Title

Cure by killing

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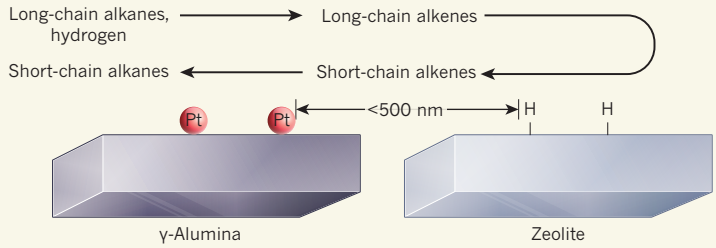

Figure 2 | Spacing of active sites in hydrocracking catalysts. Alkane molecules that have long carbon chains are reacted with hydrogen in the presence of a solid catalyst to produce shorter alkane molecules, a process called hydrocracking. The catalyst consists of a noble metal (such as platinum, Pt) on a γ-alumina support, and a porous solid called a zeolite, which contains acid sites (indicated by hydrogen atoms). The metal converts long-chain alkanes into long-chain alkene intermediates, which then diffuse to the acid sites, where they are isomerized and cracked into short-chain alkenes. These alkenes diffuse back to the platinum sites, where they react with hydrogen to form short-chain alkanes. Alkene diffusion between the metal and the acid sites is rate limiting if the distance between the sites is larger than 500 nanometres. Zečević *et al.*[2] have prepared catalysts with improved catalytic selectivity compared with conventional catalysts, by controlling the distance of the acid and metal sites on the nanoscale.

the two catalysts was comparable, and less than 1% by weight. Key to this nanoscale control is the use of specific interactions between the noble metal's precursor and its support. One of the authors' major achievements was to use advanced electron-microscopy techniques to prove that the composite catalysts had identical structural parameters, except for the location of the platinum sites.

Another highlight is the design of the catalytic experiments. By using hydrocarbon feeds with molecules of different carbon-chain length and bulkiness, the authors were able to distinguish the influence of site proximity on catalytic activity and selectivity. They show that conversion of *n*-decane and the longer *n*-nonadecane into cracking products is the same when using the catalyst in which γ-alumina supports the platinum — that is, the intimacy criterion for activity is fulfilled for these reactions. But conversion of pristane, which has bulky molecules, is lower than for the other feeds, which indicates that diffusion of reaction intermediates between the active metal and acid sites limits catalytic activity in this case.

Strikingly, however, more of the desired isomerization products and fewer unwanted side products of cracking are formed from nonadecane than from decane when using the platinum-on-alumina catalyst than when using the platinum-on-zeolite catalyst — that is, the selectivity of the reaction for desirable products is higher when using the former catalyst. But the metal and acid sites are closer together in the latter catalyst than in the former, and so the difference in product selectivity apparently contradicts conventional understanding of the intimacy criterion. This can be explained if longer hydrocarbon molecules spend a greater amount of time in the zeolite's micropores in the second catalyst, and therefore undergo multiple cracking on the acid sites.

When the platinum particles reside on γ-alumina, still in the vicinity of acid sites, the intermediate alkenes formed during hydrocracking diffuse to the zeolite and rapidly isomerize. The authors propose that reactions of the alkenes occur close to the outer surface of the zeolite crystals, from where they can easily be desorbed and transported back to the metal sites on the γ-alumina. This mechanism was previously suggested[4] by these authors to be responsible for the reactions of long-chain hydrocarbons on the outer layers of zeolites that have medium-sized pores, and is now convincingly shown to be valid for the large-pore zeolite studied by Zečević and co-workers.

It is therefore evident that catalytic activity and selectivity depend not only on the distance between the active sites and on the molecular dimensions of the reactants, but also on the accessibility of the sites to molecules and on the transfer rate of molecules between sites. These factors might be even more important when more-complex feedstocks, such as fats and oils from renewable resources, are processed, or when catalysts contain more than two kinds of active site, or complicated pore architectures[5].

In such cases, proving the benefits of nanoscale site intimacy will be considerably more challenging than for the relatively simple hydrocracking of single-component hydrocarbon feeds over a bifunctional platinum–zeolite catalyst studied here.

Two major lessons can be learnt from the present study. First, preparation strategies, modern visualization techniques and in-depth catalytic studies must be combined and directly correlated to improve the efficiency of complex multifunctional catalysts. Second, mass-transfer effects are often prominent and may even govern the conversion of reactants on solid catalysts[6]. They should therefore be carefully considered and optimized — not only for the bulk catalyst or the entire reaction chamber, but also for catalytic sites at the nanoscale. This presents a challenge for both experimentalists and theoreticians. More broadly, Zečević and co-workers' method for controlling the nanoscale structure of hydrocracking catalysts may benefit several other processes that use solid catalysts, including the conversion of renewable resources (such as fats, oils or biomass) into more-valuable products, or 'upgrading' heavy hydrocarbons to more-useful compounds. ■

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HIV

Cure by killing

Two bi-specific protein constructs have been designed that direct the body's T cells to kill HIV-infected cells. The feat provides a step on the path to removing the latent virus reservoir that persists in patients on antiretroviral therapy.

DOUGLAS D. RICHMAN

The development of combination antiretroviral therapy to suppress HIV infection and its complications has been a major achievement of modern medicine. However, these drugs do not eradicate the virus; they only suppress its productive replication cycle in infected cells. This cycle involves the integration of HIV DNA into the genome of a host cell, and the generation of new viral particles that are released by budding from the cell. A small proportion of cells survives the cell-lytic consequences of infection, and goes on to form a latent reservoir of cells that have HIV DNA integrated into host-cell chromosomes.

This reservoir can rekindle a raging infection if antiretroviral therapy is interrupted. Only strategies that eradicate the reservoir will achieve a full cure and allow patients to discontinue a treatment that is costly, inconvenient and has side effects. Two publications, from Sung *et al.*[1] in *The Journal of Clinical Investigation* and Pegu *et al.*[2] in *Nature Communications*, describe innovative molecular constructs designed to selectively kill these rare, latently infected cells.

To be targeted by drugs, infected cells need to display evidence that they are harbouring the virus. For latently infected cells, this requires the virus to be activated to restart its replication cycle. Over the past few years, approaches to the selective activation of latently infected cells have used drugs such as histone deacetylase inhibitors, which are designed to induce the transcription of viral RNA. However, only a small minority of cells responds to these compounds[3–5]. Moreover, destruction of the activated cells may rely on the patient's immune responses, but these are already impaired and could be targeted to act against the initially infecting virus, which may have mutated to escape immune recognition[6].

In attempts to circumvent these problems, Sung *et al.* and Pegu *et al.* describe conceptually similar but different protein constructs that combine the binding specificities of two different antibodies (Fig. 1). One of the antibodies binds to a broad spectrum of HIV envelope proteins, which are displayed on the surface of actively infected cells, typically $CD4^+$ T cells of the immune system; the other binds to the molecule CD3, which is displayed on the surface of all T cells. The rationale is to direct the $CD8^+$ subset of T cells, which has cell-killing (cytotoxic) ability, to kill latently infected cells that have been induced to express envelope proteins. The engagement of CD3 means that any $CD8^+$ T cell can be targeted to the infected cells, obviating the need for the T cell to specifically bind to HIV surface glycoproteins (antigens).

Both groups show that, *in vitro*, their constructs work as intended, inducing direct killing of cells that express envelope proteins, independently of the $CD8^+$ T cells' antigen specificity. The DART (dual-affinity re-targeting) construct described by Sung *et al.* also worked *ex vivo*: it killed cells taken from patients whose infection was well suppressed with antiretroviral therapy after the cells had been induced to express envelope proteins (through exposure to the protein phytohaemagglutinin or the histone deacetylase inhibitor vorinostat). Pegu *et al.* show that the CD3-binding arm of their construct not only activates cytotoxic $CD8^+$ T cells, but also serves to activate latently infected $CD4^+$ T cells to express HIV envelope proteins, thus permitting them to be killed without other inducing factors. However, additional studies will be needed to provide evidence for substantial killing of patient cells *ex vivo* using this construct.

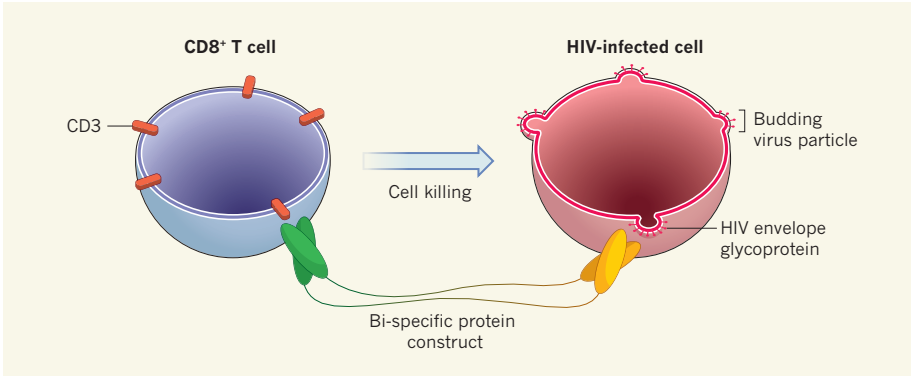

Figure 1 | Bi-specific constructs. Sung *et al.*[1] and Pegu *et al.*[2] present two different protein constructs that combine sections of two monoclonal antibodies: one antibody binds to the CD3 molecule expressed on the surface of all T cells of the immune system, and the other binds to envelope proteins of the HIV virus, which are expressed on the surface of infected cells. These constructs direct $CD8^+$ (cytotoxic) T cells to kill HIV-infected cells, regardless of whether receptors on the $CD8^+$ T cell have specificity for the virus. This targeting is designed to increase the efficiency of the anti-HIV immune response and to help clear rare, latently infected cells.

Many previous investigations have generated bi-specific antibodies against various targets, including antigens from HIV and from tumour cells. Bi-specific antibodies for killing HIV-infected cells were first described in 1991, before potent antiretroviral drug combinations existed and before a cure for HIV was a conceivable goal[7,8], and bi-specific constructs have been shown to kill cancer cells in non-Hodgkin's lymphoma[9]. However, the translation of such antibodies from an innovative molecular construct with *in vitro* activity to an effective treatment is a daunting process — and translation of the new constructs will be no exception.

There are several biological features that represent hurdles to any strategy for HIV cure[10]. Only one in a million $CD4^+$ T cells in HIV-infected individuals is latently infected, which means that these cells are a rare target and are difficult to detect and measure. So far, no agents have been identified that induce HIV antigen expression in the majority of latently infected cells while not affecting non-infected cells. Furthermore, the latent reservoir may include cells in anatomical compartments that large protein constructs cannot penetrate, such as the central nervous system and genital tract.

Moreover, every promising candidate compound faces substantial developmental hurdles before it can become an effective drug. First, the efficacy seen in *in vitro* models may not be replicated *in vivo*. Drugs can be kept at a constant concentration in cell culture, but this is difficult to achieve *in vivo*, where absorption, distribution and clearance of the compound occurs. Second, the target cells and cytotoxic cells may also not be as dense or uniformly distributed in tissues as in cell culture. A third consideration is that no envelope-binding antibody will bind to all HIV envelopes, and the possibility of the virus mutating to escape recognition by the construct must be considered.

Off-target effects may also be a concern. Pegu and colleagues' bi-specific antibody induced a rapid drop in the number of CD3-expressing cells in the bloodstream when infused into infected rhesus macaques, probably owing to the activation and redistribution of the cells. The authors' construct also activates uninfected $CD4^+$ T cells. Finally, protein constructs can induce immune responses, as documented by Pegu and colleagues in monkeys. The anti-construct antibodies generated during such responses might be toxic, and will certainly result in diminishing activity of the drug.

Research into a cure for HIV is in the early stages of a long and difficult path, and all innovative options should be welcomed and investigated. Monoclonal antibodies have been remarkably successful in treating many medical conditions, and constructs that exploit their specificity to generate bi-functional capabilities — such as those presented by Sung *et al.* and Pegu *et al.* — are one such innovation worthy of further pursuit. ■

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