HIV

Bad news for stop–start therapy?

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An HIV-infected patient who was being treated with anti-retroviral drugs in a 'stop–start' protocol has become infected with a second HIV strain, raising questions about both the treatment strategy and vaccine development.

"This is terrible news," was the response of one senior HIV immunologist at the Barcelona AIDS conference this July, on hearing the results now published on page 434 of this issue by Altfeld and colleagues[1]. These authors had studied a patient who was treated with anti-retroviral drugs soon after becoming infected with HIV-1; this therapy achieved good control of his HIV-1 levels. Later, according to the 'supervised treatment interruption' (STI) protocols developed by Walker and colleagues[2], treatment was deliberately stopped, and then restarted when virus levels rose. This stop–start procedure was repeated twice more, for a total of three treatment-free periods. Altfeld *et al.* found that, during the first two of these periods, the patient's immune system controlled replication of the virus well, if only for a few months. This stands in marked contrast to the situation seen in HIV sufferers who receive anti-retroviral drugs only late in infection; here, within two weeks of stopping therapy, virus levels normally rebound to those seen before treatment.

Viral control during STI is thought to occur through enhancement of the immune response to HIV-1, particularly the response of T lymphocytes that kill HIV-infected cells (these lymphocytes are characterized by expression of the surface protein CD8), and possibly that of 'helper' T lymphocytes (which express the alternative surface protein CD4)[2]. Indeed, the $CD8^+$ T cells from the patient studied by Altfeld *et al.* showed an excellent response, with some 6% recognizing and proliferating in response to HIV-1 proteins. His $CD4^+$ T cells also proliferated markedly when exposed to HIV proteins *in vitro* — unusual in HIV-infected people.

So what's the bad news? Things started to go wrong during the third treatment interruption. Virus levels rebounded more rapidly than before, and the response of $CD4^+$ T cells deteriorated. On further investigation, Altfeld *et al.* found that a new virus variant had emerged towards the end of the second treatment-free period. Although from the same HIV-1 'family' as the virus causing the original infection — namely the B clade, which predominates in the Western world — this new variant was quite different. The authors carried out a detailed study of 16 regions (epitopes) of HIV-1 that are recognized by $CD8^+$ T cells, and found that 7 differed by at least one amino acid between the original and the new virus; the T cells could not detect these new epitopes. The other 9 epitopes were the same, and the responses of $CD8^+$ T cells to them were maintained.

Further analysis of the sequence of the second virus showed that this variant was a distinct strain, probably a new infection (a superinfection) — indeed, the patient reported a recent episode of sexual exposure that was followed by a fever-associated illness. If this is the case, then superinfection took place despite the patient's strong HIV-specific immune response. There is, however, a small chance that the second virus was there from the beginning, possibly hiding in lymph nodes. Although in men the usual pattern is of initial infection with a single virus strain, it is not uncommon for women to be infected with multiple strains from the start — an intriguing example of gender-specific biology in HIV-1 infection[3].

But why would a superinfection be such terrible news? It is widely held that $CD8^+$ T cells play the major role in controlling HIV replication during a long-lasting infection. This notion is firmly based on evidence such as the rise in viral count that occurs after CD8-blocking antibodies are infused into macaques infected with SIV, the simian version of the virus[4], and viral mutation to escape $CD8^+$ T cells in HIV and SIV infections[5]. These data, together with findings that sex workers who are frequently exposed to HIV seem to be resistant to HIV infection and generate anti-HIV responses through $CD8^+$ T cells[6], have spurred efforts to develop preventive and therapeutic vaccines that stimulate this type of immune response. For instance, vaccinating macaques to induce SIV-specific responses of $CD8^+$ T cells enables the animals to control infection more effectively after challenge with an aggressive SIV[7]. So the findings of Altfeld *et al.*[1] might be worrying news for vaccine developers — don't they show that HIV infection can occur in the face of substantial activity of $CD8^+$ T cells?

The answer is, not necessarily. First, we do not know the denominator. Recent cases of superinfection have attracted attention[8], but there may be many more HIV-infected people who can repel an attempted superinfection. Moreover, studies of primates suggest that superinfection is rare: for instance, macaques infected with an attenuated strain of SIV that induces a strong cellular immune response are resistant to infection with more virulent strains[9].

Even if the case studied by Altfeld *et al.* turns out to be representative, there are reasons to think that the immune activity generated by a healthy person in response to an HIV vaccine will be qualitatively distinct from that of an HIV-infected person — especially one whose immune system is already damaged by more than three years of infection. For instance, immune impairment can be seen from the very earliest stages of HIV-1 infection, particularly among $CD4^+$ T cells; and increases in virus levels after treatment interruption are probably associated with preferential infection of HIV-specific $CD4^+$ T cells[10]. So the response of $CD4^+$ T cells was probably already weakened, before superinfection, in the patient studied by Altfeld *et al.* — indeed, the cells' proliferation when exposed to viral proteins *in vitro* did decline at about the time that the new virus appeared.

$CD4^+$ helper T cells may have direct anti-HIV effects, and might also influence the effectiveness of $CD8^+$ T cells. Although this patient's $CD8^+$ T cells still recognized roughly half of the epitopes in the new virus[1], including one that stimulated the strongest immune response, it seems that the cells failed to protect against superinfection. HIV-specific $CD8^+$ T cells in infected people differentiate in an unusual way: they produce little of the membrane-puncturing molecule perforin, and their ability to specifically kill infected cells is consequently reduced[11]. And the dominant response is not necessarily the most protective, as illustrated by mice infected with lymphocytic choriomeningitis virus[12]. Finally, the specificity of the responses of $CD8^+$ T cells to SIV and HIV molecules is different in vaccinated macaques[13] and highly exposed but apparently HIV-resistant sex workers[14] compared with infected animals and people[15]. So, the superinfection reported by Altfeld *et al.* might have occurred in the context of a very different immune response from that produced by vaccination of an uninfected person, in terms of T-cell specificity, the competence of $CD8^+$ T cells and the strength of $CD4^+$ helper T cells.

The new findings[1] may in fact be worse news for the STI strategy than for vaccine development. The usefulness of this strategy very early in infection remains controversial[10]. A few patients have achieved remarkable control of HIV-1 and have discontinued therapy entirely, but this seems rare[2], and as yet there has been no formal double-blind controlled clinical trial — the bedrock of evidence-based medicine. In one study of STI in a long-lasting HIV-1 infection, viral control was not improved despite the enhancement of HIV-specific immune responses[16]. The results of Altfeld *et al.* suggest that superinfection with a second HIV strain during a period off therapy could significantly undermine viral control,

so patient commitment to safe sex practices will be an important adjunct to STI. It is not clear whether superinfection is only a risk during treatment interruption: more studies on this are needed.

Altfeld *et al.*'s work[1] is a beautiful illustration of the power of modern techniques to explore the minute details of a virus-specific immune response. But the effort required will preclude studies of large numbers of patients. Certainly, this single-patient analysis raises many questions, but whether the news is bad, neutral or even good remains to be seen. Although causing a brief pause for thought, nothing here should slow or divert efforts to develop an HIV vaccine. ■

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1. Altfeld, M. *et al. Nature* **420,** 434–439 (2002).
2. Rosenberg, E. S. *et al. Nature* **407,** 523–526 (2000).
3. Long, E. M. *et al. Nature Med.* **6,** 71–75 (2000).
4. Schmitz, J. E. *et al. Science* **283,** 857–860 (1999).
5. Klenerman, P., Wu, Y. & Phillips, R. *Curr. Opin. Microbiol.* **5,** 408–413 (2002).
6. Rowland-Jones, S. L. *et al. J. Clin. Invest.* **102,** 1758–1765 (1998).
7. Amara, R. R. *et al. Science* **292,** 69–74 (2001).
8. Jost, S. *et al. New Engl. J. Med.* **347,** 731–736 (2002).
9. Daniel, M. D. *et al. Science* **258,** 1938–1941 (1992).
10. Douek, D. C. *et al. Nature* **417,** 95–98 (2002).
11. Appay, V. *et al. J. Exp. Med.* **192,** 63–75 (2000).
12. Gallimore, A. *et al. J. Exp. Med.* **187,** 1647–1657 (1998).
13. Ferrari, G. *et al. Immunol. Lett.* **79,** 37–45 (2001).
14. Kaul, R. *et al. J. Clin. Invest.* **107,** 1303–1310 (2001).
15. Vogel, T. U. *et al. J. Immunol.* **169,** 4511–4521 (2002).
16. Oxenius, A. *et al. Proc. Natl Acad. Sci. USA* **99,** 13747–13752 (2002).

Circadian rhythms

The cancer connection

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The *Per2* gene is a core component of the circadian clock in mammals. It now seems that the mouse *Per2* gene is also involved in suppressing tumours, through other genes that affect cell proliferation and death.

If the mantra in real estate is 'location, location, location', in genetics it would be 'phenotype, phenotype, phenotype'. There is simply no substitute for a detailed phenotypic analysis of a mutant strain (study of the overt manifestation of a mutated gene in the organism). This has the potential to reveal unanticipated — and sometimes truly surprising — relationships between genotype and phenotype, or between a primary phenotype and a secondary one. Such was the case for an analysis published recently in *Cell* by Lee and colleagues[1].

The organisms under study here were mice in which both copies of the *mPer2* gene were mutated — a genotype shown previously[2] to cause a strong defect in circadian rhythms. Most organisms have endogenous 'clocks' that control rhythms of physiology and behaviour with roughly 24-hour (circadian) periodicity. But the period is shortened, and rhythmicity is lost, in the *mPer2* mutant mice. This is reminiscent of the effects of the original *perS* or *per0* mutations in fruitflies, described in the 1971 landmark paper from Konopka and Benzer[3].

By observing their mutant mice for a couple of years, however, Lee and colleagues[1] made an unexpected discovery: the animals were unusually cancer prone. At six months of age they began to show excessive cell proliferation in the salivary glands, as well as teratomas — tumours that originate from germ cells and comprise a mix of cell types. Thirty per cent of the mutant mice died before the age of 16 months, half of these from spontaneous lymphomas. In contrast, such lymphomas were first found in normal mice at the age of 20 months, a highly significant difference. The mutant animals were also more sensitive to γ-radiation, as indicated by premature hair greying and hair loss, and an increased rate of tumour formation — this last effect stemming, at least in part, from a decreased likelihood of cell death (apoptosis) in response to radiation.

So, what could be the story here? The current picture of the central circadian clock in animals is of a self-sustaining transcription–translation feedback loop, involving the transcription of key clock genes, their translation into protein, and the proteins' repression of transcription of the same key genes[4] (as well as of downstream, clock-controlled genes). In fact, given the importance of post-translational mechanisms — such as protein phosphorylation and turnover — and the lack of translational control in the current picture, it might be more accurate to describe it as a macromolecular feedback loop. In any case, the mPer2 protein is a key clock component: it contributes to the circadian regulation of transcription of both *mPer2* and downstream genes[5].

All of which begs the question: is there a tight relationship between γ-radiation and clock genes? And could disruption of circadian transcriptional regulation cause the defects in cell proliferation and death (together termed cell growth) seen even in the absence of γ-radiation in *mPer2* mutant mice? In other words, is there a transcriptional cascade from clock genes, to downstream growth-control genes, to growth-effector genes? The answers all appear to be yes.

Lee and colleagues' results show that, in normal mice, the expression of several core clock genes was rapidly and potently upregulated in the liver in response to γ-radiation. But in the mutants this response was absent or severely attenuated. Even more surprising was the authors' analysis of a few key genes concerned with cell growth. They found that expression of the *Myc* gene, as judged by levels of its messenger RNA, was circadian in wild-type liver; but in the mutants the expression pattern was modestly shifted and levels of *Myc* mRNA were dramatically increased. Moreover, experiments in cultured cells suggested that *Myc* transcription is directly regulated by the circadian clock. The authors also looked at the expression of *cyclin D1* and *Gadd45α*, two *Myc*-regulated mRNAs, and found that the levels of both fluctuated in a circadian pattern in wild-type livers; in the mutants, both patterns were altered.

So Lee and colleagues propose that the key effect of inactivating *mPer2* is to derepress *Myc* expression, leading to excessive cell growth and tumour formation. The effect is exacerbated by γ-radiation, which normally upregulates clock genes and thereby presumably leads to *Myc* repression. This fails in the *mPer2* mutants. If these proposals are true, there are some testable predictions. First, overexpresssing *Myc* in an otherwise normal genetic background should have the same growth-promoting effects as mutating *mPer2*. Second, and more important, inhibiting *Myc* expression should suppress tumour formation in *mPer2* mutants.

One caveat is that all of these experiments were performed under conditions of 12 hours' light, 12 hours' darkness, so it could be that the mRNA cycles were merely light-driven, not clock-driven. In this context, it is notable that *Myc* and *Gadd45α* were not identified as cycling genes (although *cyclin D1* was) in three out of four microarray studies of liver mRNAs[6–9]. But the marked effects of the *mPer2* mutation suggest that, at the very least, there is a strong connection between cell growth and the circadian clock. The discrepancy also reinforces the importance of taking microarray data — especially negative data — with a pinch of salt. In our opinion, careful biochemical analyses are more credible.

More generally, the new results[1] have brought into proximity two previously disparate fields of study: circadian rhythms and cell-growth control. One reason why they have hitherto been infrequent bedfellows is that the mammalian circadian rhythm field has historically focused on the brain and, more narrowly, on the suprachiasmatic nucleus — the region of the hypothalmus that is essential for directing cycles of loco-