

gen receptor negative, EpCAM low and ALDH-1 high.

These new findings support the cancer stem cell (CSC) hypothesis, at least for some breast tumor intrinsic subtypes. According to this hypothesis, a cancer can arise from transformation of a normal stem cell or progenitor cell, thus giving rise to a heterogeneous population of cells. Alternatively, a differentiated cancer cell within a heterogeneous tumor may acquire stem cell-like features through acquired self-renewal mechanisms. Either way, the CSC hypothesis holds that the bulk of the tumor is composed of differentiated cells with limited proliferative potential, whereas the CSC compartment maintains the tumor and contributes to treatment resistance due to its unique biological properties¹². Melding the CSC hypothesis with the data from Lim *et al.*² suggests that MaSCs and committed luminal progenitors are the cells of origin of claudin-low and basal-like tumors, respectively (Fig. 1). Alternatively, the MaSC may still be the cell of origin for both subtypes; however, under this scenario, claudin-low tumors may be locked in this stem cell state, whereas basal-like tumors become arrested at the luminal progenitor stage.

If basal-like tumors are arrested at a specific step in luminal development and have a minor CSC or claudin-low component, then this might explain why they have a poor prognosis despite responding to chemotherapy¹³. In this scenario, it is predicted that the residual disease present after chemotherapy treatment will be enriched for cells with stem-like features, which is precisely what has been observed^{14,15}. However, isolation and better characterization of these CSCs will require further investigation. Of note, Lim *et al.*² isolated MaSCs on the basis of a low level of

expression of EpCAM, which is currently used as the antigen to identify circulating tumor cells¹⁶, along with keratin-8, keratin-18 and keratin-19, which are all either not expressed or expressed at low levels in MaSCs. Some researchers have suggested that circulating tumor cells may represent circulating CSCs; however, the data of Lim *et al.*² would argue against this.

Despite the excitement this new study is bound to elicit, investigators must keep in mind that the study of normal stem cells and CSCs has experimental limitations, including the imprecision of the markers used to purify these cells, the difficulty in performing functional reconstitution studies and the choice of functional assays. For example, the various gene expression profiles were obtained from subpopulations that were not pure cell types, a caveat that must be kept in mind when interpreting these results.

Overall, the study of Lim *et al.* improves understanding of the biological heterogeneity of breast cancer. These data suggest that a MaSC with claudin-low features progresses to a luminal progenitor state that shows characteristics of both myoepithelial (or basal) and luminal cells, which then progresses to differentiated cells with more luminal characteristics and less myoepithelial characteristics within the luminal cell lineage (Fig. 1). Much less is known about the myoepithelial and basal developmental lineage, which is an area that deserves more attention.

This study also further complicates the nomenclature issues surrounding basal-like breast tumors. Indeed, many in the field have questioned the use of the name 'basal-like' and the very existence of this subtype; however, these new analyses again identify the basal-like

features of these tumors and, importantly, their potential normal counterparts. We support the continued use of the name basal-like, as it is still accurate, given that these tumors clearly show expression of proteins and genes that are used to define morphologically identified basal epithelial cells (that is, keratin-5, keratin-6 and keratin-14). Of note, basal-like tumors do sometimes show squamous⁶ or metaplastic histology⁷ and expression similarities to lung squamous carcinomas¹⁷, thus suggesting that this differentiation cascade may also be occurring in other epithelial tumor types as well.

Irrespective of nomenclature, whether basal-like, triple negative or luminal progenitor, Lim *et al.* have advanced the field's understanding of breast tumorigenesis by providing a logical link between cancer genomics and developmental biology.

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T time in the brain

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Inflammation in neural tissue has long been suspected to have a role in stroke. A new study in mouse models of focal cerebral ischemia suggest that a stereotyped sequence of T cell infiltration and activation may underlie the progression of brain injury that can last up to days after stroke onset (pages 946–950).

Stroke remains a very challenging clinical problem. In experimental models, cerebral ischemia triggers an elevation in excitotoxic glutamate that rapidly leads to an accumulation of damaging calcium and reactive oxy-

gen species in neurons. However, in spite of remarkable advances in the molecular biology of neuronal cell death, a clinically effective neuroprotectant has not yet been developed. Numerous neuroprotection trials in acute stroke have failed¹.

Many of these early efforts, however, were focused on targets that might have short half-lives after stroke onset, such as excitotoxicity. Because it is often difficult to reach an individ-

ual quickly after stroke onset, such therapies may be difficult to implement widely.

Emerging data implicate a role for inflammation in stroke². In contrast to acute excitotoxic and oxidative stress, prolonged inflammation may offer a longer window of opportunity to block secondary events that expand infarction and brain injury. Inflammation after cerebral ischemia amplifies the initial injury by linking acute responses in glia and cytokines to a sec-

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ondary infiltration of immune cells into brain tissue (Fig. 1). A wide spectrum of cell types may be involved, comprising neutrophils, macrophages and lymphocytes. But exactly how these events are regulated remains to be fully elucidated.

In this issue of *Nature Medicine*, Shichita *et al.*³ describe a regulated sequence of an immune response in mouse models of stroke that involves a delayed infiltration of $\delta\gamma$ T cells after cerebral ischemia. The ability of these T cells to amplify the inflammatory cascade suggests that they may be a new target for stroke therapy.

To investigate the role of T cells in this process, Shichita *et al.* subjected mice to transient occlusions of the middle cerebral arteries. Examination of these brains showed that infarcts markedly expanded from day 1 to day 3. Within a day, there was an increase in macrophage numbers in the damaged tissue, accompanied by an elevation in concentration of the inflammatory cytokine interleukin-23 (IL-23). After three days, there was a secondary infiltration of $\delta\gamma$ T cells, lymphocytes with both innate and adaptive immune properties, along with a production of IL-17. Using a combination of genetic and pharmacologic manipulations, the authors nicely showed the timing and importance of these T cell signals. Early treatment with a general immunosuppressant (FTY720) reduced T cell infiltration and decreased brain injury.

Compared to wild-type mice, IL-23-deficient mice had lower numbers of infiltrating $\delta\gamma$ T cells and, consequently, smaller infarcts after focal cerebral ischemia. Elegant irradiation and bone marrow replacement experiments suggested that the initial source of IL-23 came from infiltrating macrophages instead of resident microglia. In turn, IL-17-deficient mice had less secondary brain injury and, concomitantly, an amelioration of downstream proinflammatory and neurotoxic factors.

Shichita *et al.* then showed that the delayed infiltration of $\delta\gamma$ T cells may have clinical relevance. Blocking a specific $\delta\gamma$ T cell receptor with an antibody effectively reduced three-day infarct volumes, even when treatment was initiated at 24 hours after onset of cerebral ischemia.

Taken together, these experiments delineate a sequence of events in which macrophages enter ischemic tissue soon after injury and signal with IL-23 to attract $\delta\gamma$ T cells. The secondary wave of T cells then produces IL-17 that further amplifies the inflammatory cascade, thus promoting infarction and brain damage. These results suggest that, under some conditions, the therapeutic time window for stroke might be surprisingly long, on the order of days instead of the hours previously thought. Targeting these $\delta\gamma$ T cells may provide a unique opportunity to

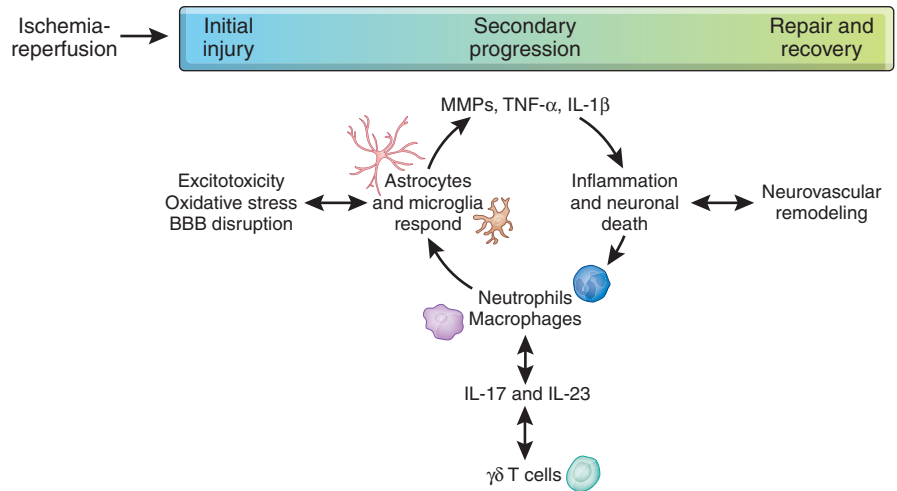


Figure 1 The sequence of events after cerebral ischemia may comprise multiple phases. Acute injury rapidly occurs due to excitotoxicity and the generation of free radicals. An inflammatory reaction to brain injury and blood-brain barrier (BBB) disruption is then triggered, involving glial and cytokine responses. Shichita *et al.*³ define a sequence of $\delta\gamma$ T cell, IL-23 and IL-17 pathways that may amplify this inflammatory cascade to increase levels of damaging mediators such as tumor necrosis factor- α (TNF- α), IL-1 β and matrix metalloproteinases (MMPs) and expand infarction over the days after stroke onset. Finally, after initial injury and secondary progression, neurovascular remodeling may eventually occur as the brain attempts to heal during stroke recovery.

prevent the secondary expansion of infarction after stroke.

Nevertheless, like any interesting study, this effort by Shichita *et al.* raises many more questions. They identified a central role for IL-23, IL-17 and $\delta\gamma$ T cells in the inflammatory cascade after stroke. However, the interwoven roles of players in the immune system suggest that other cells, cytokines and chemokines are likely to be involved. For example, depending on the balance between tolerance and sensitization to previously unencountered central nervous system antigens, lymphocytes can be either damaging or protective after ischemia⁴. A recent study suggested that regulatory T cells provide endogenous protection against ischemic brain injury by limiting the proinflammatory activities of other immune cells⁵. It has also been proposed that immune suppression occurs after cerebral ischemia^{6,7}.

What's more, there may be crosstalk between infiltrating immune cells and resident brain cells that may possess immune-like responses, including microglia, astrocytes and pericytes. Will these T cell mechanisms be different in people with hypertension, vascular disease and higher baseline inflammation? And, finally, will these mechanisms be different in mild versus severe strokes or in strokes that are reperused with therapeutics that break apart clots? Defining this complex network of interacting cells and signals in ischemic models with altered inflammatory baselines may provide more nuanced opportunities for modulation and intervention.

Runaway inflammation is obviously damaging. In their experiments, Shichita *et al.* found that blocking secondary T cell mechanisms was neuroprotective. Under some conditions, however, inflammation is triggered as an endogenous attempt by damaged tissue to heal and remodel. The same may be true in brain. Many proinflammatory signals that are upregulated after cerebral ischemia may have biphasic roles in the response, with deleterious actions during the acute phase and beneficial effects during stroke recovery⁸. For example, overactivation of *N*-methyl-D-aspartic acid (NMDA) receptors underlies excitotoxic neuronal death during early stages of stroke. But without NMDA signaling, neuronal plasticity might not take place to rebuild networks of cellular connections. Similarly, although reactive glia can release neurotoxic molecules, astrocytes and microglia can also serve as potent sources of trophic factors that aid neurovascular remodeling. Whether the inflammatory and immune pathways dissected here also possess biphasic properties may warrant further investigation.

Ultimately, the hope is to translate these promising experimental leads into clinical applications. But further studies are required to validate these hypotheses. Will these T cell pathways be operational in human stroke? The authors concede that they have not yet been able to detect $\delta\gamma$ T cells in brain tissue from individuals after stroke³. What's more, previous clinical trials targeting immune or inflammatory pathways in stroke have not succeeded^{9,10}. Caution is always important, as there can be crucial dif-

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ferences between immune responses in mouse models versus humans¹¹.

From an evolutionary perspective, blood hemostasis and immune systems are closely linked. This relationship can be best discerned in the horseshoe crab, so-called living fossils from the subclass Xiphosura that have persisted through almost 500 million years of evolution. Horseshoe crabs have merged their coagulation and innate immune responses by using a clotting system to immobilize pathogens within their hemolymph circulation¹². In mammalian systems, bidirectional signaling has also been

evolutionarily conserved, so that inflammation activates coagulation and thrombosis triggers immune responses¹³. Hence, it may not be surprising that inflammation accompanies stroke. A better understanding of how these inflammatory processes are orchestrated after cerebral ischemia may eventually allow us to design ways to prevent the secondary progression of brain injury after cerebral ischemia.

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T-ing up inflammation in fat

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Obesity generates a proinflammatory environment in adipose tissue, but the factors that initiate this inflammatory cascade have been unclear. Three studies now show that alterations in the composition of adipose tissue T cells occur early in obesity and shape the relationship between immunity and metabolism (pages 914–920, 921–929 and 930–939).

Adipocytes provide a flexible storage depot for excess nutrients, a property that creates a valuable resource during starvation. Adipocytes are also endocrine cells, secreting hormones that regulate energy intake and expenditure throughout the body. With overnutrition, however, adipocytes are pushed to the limits of their ability to store lipids and to regulate nutrient metabolism, and along with obesity comes an increase in inflammatory marker expression¹. The cells of the innate immune system regulate these processes, in particular adipose tissue macrophages (ATMs), which make up a large proportion of the nonadipose cells in adipose tissue. ATMs infiltrate fat at the later stages of obesity and can cause some of the complications of the condition, particularly, insulin resistance.

Many of the experiments that have investigated obesity-induced inflammation, however, used bone marrow transplantation to manipulate immune cells, which leaves open the pos-

sibility that bone marrow-derived cells aside from macrophages contribute to the complications of obesity.

In this issue of *Nature Medicine*, three studies show that T cells are also actively regulated in adipose tissue and contribute to obesity-induced inflammation. Remarkably, these studies provide compelling evidence that specific rearrangements in the T cell receptor (TCR) are selected for in adipose tissue T cells, suggesting that antigens in fat may communicate with the adaptive immune system.

Depending on the immune challenge, T helper cells can moderate the activity of other immune cells to generate proinflammatory T helper type 1 (T_H1) responses through phagocyte activation or humoral T_H2 responses through stimulation of B cell activity. In lean mice, resident ATMs have low inflammatory activity, restrained by T_H2 cytokines². With obesity, new macrophages are recruited to fat, and, stimulated by T_H1 signals, these macrophages secrete proinflammatory cytokines that impair insulin signaling in adipocytes. This is an early and important event in the development of type 2 diabetes, as insulin resistance in adipocytes leads to increased lipolysis and the release of free fatty acids into the circulation. These fatty acids render the liver and skeletal muscle insulin resistant and contribute to a prediabetic state.

It is unknown what regulates these activation states in ATMs, but T cells are a logical source of the T_H1 and T_H2 stimuli for the ATMs. Although lymphocytes have been

identified in adipose tissue, their role to date has been unclear.

To study this question, Nishimura *et al.*³ examined how adipose tissue T cell populations changed in adipose tissue with increasing obesity in mice. They observed an increase in the ratio of CD8⁺ to CD4⁺ adipose tissue T cells weeks before ATMs typically infiltrate fat. Winer *et al.*⁴ and Feuerer *et al.*⁵ also report similar changes in the CD8/CD4 ratio in adipose tissue T cells with obesity, although each study addresses a different component of this transition.

Nishimura *et al.* focused on the increase in CD8⁺ adipose tissue T cells and show that CD8-specific antibodies attenuate adipose tissue inflammation, ATM recruitment and insulin resistance in obesity. Coculture studies suggested that CD8⁺ T cells and adipocytes cooperate to recruit ATMs to fat. Overall, their study shows that obesity alters the properties of adipose tissue T cells before ATMs do and suggests that CD8⁺ adipose tissue T cells may initiate the inflammatory cascade that leads to insulin resistance in adipocytes.

In their study, Winer *et al.* observed that *Rag1*-deficient mice, which have reduced numbers of lymphocytes, had worse insulin resistance than control mice, suggesting that lymphocytes protect against the deleterious effects of obesity. Adoptive transfer experiments showed that CD4⁺ T cells, but not CD8⁺ T cells, normalized glucose tolerance in *Rag1*-deficient mice. T_H2 signals from the transferred CD4⁺ cells were key in the improvement

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