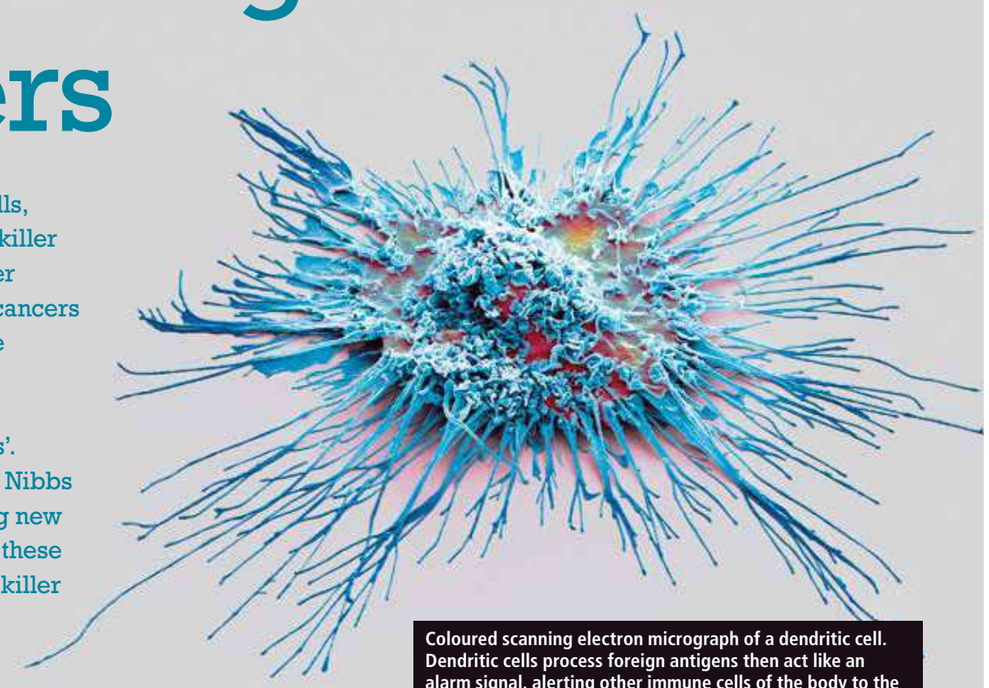
Releasing the cancer killers

Some white blood cells, appropriately called killer T cells, can kill cancer cells. Unfortunately, cancers can evolve resistance to killer T cell attack by exploiting natural 'immune checkpoints'. Immunologist Robert Nibbs explains how exciting new medicines can block these checkpoints, freeing killer T cells to kill cancers



Coloured scanning electron micrograph of a dendritic cell. Dendritic cells process foreign antigens then act like an alarm signal, alerting other immune cells of the body to the infection (×3000)

Key words

Cancer
Immunology
Killer T cells
Immune checkpoints
Immunotherapy

Cancer is a major killer. It is caused by the accumulation of mutations in DNA. Mutations in a region of DNA that is transcribed into messenger RNA and translated into protein can change a protein's amino acid sequence. Proteins altered in this way might not work normally and can increase the likelihood of getting cancer. This is particularly true if these altered proteins disrupt one of the many natural anti-cancer processes, such as properly controlled cell division. A cancer will form once cells accumulate enough DNA mutations to overcome all natural anti-cancer defences. It will grow larger, disrupt the function of the organ it is growing in, spread around the body and eventually become life-threatening.

One crucial anti-cancer defence mechanism is attack by white blood cells, particularly killer T cells — key components of the immune system. These cells identify and kill cells that contain mutated abnormal proteins. By understanding how cancers block this lethal attack, new medicines have been developed that reinvigorate killer T cells, and they show remarkable effects in some cancer patients.

Arming killer T cells

A key protein on the surface of killer T cells is the **T cell receptor** (TCR). It can bind to fragments of proteins (polypeptide **antigens**) held on the surface of other cells by a collection of proteins called the **class 1 major histocompatibility complex** (MHC1). Interactions between the TCR and MHC1–antigen complexes determine which cells killer T cells can kill. The amino acid sequence of the TCR varies randomly between killer T cells. Each is complementary to only one particular antigen. However, we have billions of killer T cells each with a different TCR so, as a population, they can recognise a huge variety of antigens. Remarkably, very few, if any, killer T cells recognise antigens that are fragments of one of our own proteins. This prevents killer T cells attacking our healthy cells. It is achieved through tolerance, where the killer T cell population only responds to antigens from proteins in microbes, and from abnormal versions of our own proteins, like those found in cancer cells.

Until they encounter a suitable MHC1–antigen complex, killer T cells cannot kill other cells. At this stage they are referred to as 'naive'. Naive killer T cells circulate in the blood but spend most of their time moving inside lymph nodes — small kidney-shaped organs each containing millions of naive T cells. We have 500 or so, each associated with a particular tissue, such as skin, lung or intestine. Inside lymph nodes, naive T cells move over specialised antigen-presenting cells called dendritic cells that have travelled there from nearby tissues. Dendritic cells are covered in MHC1 molecules, each holding an antigen generated from a protein that the dendritic cell phagocytosed while in the nearby tissue. Naive killer T cells survey these MHC1–antigen complexes. If they find one that their TCR recognises, they stop moving and stick to the dendritic cell. Signals pass through the TCR and through a molecule called **CD28** once it has bound to **CD80** or **CD86** proteins, which, like MHC1–antigen complexes, are present on dendritic cell surfaces (see Figure 1a). On receiving both signals, naive killer T cells become activated. They proliferate, making many copies of themselves, and become equipped with molecular weapons they can use to kill other cells. They then leave the lymph node in search of their first victim.

Seek out and destroy

Activated killer T cells circulate in the blood. Infected, stressed or damaged tissues and cells release small proteins called chemokines that attract activated killer T cells out of blood vessels and into tissues. In the tissue, activated killer T cells survey local MHC1–antigen complexes, rather like naive killer T cells do in the lymph node. They bind to any cell displaying an MHC1–polypeptide antigen complex complementary to its TCR. Signals are passed through the TCR but this time these signals instruct the activated killer T cell to deploy its molecular weapons to kill the bound cell (see Figure 1b). The activated killer T cell then moves on to find its next victim ('target cell'). Some activated T cells can become 'memory' T cells that stay with you your whole life. Even many years later, memory cells can rapidly destroy cells carrying MHC1–antigen complexes that their TCR recognises.

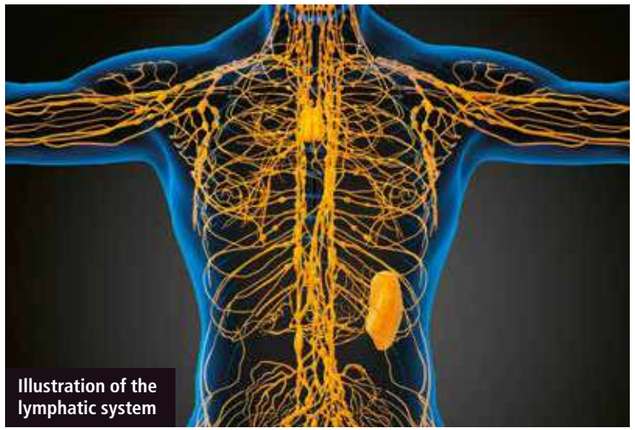
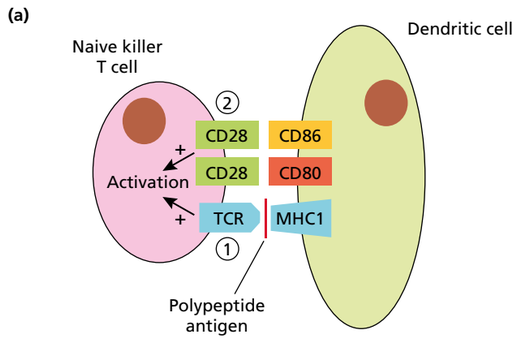


Illustration of the lymphatic system



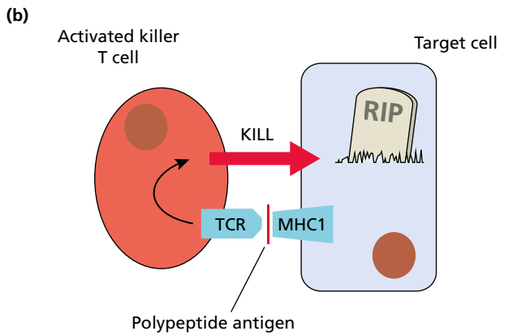


Figure 1 (a) Activation of a naive killer T cell. A naive killer T cell (pink cell) in a lymph node becomes activated when: (1) its TCR binds to a polypeptide antigen held by MHC1 on the dendritic cell (green cell), and (2) its CD28 protein binds to CD80 or CD86 on the dendritic cell. **(b)** Target cell killing by an activated killer T cell. An activated killer T cell (red cell) will kill a target cell (light blue cell) anywhere in the body if it can find it and if its TCR binds to a polypeptide antigen held by MHC1 on the surface of the target cell

Immune checkpoints control the killers

If too many activated T cells are made, or if their power is misdirected, they can damage the tissues and organs they are supposed to protect. Like cars, cells of the immune system are better controlled if there is a brake in addition to an accelerator. Many ways of restraining killer T cells have evolved. Two involve cell surface proteins called **CTLA4** and **PD1**. They are '**immune checkpoint proteins**' and play vital roles in ensuring the killing capacity of killer T cells does not get out of hand.

Terms explained

Antigens Fragments of intracellular proteins that are bound to MHC1 and presented on the cell surface to killer T cells.

CD28 A protein that binds to CD80 and CD86 and sends signals into naive killer T cells that are essential for their activation.

CD80/CD86 Proteins on the surface of dendritic cells that bind to CD28 and CTLA4.

Class 1 major histocompatibility complex (MHC1) Holds polypeptide antigens on the surface of cells and presents them to killer T cells.

CTLA4 An immune checkpoint protein that binds to CD80 and CD86.

Immune checkpoint blockade therapy (ICBT) Medicines that interfere with an immune checkpoint protein.

Immune checkpoint protein A natural inhibitor of immunological processes, such as the activation of killer T cells.

Immunotherapy Therapy that enhances the activity of white blood cells or the immune system.

PD1 An immune checkpoint protein that binds to PD-L1 and PD-L2 and can inhibit killer T cells.

T cell receptor (TCR) A cell surface protein that sends signals into killer T cells once it has bound a polypeptide antigen held by MHC1.

CTLA4 is found on immune cells called regulatory T cells. CTLA4 binds to CD80 and CD86 and removes them from dendritic cell surfaces. This prevents naive killer T cell activation in the lymph node because it prevents crucial signals being sent into the T cell via CD28 (see Figure 2a).

PD1 on activated killer T cells binds to proteins called PD-L1 or PD-L2 that can be produced on cells in infected or damaged tissues. Once bound to PD-L1 or PD-L2, PD1 sends inhibitory signals into activated killer T cells that stop them killing their target cells (see Figure 2b).

Killer T cells protect against cancer

Protective mechanisms inside cells stop cancers forming and cause cells that acquire DNA mutations to die. Dendritic cells can phagocytose proteins released from dead cells, load antigens from these proteins onto their surface MHC1 molecules and migrate to nearby lymph nodes. Many of these antigens will be from normal proteins, so will not activate naive killer T cells because of the tolerance mechanisms mentioned earlier. However, some antigens will have come from proteins with an abnormal amino acid sequence caused by DNA mutation. These are called neo-antigens, from the Greek 'neos' meaning 'new'. Killer T cells with a TCR that is complementary to an MHC1–neo-antigen complex can become activated and armed for killing. These activated killer T cells seek out and destroy any potentially cancerous cells that contain the mutated protein. In this way, killer T cells protect us against cancer.

Cancers evolve evasion strategies

Mutation and selection causes cancers to adapt to their environment. For example, they can avoid being killed by T cells by producing PD-L1 or PD-L2, which bind to PD1 on activated killer T cells and prevent them attacking. Other cancers release molecules that attract regulatory T cells: these cells bring CTLA4 into the cancer, which strips CD80 and CD86 off dendritic cells and so upon their migration to the lymph node they are unable to activate naive killer T cells. With killer T cells under control, the cancer continues to grow.

Immune checkpoint blockade therapy

If cancers exploit immune checkpoints to evade killer T cells, then can blocking immune checkpoints free killer T cells to kill again? Researchers spent many years studying animal models of cancer to find out. They focused mainly on melanoma, an aggressive form of skin cancer caused by repeated sun damage. Melanoma typically contains many DNA mutations so produces a lot of neo-antigens that activate killer T cells. Researchers generated antibodies that could either stop CTLA4 from binding to CD80 or CD86 (see Figure 3a), or interfere with PD-1 function (see Figure 3b). These antibodies had impressive effects in animal models of cancer: they stopped growth of some cancers, made others smaller and, occasionally, made them disappear.

Would cancers in people respond to these antibody-based **immune checkpoint blockade therapies** (ICBTs)? Antibodies were formulated for use in humans, clinical trials were set up, and patients with advanced melanoma were recruited to undertake the treatment — many were only expected to survive for a few months. The first antibody to be tested was ipilimumab, commonly referred to as 'Ipi', which blocks CTLA4 function. Some patients' cancers responded well to this new treatment. Life expectancy was extended and, remarkably, a few patients' cancers disappeared. Some have now been cancer-free for over 10 years — cured of a normally fatal disease. Treatment with Ipi has side effects, not surprisingly given the role of CTLA4 in a healthy immune system, including colitis, which is a painful and destructive inflammatory disease

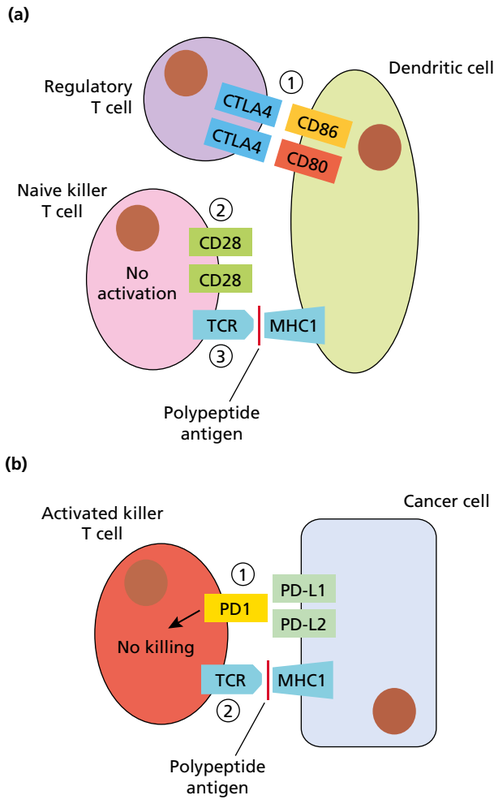


Figure 2 The function of immune checkpoint proteins CTLA4 and PD1. **(a)** CTLA4: (1) CTLA4 on a regulatory T cell (purple cell) binds to CD80 and CD86 on a dendritic cell (green cell). (2) This prevents signals being passed through CD28, so the naive killer T cell (pink cell) cannot become activated, even if its TCR (3) binds to a polypeptide antigen held by MHC1 on the dendritic cell. **(b)** PD1: (1) Once bound to PD-L1 or PD-L2, PD1 sends signals into an activated killer T cell (red cell) that prevent it from killing its target cell (blue cell), even if its TCR (2) binds to a polypeptide antigen held by MHC1 on the target cell

in the intestine. But these are manageable and the benefits outweigh the disadvantages.

A new era of cancer immunotherapy

The success of Ipi was only the start. Antibodies blocking PD-1 or PD-L1 were developed. Once again, the life expectancy of patients with advanced melanoma was extended when they received these antibodies, with some showing long-term melanoma-free survival. These medicines seemed more effective than any other available treatments, including Ipi. What is more, blocking both CTLA4 and PD-1 was even more effective than interfering with just one immune checkpoint. PD-1 blockade also works in some patients with cancer in their kidneys, bladder, lungs, liver, stomach, colon or head and neck area, and those with Hodgkins

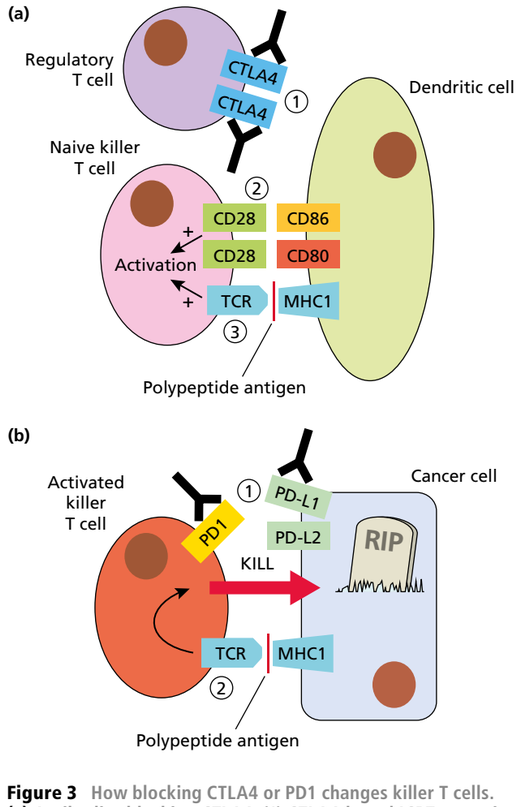
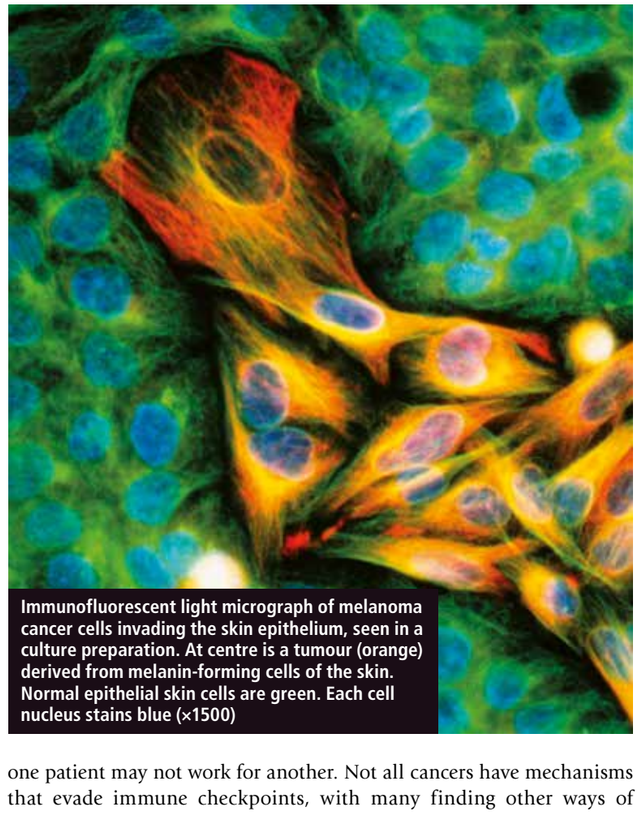


Figure 3 How blocking CTLA4 or PD1 changes killer T cells. **(a)** Antibodies blocking CTLA4: (1) CTLA4-based ICBTs contain antibodies (black shapes) that bind to CTLA4 on a regulatory T cell (purple cell). (2) This frees CD80 and CD86 on the dendritic cell (green cell) to bind to CD28 on the naive killer T cell (pink cell). Signals are passed through CD28, which along with those from the TCR (3) lead to the activation of the naive killer T cell. **(b)** Antibodies blocking PD1 or PD-L1: (1) ICBTs aimed at stopping PD1-mediated signals contain antibodies (black shapes) that bind to PD1 or PD-L1. They prevent PD1's inhibitory signals from interfering with the ability of an activated killer T cell (red cell) to kill its target cell (light blue cell) once its TCR (2) has recognised and bound to a polypeptide antigen held by MHC1 on the target cell

lymphoma, a cancer that develops from white blood cells. For decades it was hoped that new medicines could be made that unleash the power of the immune system against cancer. There have been many disappointments, but with the advent of ICBTs, we have finally entered the era of cancer immunotherapy. The importance of these advances has recently been recognised by the Nobel prize committee who awarded the 2018 Nobel prize in physiology and medicine to James Allison and Tasuku Honjo for their discovery that blocking immune checkpoints was an effective cancer therapy.

Towards personalised medicine

Not all cancers respond to ICBT. One reason is that each patient's cancer is unique, so what might successfully treat



Immunofluorescent light micrograph of melanoma cancer cells invading the skin epithelium, seen in a culture preparation. At centre is a tumour (orange) derived from melanin-forming cells of the skin. Normal epithelial skin cells are green. Each cell nucleus stains blue (×1500)

one patient may not work for another. Not all cancers have mechanisms that evade immune checkpoints, with many finding other ways of avoiding attack by killer T cells. It may be possible to develop medicines that disrupt these alternative ways that cancers avoid killer T cells. It also makes sense to try to identify patients who are likely to respond to ICBT before starting treatment. This would avoid unnecessary side effects and save money. Unsurprisingly, patients whose cancers contain PD-L1 protein and killer T cells often respond well to ICBTs that block PD-1 or PD-L1. By carefully analysing a patient's cancer, it might be possible to design personalised treatments to meet their specific needs. This 'personalised medicine' approach will be a major part of healthcare in the future.

Future prospects

There is some way to go before the impact of ICBTs is fully appreciated. Other types of cancers may also respond to these new medicines, and hundreds of clinical trials are now in progress. ICBT might also improve the effectiveness of other cancer therapies. Chemotherapy and radiotherapy kill cancer cells. Dead cancer cells are a rich source of neo-antigens so should activate killer T cells. ICBT would be expected to enhance the killing properties of these activated killer T cells to improve their ability to kill any cancer cells that survive other therapies. It is an exciting time for cancer immunotherapy and doctors have been handed new, powerful weapons to aid their fight against cancer.

Robert Nibbs is a professor at the Centre for Immunobiology in the Institute for Infection, Inflammation and Immunity at the University of Glasgow. His research focuses on understanding the mechanisms controlling white blood cell migration in health and disease, particularly in cancer.