, this study shows that preexisting SIV-specific CD4⁺ T cells did not exacerbate SIV infection, raising the possibility that they may have contributed to the control of viral replication.

The eight vaccinated macaques that became infected had equivalent plasma virus loads to control unvaccinated macaques. The authors ascribe this observation to potentially low numbers of SIV-specific CD8⁺ T_{CM} cells in lymph nodes¹, because the persistent expression of SIV antigens by the recombinant RhCMV drives CD8⁺ T_{CM} cells to differentiate to CD8⁺ T_{EM} cells. Furthermore, the authors make the case that the current HIV vaccines do not protect from infection but merely limit the extent of viral replication, because they can express SIV antigens transiently and therefore elicit mainly CD8⁺ T_{CM} cells. Indeed, in macaques vaccinated with conventional T cell vaccines, other groups have found an inverse correlation between virus levels and the frequency of SIV-specific CD8⁺ T_{CM} cells but not T_{EM} cells^{6,7}. Future experiments could obtain direct evidence for this hypothesis. The introduction of SIV directly into the bloodstream of RhCMV-SIV-vaccinated macaques would bypass the frontline defense of CD8⁺ T_{EM} cells and should abolish protection.

Repeated low-dose challenge exposure to pathogenic SIV has seldom been used in pre-clinical trials. It is possible that the method of challenge exposure to pathogenic SIV used in the study by Hansen *et al.*¹, rather than the subtype of the CD8⁺ T cells elicited by the vaccine, accounts for the protection from SIV acquisition. In fact, Van Rompay *et al.*⁸ have shown that systemic immunization with T cell vaccines whose expression wanes over time, such as MVA-SIV and ALVAC-SIV, protected macaques from mucosal acquisition of pathogenic SIVmac251 administered at repeated low doses (six of seventeen of MVA-SIV-immunized macaques and ten of sixteen ALVAC-SIV-immunized macaques were protected).

Future efforts should test the relative efficacy of various prototype vaccines after either repeated low-dose or high-dose challenge exposure to pathogenic SIV. In such studies, both SIV-specific CD4⁺ and CD8⁺ T_{CM} and T_{EM} cell numbers should be carefully measured at mucosal sites and in lymph nodes, and attempts to correlate their frequency and antigen specificity to protection should

include as a direct approach as possible.

Lastly, because most HIV infection occurs by heterosexual transmission, and women are more susceptible to HIV infection than men, it would be important to know whether the RhCMV vaccine also induces SIV-specific CD8⁺ T_{EM} cells and CD4⁺ T_{EM} cells in the female genital tract.

The work of Hansen *et al.*¹ has catalyzed a

badly needed discussion about how to induce the right types of T cells to protect against HIV. The macaque model of SIV infection is central for the understanding of the nature and location of protective T cell responses induced by different vaccine modalities⁹. Hopefully, the answers to these questions will guide future efforts in the development of effective T cell vaccines for HIV.

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Oxygen sensor boosts growth factor signaling

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Activation of hypoxia-inducible factor, a molecule central to oxygen sensing, can promote the survival and growth of tumor cells. New experiments dissect a pathway behind this effect—upregulation of the epidermal growth factor receptor (pages 319–324).

Several factors can contribute to poor prognosis for an individual with cancer and can contribute to resistance to conventional cancer therapy. Two well known factors are tumor hypoxia and overexpression of epidermal growth factor receptor (EGFR), a molecule that triggers signal pathways to regulate cell growth and contribute to oncogenesis. Previous work has shown that tumor hypoxia can upregulate signaling through EGFR and other receptor tyrosine kinases (RTKs).

New findings in this issue of *Nature Medicine* show how these two factors are even more intertwined. Wang *et al.*¹ report that hypoxia prolongs the half-life of EGFR by delaying the formation of endosomes that cause the destruction of this molecule. This effect is mediated by hypoxia-inducible factor (HIF), a transcription factor central in the hypoxic response.

Tumors usually have abnormal blood vessels and poor blood flow, and thus they frequently experience a decrease of oxygen tension to below the normal level². When severe and prolonged, such hypoxia can lead to cell death. But cancer cells have adapted a variety of biological responses and signaling mechanisms to survive and even grow in a hypoxic environment, which leads to an aggressive tumor phenotype.

One of the hypoxia responses is stabiliza-

tion of HIF. When cells are under hypoxic conditions, HIF translocates from the cytoplasm into the nucleus, where it binds hypoxia response elements in various target genes³. HIF subsequently regulates the transcription of many genes that have important roles in promoting angiogenesis, cell proliferation, cell survival and reprogramming of cancer cell metabolism, leading to tumor progression and cancer metastasis⁴. HIF1 is overexpressed in human cancers, and its expression has been correlated with increased cancer metastasis and poor prognosis⁵. Therefore, HIF1, as an integral part of the oxygen-sensing pathway, contributes to the survival and growth of tumor cells in a hypoxic environment. However, the major events that mediate these hypoxia-induced cellular responses in response to HIF1 activation are unknown.

EGFR is overexpressed or highly activated in many types of human cancers of epithelial cell origin, which account for 80% of all solid tumors. Upon EGF ligand binding, EGFR dimerizes, autophosphorylates and triggers cascades of downstream signaling pathways that enhance cancer cell proliferation and survival⁶. Consistently, overexpression of EGFR in human cancers is associated with increased metastasis, therapeutic resistance and poor prognosis⁷. Therefore, EGFR has been recognized as an ideal cancer therapeutic target, and EGFR-targeting agents, including EGFR-specific antibodies and tyrosine kinase inhibitors, have been used for treating individuals whose tumors overexpress EGFR or have a mutant EGFR^{8,9}.

Wang *et al.*¹ were intrigued by findings that tumor hypoxia translationally upregu-

lates EGFR and enhances EGFR signaling¹⁰—which can be regulated by EGFR turnover. They speculated that HIF might also somehow mediate EGFR signaling and discovered that tumor hypoxia-mediated activation of HIF transcriptionally represses rabaptin-5 (encoded by *RABEP1*, ref. 1), a key effector of Rab5-mediated endosome fusion¹¹. As a result, endocytosis slows, inhibiting degradation of EGFR.

The findings suggest that increased EGFR signaling via decreased EGFR degradation may mediate many of the hypoxia-induced cellular responses that occur upon HIF activation.

The authors set out to examine how the oxygen-sensing pathway regulates the endocytic and degradation pathways. The authors modulated HIF by using cells with or without Von Hippel-Lindau (VHL) protein, a component of the HIF E3 ligase for HIF degradation and a major negative regulator of HIF proteins¹². Loss of VHL increased activity of HIF and delayed EGFR turnover, prolonging the activation of EGFR to enhance EGFR downstream signaling.

As endocytosis is one of the major pathways for RTK turnover, they next investigated whether loss of VHL prolonged EGFR turnover by inhibiting the endocytic and degradation pathways¹. They focused on the Rab proteins¹, which interact with rabaptin-5 to control sequential early and late endosomal fusion events that mediate endocytosis and the subsequent degradation of internalized RTKs in lysosomes; Rab5, in particular, is a well known activator for early endosome fusion. They found that HIF upregulation inhibits Rab5-mediated early endosome fusion and

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