retroviral sequences called retroelements[5,6]. The fifth protein was SETDB1, a methyltransferase enzyme that can modify histone proteins and interact with the HUSH complex to block the transcription of newly integrated retroviral DNA[4–6].

The authors tested the function of each of these proteins in human cells grown *in vitro* that were infected with murine leukaemia virus, a model retrovirus that was used in early attempts at retroviral gene therapy[7]. They found that NP220 binds unintegrated retroviral DNA (Fig. 1) and, on binding, recruits the HUSH complex. This complex then recruits SETDB1 to deposit methyl modifications on histone proteins bound to viral DNA. These histone modifications are associated with the suppression of gene expression. Zhu and colleagues also discovered that NP220 recruits two enzymes, called HDAC1 and HDAC4, from a family of enzymes called histone deacetylases, which catalyse the removal of acetyl groups from histones. A decrease in the level of histone acetylation can repress gene expression.

The authors tested the effect of depleting either NP220 or HDAC1 and HDAC4 in human host cells infected with different types of retrovirus. For example, they tested HIV, which is from a different genus of retroviruses from that of murine leukaemia virus, and found that the depletion of these components caused an increase in gene expression from the unintegrated retrovirus. However, the depletion of HUSH-complex components or SETDB1 did not cause increased expression of the unintegrated viral sequence in this scenario. A similar pattern of results was obtained when the authors conducted the same type of study using Mason–Pfizer monkey virus, which belongs to yet another genus of retroviruses. This suggests that the role of NP220 is evolutionarily conserved, but that the proteins that might act with NP220 to silence viral gene expression can vary depending on the retrovirus.

NP220 can bind sequences in double-stranded DNA that are rich in the nucleoside cytidine[3]. Zhu and colleagues report that there are sequences rich in the DNA building block cytidine in repeat sequences called long terminal repeats (LTRs) at the ends of murine leukaemia virus, HIV and Mason–Pfizer monkey virus sequences, and that these cytidine-rich LTR sequences can serve as binding sites for NP220. When the authors tested the effect of depleting NP220 in human cells infected with Rous sarcoma virus (from another retrovirus genus), which has cytidine-poor LTRs, this depletion did not affect the gene expression of unintegrated viral DNA. This suggests that NP220 needs cytidine-rich DNA sequences to bind and silence viral genes. However, the NP220-independent silencing of gene expression of unintegrated Rous sarcoma viral DNA was found to be SETDB1 dependent because the deletion of the gene encoding SETDB1 led to increased gene expression of the viral DNA.

It would be interesting to learn more about how unintegrated Rous sarcoma virus is silenced through this NP220-independent mechanism. Together, these studies reveal that NP220 or its interacting partners can silence unintegrated DNA from a range of retroviruses.

The HUSH proteins and SETDB1 are involved[4–6] in the transcriptional silencing of integrated retroviral elements. Whether NP220 also acts to silence the expression of integrated retroviral DNA remains to be determined. This is a possibility, given that the cytidine-rich binding motifs for NP220 are preserved in viral LTRs after their integration into a host genome. Clearly, there is more to investigate about the silencing mechanism uncovered by Zhu and colleagues.

The high level of evolutionary conservation of NP220 proteins across vertebrate species is noteworthy. The origin of retroviruses is at least as ancient as that of vertebrates[8]. Perhaps NP220 represents an ancestral defence system to tackle invading retroviruses. But, in turn, some retroviruses could have evolved mechanisms to evade such gene silencing, which might explain why Rous sarcoma virus has lost cytidine-rich LTRs that would provide NP220 binding sites. Zhu and colleagues' study might inspire new ways to prevent the silencing of foreign DNA for use in gene-therapy applications. Importantly, it also provides clues to the evolutionary arms race between humans and retroviruses, and offers insights into a mechanism that might offer protection against disease-causing viruses. ■

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IMMUNOTHERAPY

Adrenaline fuels a cytokine storm

Attempts to boost the body's antitumour immune responses can trigger a harmful inflammatory reaction called a cytokine storm. New insights into the mechanisms involved might help to prevent this problem. SEE LETTER P.273

STANLEY R. RIDDELL

Many newly developed, potent cancer therapies aim to harness an immune response to target tumours[1]. However, a common problem with such immunotherapy approaches is the development of a severe inflammatory response called a cytokine storm[2,3], in which levels of proteins called cytokines become abnormally high. This results in fever, low blood pressure, heart problems and, in some cases, organ failure and death. There is therefore great interest in understanding the underlying mechanisms to develop ways of preventing cytokine storms without altering the effectiveness of anti-cancer treatments. On page 273, Staedtke *et al.*[4] reveal that the protein ANP can block cytokine storms, and they uncover a self-amplifying production loop in immune cells that generates a class of molecule called catecholamines, which includes the hormone adrenaline (also known as epinephrine). They report that this catecholamine production helps to initiate and maintain cytokine storms.

When immune cells recognize a molecule that indicates a possible threat, they release cytokines that promote inflammation and orchestrate host defence[5]. One antitumour treatment that can trigger a cytokine storm uses a bacterium called *Clostridium novyi*-NT, which tracks to the low-oxygen environments found in certain tumours and releases spores that cause tumour-cell death[6]. Determining the correct bacterial dosage is difficult, and mice that have large tumours and receive a high dose of *C. novyi*-NT often develop a fatal cytokine storm that cannot be prevented by using inhibitor molecules to block the actions of cytokines or their receptors[6].

To determine whether some known anti-inflammatory proteins could block a cytokine storm, Staedtke and colleagues engineered *C. novyi*-NT to secrete anti-inflammatory proteins and tested whether any of these bacteria could treat tumours effectively without

causing severe toxicity owing to high cytokine levels. Their experiments revealed that ANP can dampen a cytokine storm. Mice treated with ANP-expressing *C. novyi*-NT had lower levels of proinflammatory molecules, including cytokines, in their bloodstream, and lower levels of organ infiltration by immune cells called myeloid cells that are associated with a cytokine storm, compared with mice given *C. novyi*-NT that had not been engineered to express ANP.

To determine how ANP decreased cytokine storms in their model system, Staedtke and colleagues characterized the differences between mice treated with the ANP-expressing *C. novyi*-NT and those that received non-engineered bacteria. This revealed that the dampened immune response linked to ANP was accompanied by a decrease in the level of catecholamines in the animals' bloodstream. Catecholamines such as adrenaline are best known for their role as part of the 'fight or flight' response to acute stress, in which they are released by certain neurons and by the adrenal gland. The idea that catecholamines might act to promote cytokine storms seems counter-intuitive, given that molecules of this class are used routinely to treat the low blood pressure associated with cytokine storms. However, it was known[7] that two types of immune cell — macrophages and neutrophils — produce catecholamines in response to inflammatory stimuli such as the molecule lipopolysaccharide (LPS), which is a hallmark of many types of bacterial infection.

To investigate whether catecholamines might have a key role in driving strong inflammatory responses, Staedtke *et al.* gave mice LPS and also gave a subset of these animals adrenaline. The animals that received adrenaline and LPS had higher cytokine levels and mortality than did those that received only LPS. Conversely, when the authors gave LPS to mice whose macrophages had been engineered to lack an enzyme called tyrosine hydroxylase (which is needed to make catecholamines), the animals had better survival rates and lower levels of cytokines and catecholamines than did LPS-treated mice that had macrophages with intact tyrosine hydroxylase. When the authors treated mice with a drug that blocks a receptor for catecholamines called the α1 adrenergic receptor, this interference with catecholamine signalling reduced inflammation when the mice were treated with LPS, compared with LPS-treated mice that did not receive the drug.

The authors also demonstrated the importance of catecholamines in initiating cytokine storms induced by bacteria in a different model system of severe bacterial infection. In both settings, the authors found that animals given metyrosine, a drug that inhibits tyrosine hydroxylase, had lower catecholamine and cytokine levels and increased survival rates compared with mice that did not receive the inhibitor.

The next key question was whether catecholamine release has a function in cytokine storms that arise from immune-cell activation for reasons other than encounters with a bacterium. Immune cells called T cells that have been triggered to launch an immune response can also make catecholamines[8]. Certain immunotherapy approaches aim to generate such activated T cells by the administration of antibodies that can activate T cells or by the introduction of engineered T cells (called chimaeric antigen receptor (CAR) T cells) designed to target tumour cells. These approaches can cause a cytokine storm[9,10]. To test whether catecholamines might have a role in such cytokine storms, the authors administered a T-cell-activating antibody to a group of mice, and treated a subset of the mice with metyrosine. The animals that received the inhibitor had improved survival and lower cytokine levels than the mice that did not receive metyrosine.

The authors then studied human CAR-T cells that were grown *in vitro* together with the type of blood cancer cells that activate them. The medium from these cell cultures contained catecholamines and cytokines, and the levels of these molecules increased if adrenaline was added to the culture, providing support for a model of a self-amplifying response driving their production.

The authors went on to give CAR-T cells to mice carrying tumours. A subset of the mice were given ANP or metyrosine before receiving the CAR-T cells, and these animals' cytokine levels were lower than were those of the mice that received only CAR-T cells. However, this difference did not affect the efficiency of the antitumour treatment, suggesting that toxicity due to cytokines is independent of the antitumour effects of this treatment.

Staedtke and colleagues provide compelling evidence for a self-amplifying circuit of catecholamine release by immune cells in the initiation of a cytokine storm (Fig. 1). However, determining the details of this circuitry will require additional studies. For example, how immune-cell activation drives an increase in catecholamine levels and how catecholamines boost cytokine production is unknown and should be investigated. Another mystery is which types of adrenergic receptor are crucial for the effects of catecholamines on cytokine levels in humans. ANP has anti-inflammatory properties[11], but how it inhibits catecholamine production is another key unanswered question that deserves future study.

The authors' findings might lead to new strategies to tackle cytokine storms during immunotherapy. Mouse models of CAR-T cell immunotherapy indicate that the activation of myeloid cells has a key role in driving cytokine storms — pre-emptive blockade of the action of certain cytokines or their receptors by antibodies or other approaches can effectively prevent the storms[12,13]. However, Staedtke *et al.* now also identify a central role for catecholamine production in the generation of cytokine

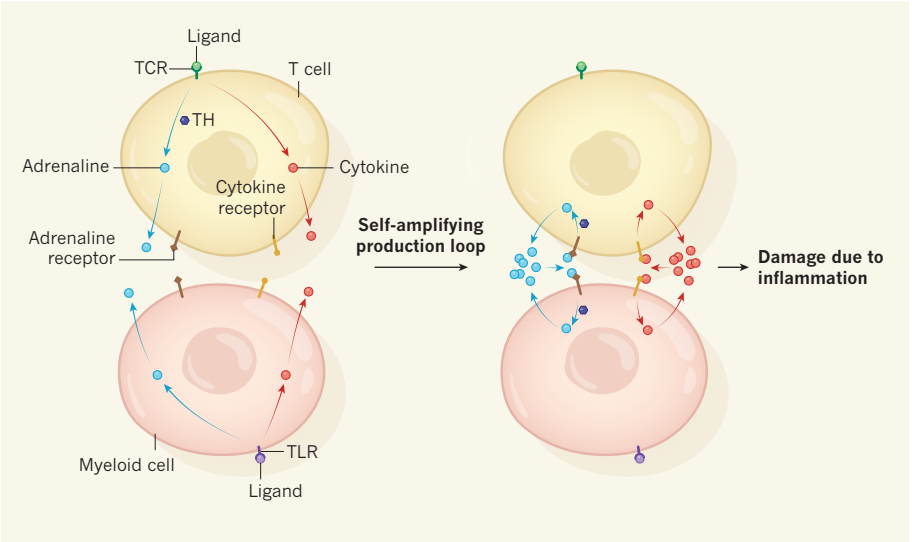

Figure 1 | The pathways driving the harmful inflammatory response called a cytokine storm. Immunotherapy treatments try to boost the responses of immune cells such as T cells against tumours. However, toxicity can occur if immunotherapy triggers a cytokine storm, in which the levels of immune-signalling proteins called cytokines become abnormally high and cause tissue damage. Staedtke *et al.*[4] report studies, using mice and human cells, which reveal that a class of molecule called catecholamines, which includes the hormone adrenaline, has a key role in driving cytokine storms. A T cell can become activated if a ligand molecule binds to the T-cell receptor (TCR), and an immune cell called a myeloid cell can be activated if a ligand binds to its Toll-like receptor (TLR). The activation of these cells leads to the production and release of cytokines, as well as to the production and release of adrenaline. The enzyme tyrosine hydroxylase (TH) catalyses the first step needed for adrenaline production. The authors' work supports a model suggesting that when adrenaline and cytokines bind their respective receptors on immune cells, this increases the production of these molecules through a self-amplifying loop and causes a cytokine storm. Staedtke and colleagues report that if tyrosine hydroxylase is inhibited by the drug metyrosine, it can help to limit cytokine storms (not shown).

storms, and show that ANP and metyrosine, which are approved for use in the clinic in other contexts, might be effective in preventing this complication. It is generally assumed that the production of cytokines and their role in the activation of immune cells contributes to the efficiency of antitumour immune responses[14]. To ensure that antitumour effects are not diminished, it will be necessary to proceed cautiously when testing whether targeting catecholamine synthesis can reduce cytokine storms in a clinical setting. ■

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EVOLUTION

Finches choose parent lookalikes as mates

A preference for mating with similar individuals can have a key role in speciation. Research on Darwin's finches suggests that individuals might use the likeness of their parents as a guide for choosing mates.

LEWIS G. SPURGIN & TRACEY CHAPMAN

New species form when groups of individuals in a population become reproductively isolated and can no longer mate with each other to produce living, healthy offspring. For decades, evolutionary biologists have sought to understand the links between an individual's choice of mate and reproductive isolation between populations and species[1]. Writing in *Proceedings of the National Academy of Sciences*, Grant and Grant[2] provide evidence suggesting that two species of Darwin's finch learn features of their parents early in life and use this knowledge to inform their choice of mate in adulthood, a process known as sexual imprinting. Their study raises fascinating questions about the roles of learning and genetics in mate choice, and how matings between similar individuals (assortative mating) drive the evolution of new species.

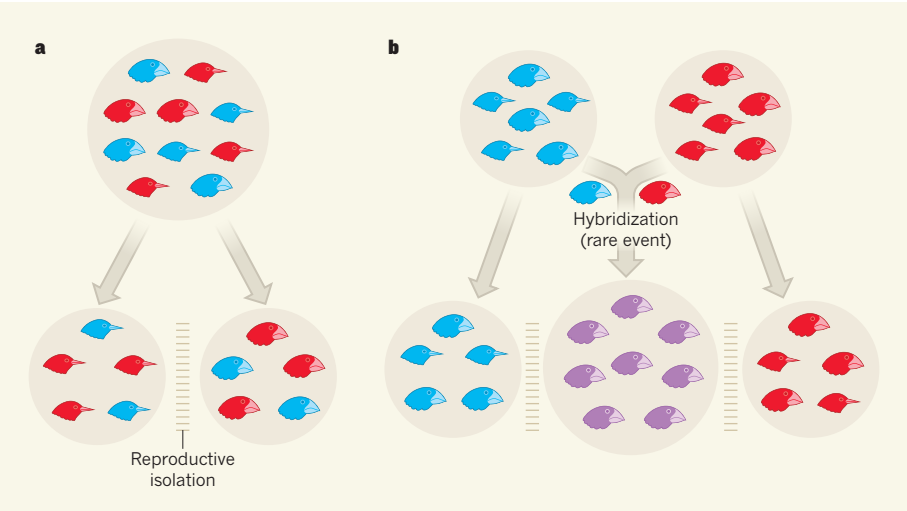

Figure 1 | Potential contributions of sexual imprinting to speciation. Grant and Grant[2] report that, in Darwin's finches, sexual imprinting and assortative mating — learning parental features early in life and using this to choose a mate — can reduce the likelihood of dissimilar individuals mating with each other. Reproductive isolation caused by these and other factors might contribute to the evolution of new species in two ways. **a**, Speciation by fission involves the splitting of one species into two. In this example, imprinted mating preferences for beak size promote the formation of two new species with either small or large beaks. **b**, In speciation by fusion, a rare hybridization event between individuals of different species, followed by an imprinted preference to reproduce with similar individuals, promotes the formation of a new species alongside the two original ones.

Darwin's finches live in the Galapagos archipelago. They are an iconic group of approximately 15 bird species that have contributed hugely to our understanding of natural selection and speciation[3–5]. Previous work has shown that the cultural inheritance of song can promote reproductive isolation between different species of Darwin's finch[6]. However, it was not known whether sexual imprinting based on morphological features such as body size and beak characteristics could similarly promote reproductive isolation, or play a part in the rare cases of mating between species that produce hybrid individuals.

If sexual imprinting is key in directing mate choice, then individuals should choose mates that resemble their parents, and also themselves. In addition, if sexual imprinting contributes to matings between species, then the parents of the hybrid individuals that result from such matings should more closely resemble the other species than their own. To test these hypotheses, Grant and Grant analysed 22 years of data on body size, beak size and beak shape in two finch species — *Geospiza fortis* and *Geospiza scandens* — living on the same island.

Grant and Grant found significant positive associations between certain features of the birds' chosen mates and those of their parents. For *G. fortis*, the body size of the chosen mate was strongly correlated with the body size of the chooser's father, but weakly correlated with that of the chooser's mother. The researchers did not explicitly test whether this imprinting was stronger in male or female offspring. For *G. scandens*, the beak length of male mates chosen by females was significantly associated with the beak shape and length of the female's father, although there were no other significant associations. Grant and Grant suggest that these imprinting patterns can promote assortative mating by body size and beak length in both species.

For matings between species, the results were less straightforward. In some cases, hybridizing individuals or their fathers from a given species were similar in size and shape to individuals from the other species. Although such results are intriguing, hybridization events are rare and sample sizes are small, so