affects the ability of boreal forests to act as a carbon sink at the landscape scale. Their work also highlights the importance of considering below-ground stores of terrestrial carbon to understand ecosystem responses to climate change.

Walker and colleagues' results suggest that certain boreal forests are about to reach a tipping point in their resilience to fire, beyond which the carbon-storage function of this ecosystem will change. This is a major concern, not only because it will increase the rate at which carbon dioxide is released to the atmosphere, but also because these forests provide multiple benefits. Boreal forests constitute the largest terrestrial biome, with some of the world's largest intact areas of untouched forest, and they harbour unique biodiversity. Moreover, they are sources of timber products and contribute greatly to global air quality and climate regulation. Carbon loss and removal of organic soils by fire will transform this ecosystem, with unknown consequences.

For example, increased fire frequency might alter species composition and biodiversity, and will probably affect soil fertility as a result of carbon removal. Boreal forests could therefore become less productive than they are at present, and more vulnerable to other stresses, such as insect attacks and droughts, which are likely to become more frequent as the climate changes. These effects, in turn, will further decrease the carbon-sink function of boreal forests and adversely affect the economies of countries that rely on timber harvests. Because it is extremely difficult to predict what, when and where changes will happen, these effects present a challenge for forest managers and policymakers[6].

Walker and co-workers' study demonstrates that there is an intimate link between soils and climate. The authors suggest that the increased frequency of fires in boreal forests is resulting in a positive feedback loop: a higher incidence of fires means that younger forests will be affected, increasing the probability that legacy carbon will be released from soil (assuming that the intensity of the fires does not change). Legacy carbon has been sequestered for hundreds of years; its release would further increase atmospheric $CO_2$ concentrations, thereby accelerating climate change.

The effects of wildfires reported by Walker *et al.* are specific to organic soils that have accumulated large amounts of organic matter because low temperatures and/or high water tables have protected them from microbial decomposition. Such soils are widespread in boreal regions, but are also found in temperate and tropical climates[7]. These organic soils, which contain more than 20% carbon, are very different from the relatively carbon-poor mineral soils that underlie most temperate and tropical forests and grasslands.

Disturbances such as increasing temperatures and fire will trigger much bigger carbon losses from boreal organic soils than from mineral soils in temperate and tropical regions[7]. This is because a large proportion of the organic matter in mineral soils is protected from microbial decomposition by its interaction with soil minerals, preserving it as temperatures increase, and because mineral soils have low heat conductivity, which usually protects organic matter from fire. However, if the organic soil layers of boreal forests are reduced in the future, more attention will need to be paid to the organic carbon in the underlying mineral soils. Mineral soils under boreal forests store substantial amounts of carbon[6], but Walker *et al.* did not take this into account in their analysis.

Because of the contrasting processes that protect carbon in organic and mineral soils, different strategies are needed to manage their carbon-sink function. For organic soils, it will be necessary to maintain low temperatures, high moisture levels and low fire-return intervals[4,7]. This will require immediate, aggressive reductions in greenhouse-gas emissions from all sectors of society to minimize further increases in atmospheric $CO_2$ concentrations and climate change. To improve the carbon-sink function of mineral soils, sustainable management practices for forestry and agriculture must be adopted. In both cases, awareness of the benefits of soil carbon must be raised, and collaboration is needed at all levels from everyone who uses soils, and from those whose activities might damage soils. ■

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IMMUNOLOGY

# Regulators to the rescue

**A type of immune cell called a CD8 T cell, which usually kills disease-causing agents, has been found instead to suppress self-reactive immune cells, thereby offering protection against an autoimmune disease in mice.** SEE ARTICLE P.481

HYE-JUNG KIM & HARVEY CANTOR

The immune system has evolved complex mechanisms that allow rapid and destructive responses to microbial intruders while sparing the host's own tissues. Regulation of this delicate balance depends mainly on the immune system's two major types of T cell, which are distinguished by the protein — either CD4 or CD8 — that is expressed on their surface. They are called CD4 T cells and CD8 T cells, respectively. The task of CD8 T cells has generally been assumed to be to kill cells infected with microbial invaders and to destroy foreign or abnormal cells. However, Saligrama *et al.*[1] report another role on page 481: CD8 T cells can inhibit self-reactive CD4 T cells and quell autoimmune disease in a mouse model of multiple sclerosis.

In previous work[2], researchers from the present group showed that, if people with coeliac disease were exposed to gluten proteins (a major type of trigger, called an allergen, in this autoimmune disorder), it activated not only CD4 T cells that could specifically recognize gluten, as expected, but also a subset of CD8 T cells. Exactly what the latter were doing, however, was unclear. Saligrama and colleagues now report their investigation into whether a similarly coordinated T-cell response might be detected in experimental autoimmune encephalomyelitis (EAE), which is a mouse model of multiple sclerosis. This autoimmune model can be induced by injecting the protein myelin oligodendrocyte glycoprotein (MOG), a component of the fatty coating of nerve cells called myelin, into mice. The authors identified populations of both CD4 and CD8 T cells (among other immune cells) that proliferated vigorously after immunization with MOG, generating clones of cells (Fig. 1a).

For each of these mobilized populations, Saligrama *et al.* identified the T-cell antigen receptors (TCRs, the proteins on T cells that recognize foreign or self peptide fragments known as antigens), and attempted to identify antigens that the TCRs could recognize. Such recognition causes T-cell activation and proliferation. The authors found that the CD4 T cells in question recognized MOG-derived peptide fragments and so were primed to specifically attack myelin-coated nerve cells and cause disease. But the CD8 T-cell clones did not recognize MOG, and none of about 350 myelin-derived peptides tested could activate their TCRs. So how were these cells being activated?

To find out, the authors generated a library of roughly $10^8$ different peptides, each embedded

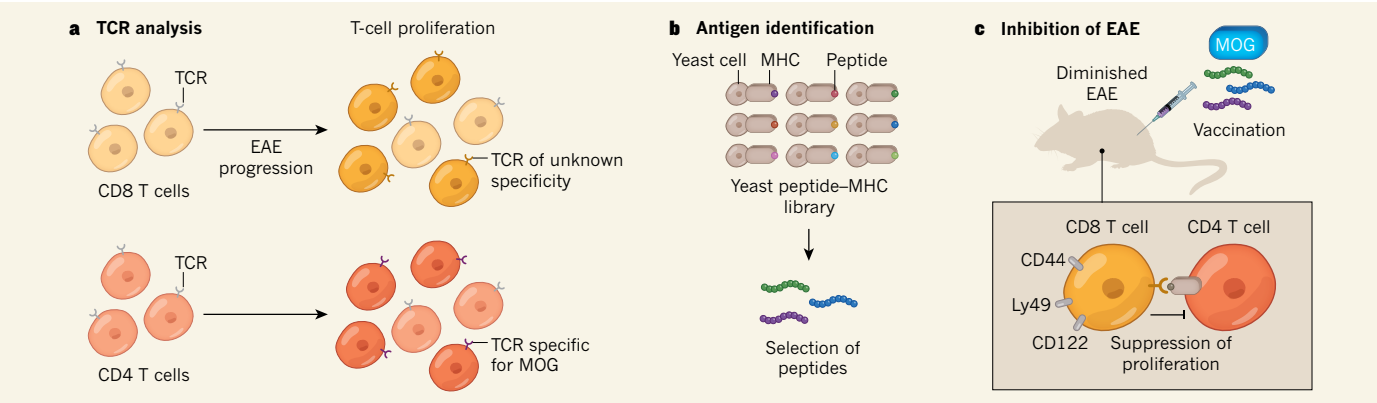


**Figure 1 | A regulatory function for T cells that express the protein CD8.** Saligrama *et al.*[1] studied a model of multiple sclerosis called experimental autoimmune encephalomyelitis (EAE), which is induced by injecting the protein myelin oligodendrocyte glycoprotein (MOG) into mice. **a**, Immune cells called CD8 and CD4 T cells express T-cell antigen receptors (TCRs) on their surface that recognize peptide fragments called antigens. If antigen recognition occurs, the T cell proliferates. In EAE, as the condition progressed, this was accompanied by the proliferation of both CD4 T cells (dark red) that recognize MOG and CD8 T cells (dark orange) that recognize an unknown target. **b**, Using yeast cells, the authors screened a library of peptides bound to mouse major histocompatibility complex (MHC) molecules, which enabled the identification of peptides that are recognized by the TCRs of the CD8 T cells. **c**, The vaccination of mice with both MOG and these peptides diminished the severity of EAE, compared with that seen in control animals. This effect was associated with the proliferation of CD8 T cells that express the proteins CD44, CD122 and Ly49. These cells suppress the proliferation of CD4 T cells in a process that involves the recognition of antigens presented by MHC proteins.

in a class Ia major histocompatibility complex (MHC) protein — an essential component of the immune system that displays antigens to T cells. The authors used TCRs from CD8 T cells as the bait with which to capture the corresponding antigens in this peptide–MHC library (Fig. 1b). This approach to defining TCR-binding peptides has not been widely adopted because it is tricky to generate large libraries that display individual peptide–MHC complexes with enough structural fidelity and stability to allow sensitive and efficient antigen screening[3]. Saligrama and colleagues' technique overcomes these obstacles. The strength of their approach can be appreciated by a glance at the numbers: a screen of around $5 \times 10^8$ different peptide–MHC complexes, using a single TCR, identified a dozen peptide–MHC complexes that could bind the TCR, akin to finding a small needle in a large haystack.

None of the peptides identified after numerous rounds of screening came from mouse proteins, and the authors call them 'surrogate' peptides, to indicate that they probably stand in for self peptides normally present in the body. To determine how T cells that recognize these peptides contribute to EAE, the authors immunized mice with a mixture of these surrogate peptides and MOG. CD8 T cells that recognized the peptides used for vaccination proliferated and suppressed the proliferation of the CD4 cells that promote EAE, possibly by recognizing peptide–MHC complexes displayed on MOG-reactive CD4 T cells (Fig. 1c). The peptides that these CD8 T cells recognize in the body are not yet defined. Cells such as these CD8 T cells that can suppress an immune response are called regulatory cells. Whether the initial proliferation of these regulatory CD8 T cells also depends on peptides presented to them by CD4 cells, or indeed by other types of immune cell, is unknown.

Progress in identifying and sequencing the TCRs that are expressed by specific T-cell clones has led to the development of important TCR-based therapeutics. However, the identity of the peptide–MHC complexes that bind to those TCRs has generally been elusive. An essential feature of Saligrama and colleagues' approach involves modifying the peptides to increase their affinity for class Ia MHCs, ensuring that most of the peptides in the library are bound to MHCs. These modifications might also increase the strength of binding of peptide–MHC complexes to TCRs, and hence their ability to stimulate regulatory CD8 T cells. An analogy might be seen with CD4 regulatory T cells. Earlier research[4] suggested that mutations that increase the binding of peptides to MHC class II proteins and the T-cell-activating effect of self peptides derived from insulin resulted in peptide–MHC complexes that stimulated the differentiation of insulin-specific CD4 regulatory T cells. Perhaps the strength of binding of peptide–MHC complexes to TCRs has a similar stimulatory effect on CD8 regulatory T cells.

One downside of the authors' approach could be a bias towards studying cells that recognize peptide–MHC complexes of the class Ia type in particular. Other studies[5] have found that CD8 regulatory T cells similar to those found here also recognize complexes of peptides with MHC proteins from class Ib. It would be interesting to know whether the CD8 regulatory T cells identified by Saligrama *et al.* consist of two different cellular lineages, which act in a complementary way to block autoimmunity by monitoring class Ia or class Ib MHCs expressed in different tissues.

The authors' further investigation of the CD8 T-cell population that dampened the CD4 autoimmune response boiled down to studies of whether or not these CD8 T cells are a specialized lineage of cells that is distinct from 'effector' CD8 T cells — those that are genetically programmed to respond to microbial intruders. The authors found that regulatory activity was invested in a small subpopulation (less than 5%) of CD8 T cells, which express a specific triad of proteins (CD44, CD122 and Ly49) on their surface[6]. RNA analysis of this subpopulation indicates a profile that is distinct from that of most typical CD8 effector cells, and that shares features with what are termed natural killer T cells and with CD8 regulatory cells identified in other autoimmune settings[5].

Like CD4 T cells, CD8 T cells might be divided into an effector-cell lineage that targets microbes, and a regulatory-cell lineage that subdues self-reactive CD4 T cells. The tracing of isolated cells by their surface markers has been instrumental in defining the regulatory lineage of CD4 T cells. Saligrama and colleagues' approach might prove equally revealing in efforts to define a regulatory lineage of CD8 T cells.

Finally, Saligrama *et al.* observed coordinated mobilization of CD4 and CD8 T cells in people with recent-onset multiple sclerosis, suggesting that their findings in mice might also apply to humans. The identification of cell-surface markers that could be used to reliably isolate putative human CD8 regulatory T cells should offer insight into whether such cells contribute to human autoimmune disease. Moreover, defining the antigens that such cells recognize could pave the way to new clinical treatments. ■

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ENGINEERING

# Droplet motion electrically controlled

**The movement of small droplets on a substrate is governed by surface-tension forces. A technique that can tune the surface tension of robust oxide substrates for droplet manipulation could open up many applications.** SEE LETTER P.507

FRIEDER MUGELE

Many plants and insects control their interactions with raindrops and other forms of ambient humidity using surface patterns of hydrophobic and hydrophilic regions[1]. In artificial devices, the ability to switch between such patterns enables the complex manipulation of droplets for applications including biomedical lab-on-a-chip systems[2,3], optofluidic lenses[4] and displays[5], and energy-harvesting systems[6]. A method called electrowetting-on-dielectric (EWOD) is arguably the most mature and versatile tool for achieving such functionality[7]. However, its commercial success has been limited, because the required hydrophobic dielectric (electrically insulating) surfaces gradually degrade. On page 507, Li *et al.*[8] report an approach to tune the wettability of chemically robust hydrophilic surfaces by electrically controlling the adsorption and desorption of molecules called surfactants. If this technology is successful, it might help to turn some previous EWOD-based systems into reliable devices.

The wettability of a solid surface for a particular liquid is determined by the chemical properties of the materials involved. If molecules of the liquid and the solid strongly attract each other, the liquid will cover as much of the solid surface as possible. As a result, there will be a small contact angle — the angle between the liquid surface and the solid surface at the point at which these surfaces meet. In the case of water, such a solid (for example, clean glass or silicon oxide) is hydrophilic. By contrast, if the attraction between water and the solid is weak (for instance, in the case of the non-stick pan coating polytetrafluoroethylene), the surface is hydrophobic and water will bead off.

For hydrophobic surfaces, the tension (force per unit length) at the interface between the solid and the liquid is larger than that at the interface between the solid and the surrounding gas (Fig. 1). For hydrophilic surfaces, the opposite applies. At equilibrium, the contact angle adjusts itself in such a way that the difference between the solid–liquid and solid–gas interfacial tensions is balanced by the horizontal component of the liquid–gas interfacial tension.

Tuning the wettability requires the balance between these surface-tension forces to be manipulated. In EWOD, this is achieved by applying a voltage between a droplet on a thick, hydrophobic dielectric layer and an electrode that is positioned underneath the layer. This voltage generates an electric force that, along with the solid–gas interfacial tension, pulls on the droplet and thereby reduces the contact angle (Fig. 1a). The combination of EWOD and patterned electrodes allows for complex droplet operations such as transport, splitting, merging and mixing[2,3].

The success of EWOD crucially depends on the stability and the chemical inertness of the dielectric layer. Almost two decades of applied research have focused on optimizing these layers, based on the principle that they should be hydrophobic and as thin as possible, but also should block any voltage-induced electric current that would degrade performance. This has led to layers of polytetrafluoroethylene-like fluoropolymers being a gold standard in the field. Notwithstanding impressive successes, the intrinsically high tension of any interface between a hydrophobic layer and water makes such surfaces prone to adsorption of solutes and to other degradation processes on continued exposure to water. This limitation has become the main bottleneck for the commercialization of the technology.

Li and colleagues avoid this inherent problem of EWOD by using a hydrophilic silicon oxide surface that has an intrinsically small solid–liquid interfacial tension. They tune the wettability of this surface using electrically controlled, reversible adsorption of surfactants (Fig. 1b). These molecules consist of a hydrophobic tail and a hydrophilic head. Their adsorption on the hydrophilic surface reduces the solid–gas interfacial tension and thereby increases the contact angle. For this reason, the authors refer to their approach as

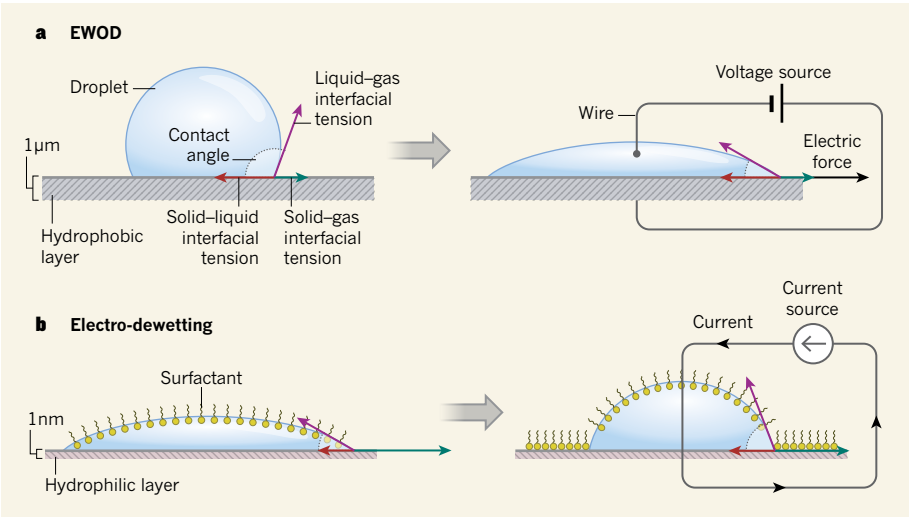


**Figure 1 | Droplet manipulation.** **a**, In a method called electrowetting-on-dielectric[7] (EWOD), a droplet is placed on a micrometre-thick hydrophobic layer. The solid–liquid interfacial tension (force per unit length) is larger than the solid–gas interfacial tension. The difference between these tensions is balanced by the horizontal component of the liquid–gas interfacial tension. As a result, the contact angle between the solid and the liquid is large. An applied voltage generates an electric force that causes this angle to decrease. **b**, Li *et al.*[8] report a technique dubbed electro-dewetting, in which a droplet is placed on a nanometre-thick hydrophilic layer. The solid–liquid interfacial tension is smaller than the solid–gas interfacial tension and the contact angle is small. The droplet contains charged molecules called surfactants that have a hydrophobic tail and a hydrophilic head. Under an electric current, surfactants are adsorbed on the hydrophilic layer. These regions are rendered hydrophobic, which causes the contact angle to increase.