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Finding latent needles in a haystack

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Carvell and colleagues took DNA samples from 537 queens and 2,101 worker bees throughout the 20-km² landscape over 2 years.

One challenge in understanding how landscapes influence social insects such as bumblebees is that surveys of individuals do not necessarily provide accurate information about the number of colonies, which is important for predicting population trajectories. Carvell *et al.* used genetic markers to link the surveyed bees to their colonies; because bumblebees from the same colony are sisters, genetic relatedness can reveal which samples are taken from foragers of the same colony. By documenting the coordinates at which each bee was captured, it is then possible to triangulate the approximate location and foraging range of each nest, and to estimate nest density across the landscape. In addition, the authors go a step further than previous genetic studies that analysed bumblebee-colony density and foraging distance within single seasons, tracking relatedness from queen to worker to queen, both within generations and between them, and analysing the dispersal of queens across the landscape over the years.

Carvell and colleagues' primary finding is that the quality of the habitat that surrounds nest sites affects lineage survival between years (Fig. 1). Beneficial features include: mixed semi-natural vegetation (for example, field margins sown with flowers); spring flowers from trees and hedgerows that are good for queens early in the season; and summer flowers that are good for workers. By examining the spatial distributions of these resources around estimated colony locations, the authors quantify this finding, suggesting that having sufficient floral resources within about 1 km of each colony increases queen production and survival. A comparison of samples taken across seasons also clearly demonstrates the value of persistent floral resources for queens and workers throughout the colony life cycle, and of suitable hibernation and nesting sites to facilitate successful overwintering and founding of the next generation.

The results extend those from other studies^{7,8}, which found that having semi-natural habitat and floral richness at similar spatial scales promotes colony growth, density and persistence within a season. No previous studies, to my knowledge, have tracked colony and queen survival within and between generations — a major component of year-to-year bumblebee population dynamics.

One unanswered question is whether remaining in the same patch of land over generations is necessarily ideal for a bumblebee colony. If high-quality restored habitat is nested within a poor-quality landscape that impedes queen emigration or immigration from other high-quality patches, this could promote inbreeding in the long term. Carvell *et al.* find some evidence that high levels of non-crop land cover can promote queen

dispersal into the broader landscape, but this is a subject in need of more research.

Given the large geographical ranges in which individual bumblebee species are found and the potential for long-distance queen dispersal, even the 20-km² landscape studied by Carvell and colleagues represents only a small fraction of each species' total population⁹. One could therefore imagine that a network of restored habitats across a broader agricultural landscape, each with a high density of season-long floral resources, would both maintain connectivity and boost local pollinator populations. But establishing the benefit of such a management scheme for pollinators and crops will require the non-trivial effort of expanding and replicating the current study in other landscapes.

Declines in bumblebee populations are increasingly recognized by both the public and policymakers, resulting in several notable conservation actions — including the recent proposed federal listing of a bumblebee species as endangered in the United States¹⁰ (although at the time of writing, this listing has been delayed). Carvell *et al.* have provided valuable information for landscape managers about restoration activities that could complement such conservation strategies by enhancing agricultural landscapes for wild pollinators.

Their study focuses on land-use effects for just three species, but the detailed data set provides a framework that can be expanded to more species and larger areas. It could also be used to investigate the effects of pesticides, pathogens and other factors on multigenerational life-cycle dynamics, with the goal of identifying a realistic way to optimize land-use heterogeneity for bumblebee populations. ■

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HIV

Finding latent needles in a haystack

Antiretroviral therapy can keep HIV at bay, but a few cells remain infected, so the disease cannot be cured. The discovery of a protein that marks out these infected cells will facilitate crucial studies of this latent viral reservoir. SEE LETTER P.564

DOUGLAS D. RICHMAN

The development of combination antiretroviral therapy, which suppresses the replication of HIV in immune-system cells called CD4 T lymphocytes, is a major achievement of modern medicine¹. However, a small proportion of these CD4 T cells retains the virus despite ongoing therapy, forming a latent HIV reservoir that can reactivate if treatment is stopped. Ways of eradicating this reservoir are an intense focus of research, but efforts have been hampered by the difficulty of analysing latently infected cells — of the order of one in a million CD4 T cells in an individual receiving antiretroviral therapy is latently infected, and markers for these cells have been lacking^{2,3}. On page 564, Descours *et al.*⁴ have identified just such a marker for about half of the latently infected CD4 T cells in the blood.

During its replication cycle, HIV generates, through reverse transcription, a DNA version of its RNA genome, which integrates into a host-cell chromosome. Replication of the HIV genome in CD4 T cells produces viruses that destroy the host cells. The rare cells that survive infection constitute the latent reservoir. HIV DNA remains integrated in their genomes, but might not produce viral RNA or protein.

To analyse this reservoir, the investigators developed an *in vitro* model of HIV latency using CD4 T cells infected with an HIV-derived genetic construct that produced green fluorescent protein (GFP) as a marker of infection. An important point to note when considering this study is that the cells used were in a resting state — they were not activated or proliferating as are most CD4 T cells propagating high levels of HIV. An unresolved

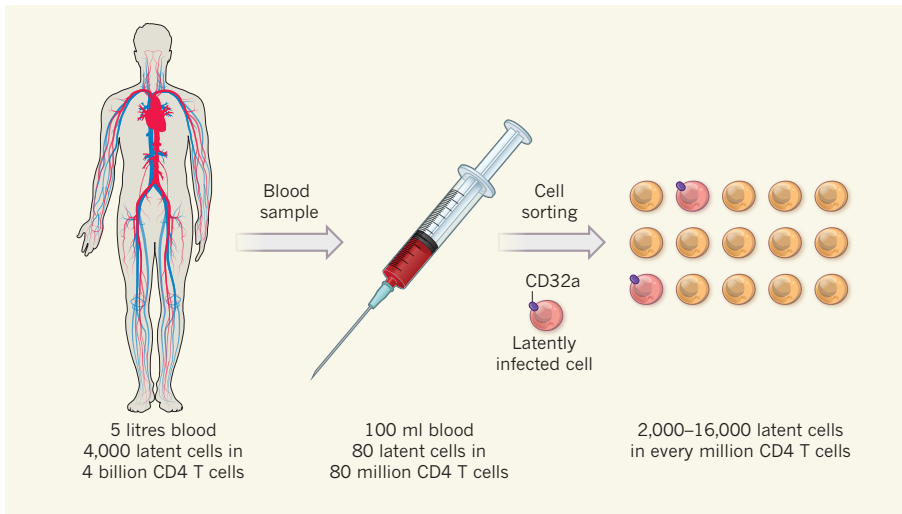


Figure 1 | Hunting down an elusive cell population. A person chronically infected with HIV and receiving antiretroviral therapy has about 200 billion immune cells called CD4 T cells, of which the order of one in a million remains infected with a latent, replication-competent form of HIV despite treatment^{2,3}. About 2% of the body's CD4 T cells (about 4 billion) are present in the approximately 5 litres of blood in an adult — 100 millilitres, as might be extracted in a blood sample in human studies, therefore contains roughly 80 million CD4 T cells, of which only about 80 are latent cells. Descours *et al.*⁴ identified the protein CD32a as a marker of about 50% of all latently infected CD4 T cells. By using a sorting technique to separate cells expressing the protein from other cells, they can selectively concentrate the latent population by more than 1,000-fold to between 2,000 and 16,000 in a million CD4 T cells, making many new experimental studies of this population feasible.

controversy has been whether latency results from infection of resting cells or (in line with the predominant view) is a rare consequence of a cell surviving active HIV replication, and then converting to a resting state⁵. The current study should prompt more investigation to address this issue.

Descours *et al.* screened the infected, GFP-expressing cells using ultradeep RNA sequencing to search for host gene transcripts that were increased in this population compared with uninfected cells. They identified 103 differentially expressed genes, including 16 that encode transmembrane proteins, which make cell markers that are readily amenable to identification and cell-sorting techniques. Of these, the most highly expressed was *FCGR2A*, which encodes the protein CD32a.

CD32a is a low-affinity receptor for a fragment, Fc, of immunoglobulin G (IgG) antibodies, and, like other Fc receptors, is expressed on the surface of dendritic cells and macrophages⁶. These immune cells internalize Fc receptors, along with their bound IgGs and the specific antigen molecules that associate with the IgGs, and then present the processed antigens to lymphocytes. This triggers an immune response specifically against cells expressing that antigen⁶. Descours and colleagues showed that CD32a is also expressed on about 50% of latently infected CD4 T cells — but not on uninfected T cells or those with an active HIV infection. CD32a can be bound by a commercially available antibody, enabling the authors to separate CD32a-expressing CD4 T cells from other cells.

This highly selective marker for latently infected CD4 T cells could at last allow researchers to investigate the mysterious mechanisms of latency, without needing to find the one cell of interest in a million (Fig. 1). The marker might also be helpful in analysing the success of candidate drugs that aim to target this reservoir. Isolation of CD32a-expressing CD4 T cells would concentrate the population of latently infected cells by about 1,000 times. But although this process would

“This highly selective marker for latently infected T cells could at last allow researchers to investigate the mysterious mechanisms of latency.”

remove the vast majority of irrelevant cells, a large amount of blood would still be needed to obtain enough latently infected cells for analysis — 100 millilitres or more, depending on the type of investigation. As with any breakthrough, new questions arise and new experiments become feasible. First, Descours and colleagues raise an interesting question: can CD32a be used to selectively target the rare, latently infected cells? This might be a basis for strategies aimed at eradicating the latent reservoir. One problem, however, is that CD32a is a marker for only 50% of the reservoir, whereas the eradication of latent HIV would require a much greater reduction in the number of latently infected cells in the body.

Moreover, targeting CD32a would also make the antigen-presenting cells that normally express CD32a vulnerable to destruction, which might well cause unwanted or harmful side effects.

Second, the authors studied CD4 lymphocytes from the blood, but these circulating cells account for 2%, at most, of the CD4 T cells in the body⁷. It remains to be seen whether CD32a is as good a marker for latently infected cells in the lymph nodes, bone marrow, gut and other tissues. Perhaps more markers could be identified from the 103 differentially expressed genes found in the researchers' screen — analysis of these proteins in combination with CD32a might increase the total proportion of identifiable latent cells.

Third, does the presence of CD32a vary between latently infected CD4 T-cell subsets? CD4 T cells differentiate into a complex variety of specialized subsets, some of which may not express CD32a when latently infected. It would also be interesting to characterize the mechanism by which latent HIV — thought not to be transcribed — can increase transcription of *FCGR2A* and other differentially expressed genes. Such an insight might help to reveal how HIV latency is maintained or reversed. Another question that merits careful investigation is whether CD32a is ever expressed on lymphocytes other than those latently infected with HIV.

Finally, the antibody used by the authors does not react with the CD32a protein found in rhesus macaques, which are used as a model of latency with simian immunodeficiency virus. An alternative antibody must therefore be produced if this finding is to be exploited for studies in monkeys.

A good understanding of HIV latency and interventions to manage it would provide remarkable benefits, but the field has so far progressed too slowly to capitalize on this. This potentially seminal study promises to facilitate the study of HIV latency and accelerate the generation of insights and therapeutic approaches. ■

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