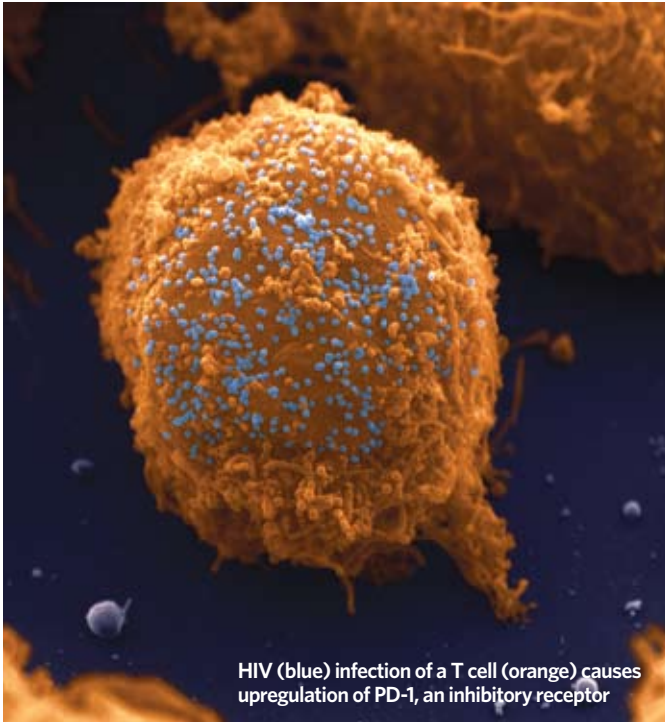


OCTOBER 2008

# The Literature



HIV (blue) infection of a T cell (orange) causes upregulation of PD-1, an inhibitory receptor

## HOT PAPERS

### Impeding PD-1

The discovery that blocking an inhibitory immune receptor restores T cell function in HIV sheds light on immune dysfunction.

By Megan Scudellari

**P**rogrammed death I (PD-1), an inhibitory receptor on immune system cells, has long been known to play an important dual role in immune regulation: Preventing the immune system from attacking the self and keeping an activated immune system in check.

In 2006, however, Rafi Ahmed, of the Emory University School of Medicine, and colleagues discovered that the PD-1 pathway could also be exploited by pathogens to repress normal T cell function during chronic viral infection. In mice infected with lymphocytic choriomeningitis virus (LCMV), Ahmed found PD-1 upregulated in functionally-impaired CD8 T cells. The team then showed that preventing PD-1 activation by blocking its ligands with antibodies restored T cell function and decreased viral load in the mice.<sup>1</sup> Scientists had previously believed that T cells suffered irreversible dysfunction during chronic disease. Suddenly, there was the possibility of rescue.

The research was immediately extended to human chronic viral disease. Seven months after Ahmed's paper was published, a team of researchers led by Bruce Walker at Partners AIDS Research Center (PARC) in Boston published this month's Hot Paper. They showed that PD-1 is up-regulated on the surface of T cells cultured from individuals infected with HIV, and its expression correlates positively with viral load and inversely with CD4 T cell count.<sup>2</sup> T cells that unsuccessfully attack the virus start to express high amounts of PD-1, says Gordon Freeman, a professor of medicine at Harvard Medical School and co-author on the paper. "It's like putting a red flag on a T cell saying bind me and stop me;" once PD-1 is expressed, ligands PD-L1 and PD-L2 bind the receptor, activating inhibitory signals in the cell.

As in Ahmed's study, Walker's investigation found that blocking the pathway with an antibody to PD-L1 restored function to exhausted T cells in vitro. "The results from the HIV study were very robust and very exciting," says John Wherry, a co-author on the paper from the Wistar Institute in Philadelphia. Shortly after, many labs began HIV studies of PD-1, says Wherry, and the data began piling up.

### Other inhibitors

In 30 to 40 subsequent papers, says Ahmed, upregulated expression of the PD-1 pathway has been firmly documented as a mechanism of T cell dysfunction in the three main human chronic viral infections – HIV, hepatitis B, and hepatitis C – as well as SIV infection in non-human primates.

Further investigations of PD-1 expression on exhausted T cells led to the discovery of a plethora of inhibitory pathways active during chronic infection. Last year, Ahmed and Wherry examined the molecular signature of T cell exhaustion by comparing gene expression profiles of infected and functional CD8 T cells in mice.<sup>3</sup> Microarray data not only revealed "striking" levels of PD-1 expression on the chronically infected T cells, says Wherry, but expression of a whole set of inhibitory genes, including those for known inhibitory receptors like 2B4, Ly49 family members, and GP49B. "The immune system has evolved multiple layers of negative regulation," says Wherry.

Around the same time, researchers identified a second reversible pathway involved in HIV T cell inhibition.<sup>4</sup> In the study, Daniel Kaufmann, an instructor at PARC, and colleagues showed that blocking CTLA-4, another inhibitory immunoregulatory receptor, ▶

Data derived from the Science Watch/Hot Papers database and the Web of Science (Thomson ISI) show that Hot Papers are cited 50 to 100 times more often than the average paper of the same type and age.

C.L. Day et al., "PD-1 expression on HIV-specific T cells is associated with T cell exhaustion and disease progression," *Nature*, 443:350–4, 2006. (Cited in 201 papers)

## THE LITERATURE

can also reverse T cell dysfunction in chronic infection. They noted that in HIV infection, CTLA-4 is selectively upregulated in CD4 T cells but not CD8 cells, demonstrating that different types of T cells utilize different combinations of inhibitory pathways.

### Missing pieces

How PD-1 blocks T cell activation is still an open question, says Ahmed. Researchers know that PD-1 has two tyrosine molecules on its cytoplasmic tail that get phosphorylated when PD-1 binds a ligand. The tail then binds phosphatases in the T cell, triggering a downregulation of antigen receptor signaling. But few additional details are known, says Freeman.

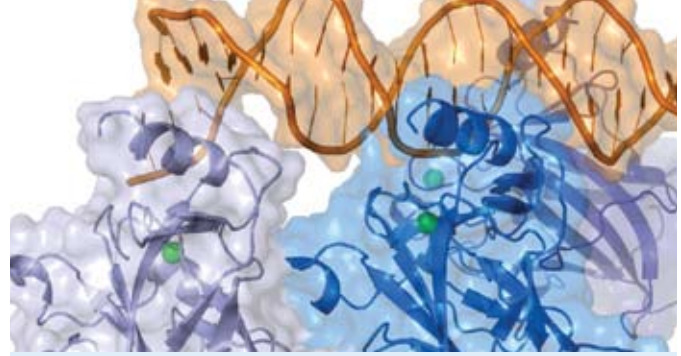
Recent research seems only to be further muddying the waters. Last year Freeman and colleagues implicated an additional co-stimulatory molecule in the inhibition pathway, B7-1.<sup>5</sup> B7-1 binds the PD-1 ligand, PD-L1, as well and also induces an inhibitory signal in T cells. This suggests that B7-1 and PD-1 may compete for binding of PD-L1. "It complicates the whole pathway in vitro," says Ahmed. Freeman agrees, and notes that because PD-L1 binds two inhibitory receptors, it may play a more crucial role in immune response than previously believed. This also may explain the greater immune salvaging effect of an anti-PD-L1 blockade compared to an anti-PD-L2 blockade, a result previously attributed to differential expression of the molecules.

Also, this past July researchers revealed the crystal structures of the PD-1/PD-L1 and PD-1/PD-L2 complexes.<sup>6,7</sup> The structural data shows the binding interfaces between PD-1 and its two ligands, vital information for developing improved ways to manipulate their interaction: Some research groups are already using this structural data to develop ligands with higher affinity as immunosuppressants, says Freeman.

But before investigating molecular mechanisms, researchers are focused on turning the research into therapy. The next step is to determine the side effects of blocking the PD-1 pathway in vivo. "All indications are if you block it, the immune system will be more active. The big question is if you do that, is it safe?" says Freeman. Ahmed and his team are beginning experiments in non-human primates with SIV. He expects results on in vivo blockades to be published within the next 6-12 months. "Those papers will be extremely critical in taking [the research] to the next step," says Ahmed. ■

### REFERENCES

1. D.L. Barber et al., "Restoring function in exhausted CD8 T cells during chronic viral infection," *Nature*, 439:682-7, 2006.
2. C.L. Day et al., "PD-1 expression on HIV-specific T cells is associated with T cell exhaustion and disease progression," *Nature*, 443:350-4, 2006. (Cited in 201 papers)
3. E.J. Wherry et al., "Molecular signature of CD8+ T cell exhaustion during chronic viral infection," *Immunity*, 27:670-84, 2007.
4. D.E. Kaufmann et al., "Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction," *Nat Immunol*, 8:1246-54, 2007.
5. M.J. Butte et al., "Programmed Death-1 Ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses," *Immunity*, 27:111-22, 2007.
6. D.Y. Lin et al., "The PD-1/PD-L1 complex resembles the antigen-binding Fv domains of antibodies and T cell receptors," *Proc Natl Acad Sci*, 105:3011-6, 2008.
7. E. Lazar-Molnar et al., "Crystal structure of the complex between programmed death-1 (PD-1) and its ligand PD-L2," *Proc Natl Acad Sci*, 105:10483-8, 2008.



## HOT PAPER IN GENOMICS

# Factor tracker

### The paper:

C.-L. Wei et al., "A global map of p53 transcription-factor binding sites in the human genome," *Cell*, 124:207-19, 2006. (Cited in 184 papers)

### The technique:

Yijun Ruan, of the Genome Institute of Singapore, and colleagues wanted a better way to study where transcription factors (TFs) bind to DNA. They developed a genome-wide technique using chromatin immunoprecipitation (ChIP), which isolates DNA bound to proteins such as transcription factors. They then sequenced the DNA using a novel paired end ditag (PET) method which tags both 3' and 5' end of the DNA.

### The finding:

Using the tool called ChIP-PET, on p53, Ruan et al. identified 542 binding sites, 98 of which were located on previously unidentified genes.

### The follow-up:

Since 2006, ChIP-sequencing methods, like ChIP-PET, have become commonly used, says Mark Biggin, at the Ernest Orlando Lawrence Berkeley National Laboratory, for studying whole genome binding of TFs controlling development, speech, and language. Other researchers have gone on to show that only 160 of the 542 sites in this study are actual p53 binding sites that control 122 genes (*Nat Rev Mol Cell Biol*, 9:402-12, 2008).

### The future:

To examine the remaining 382 binding sites not associated with genes, called distal binding sites, Ruan's group developed a method called ChIA-PET, short for chromatin interaction analysis. By capturing the distal TF binding sites that loop around to interact with other regions of DNA, Ruan hopes to uncover whether these sites are merely parking spaces for inactive TFs or act on DNA directly.

—Edyta Zielinska

	Binding sites	No. Associated Genes
Previous studies	~50-100	24
ChIP-PET	542	122 (98 novel)
Riley et al. validation	160	122