

The Highest Mountain – T- Cell Technology

Dr Bryan McIntosh, Senior lecturer, School of Health, University of Bradford

Dr. Michael Fascia, Research Fellow, School of Medicine, University of Edinburgh

Abstract

T cells have the capacity to eradicate diseased cells, but tumours present considerable challenges that render T cells ineffectual. Cancer cells often make themselves almost 'invisible' to the immune system, and they sculpt a microenvironment that suppresses T cell activity, survival and migration. Genetic engineering of T cells can be used therapeutically to overcome these challenges. T cells can be taken from the blood of cancer patients and then modified with genes encoding receptors that recognize cancer-specific antigens. Additional genes can be used to enable resistance to immunosuppression, to extend survival and to facilitate the penetration of engineered T cells into tumours. Using genetic modification, highly active, self-propagating 'slayers' of cancer cells can be generated.

Introduction

Antibody-based drugs have become a mainstay of cancer treatment, but their use is limited to the small fraction of cancer targets that present as whole proteins on the cell surface (Mosmann, Cherwinski, & Bond, 1986). Most cancer targets are hidden inside cancerous cells where antibodies cannot reach them. By contrast, T cells employ T Cell Receptors (TCRs) which target the peptide antigens found on the cell surface as a marker of cancer and viral infection inside the cell. TCRs can potentially target any of the markers that are hallmarks of cancer and enables the development of drugs against cancers for which no antibody targets are known.

Unfortunately, the TCRs found naturally on the surface of killer T cells are primarily designed to recognise virally infected cells, and are often not sensitive enough to recognise cancer, which is then ignored by the immune system (Hirota et al., 2011). The issue of cancer recognition has been overcome by boosting the ability of cancer-specific TCRs to bind to their targets with several million fold higher affinity than natural TCRs. These high affinity, soluble TCRs have been equipped with the ability to activate all the T cells of the body to kill cancer, even those that would normally not recognise cancer, thereby bringing the entire weight of the immune system to bear against the disease.

These, engineered TCRs, called ImmTACs (Immune Mobilising mTCR against Cancer) created by Oxfordshire biotechnology company, Immunocore (www.immunocore.com), mobilise T cells to kill cancer cells and overcome immune tolerance to cancer. In this paper we will describe how this process is applied.

T Cells

Helper T (T_H) cells are critical to coordinating the activity of the immune response. The chemical messages they secrete (cytokines) stimulate the non-specific immune response to continue, and strengthen and boost appropriate specific responses. Helper T cells have sometimes been called the "conductors" of the immune system because they coordinate activity like the conductor of a symphony (Larsen *et al.*, 2011). They have also been called the "generals" of the immune system because they call up troops of B cells, cytotoxic T cells, and other helper T cells to go into battle against invading pathogens (Fig. 1).

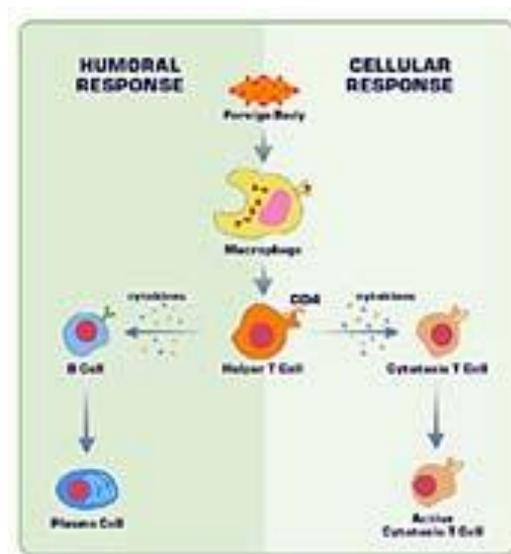


Figure 1. Helper T cells regulate both humoral and cellular immunity

Macrophages alert helper T cells to the presence of pathogens. These phagocytic macrophages engulf bacteria and viruses, and can display foreign antigens - the identifying proteins of the bacteria or viruses - on the surface of their cell membrane (Said *et al.*, 2010). Embedded within the macrophage cell membrane is a molecule produced by the human leukocyte antigen (HLA) complex. The helper T cells bind simultaneously to the foreign antigen and the HLA molecule. Only T_H cells with receptors that match those of the foreign antigen on the activated macrophage are able

to bind and respond to the call to action. Once bound, the 'helper' T cell proliferates to form a clone of cells, each capable of recognizing the same antigen. The members of the 'helper' T clone, the generals, generate the chemical signals that attack the foreign body (Saraiva *et al.*, 2009).

Some signals sent by helper T cells stimulate cytotoxic T cells (T_C). Cytotoxic T cells (also known as killer T cells) bind cells that have been altered, such as by viral infection; they avoid healthy cells. Surface antigens on the altered cell perform the binding. These antigens are specific to the offending agent, and match receptors in the membrane of the specific T_C cell. In addition, the T_C cell simultaneously binds an MHC molecule on the surface of the infected cell. Once bound by both the foreign antigen and the HLA molecule, the cytotoxic T cell secretes a chemical called "perforin," which destroys the offending cell (Fig. 2).

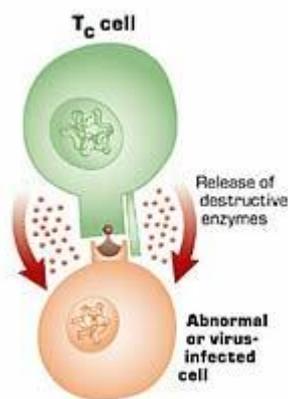


Figure 2. A cytotoxic T cell attacking a host cell that is expressing foreign antigens

Helper T cells stimulate the production of antibodies. Chemical signals from helper T cells stimulate the production of B cells specific to an infecting pathogen, and then stimulate the B cells to differentiate into plasma cells. The plasma cells are factories for the production of antibodies, which are specific to given pathogens circulating in blood or lymph (Hu, 2007). Antibodies work by blocking the receptors that allow pathogens to attach to target cells, or by creating clumps of bacteria. Clumping makes the job of phagocytes easier, as they will more readily engulf bacteria in clumps. Bound antibodies sometimes serve as tags, called opsonins, enhancing phagocytosis. Antibody binding can

also initiate a cascade of biochemical reactions, activating a set of chemicals known as complement. Activated complement components can form holes in bacterial membranes and enhance inflammation (Rang, 2003).

Nature of Research

Harnessing the power of the immune system's killer T cells is a key to the treatment of certain cancers. The T-cells of the blood are designed to seek out and kill invading pathogens, such as viruses and bacteria. T-cells are not naturally effective at seeking and destroying cancer cells (Harrington *et al.*, 2005). Immuno-oncology represents a sea change in terms of cancer treatment. Cancer in the past has been largely treated by surgery, chemotherapy or radiotherapy. However, all are burdened with the inherent problem of how to spare healthy tissue from irreparable damage while ensuring that every cancer cell is destroyed, deactivated or removed (Nakayamada *et al.*, 2012).

This notwithstanding, there is another approach based on the immune system, a complex web of cells, tissues and organs that constantly strive to keep the body free of disease, which almost certainly includes keeping cancerous cells in check. Jones *et al.* (1999) noted that the immune system plays a key role in cancer prevention. The immune system has two basic ways of fighting invading pathogens and the body's own cells that have been affected. One involves the release of free-floating proteins, or antibodies, that lock on to an invader, triggering other immune cells to come in and sweep them away. A second is cellular immunity, where T-cells seek out and destroy invading pathogens.

This research has allowed the creation of a process where protein molecules attach themselves to cancer cells, strongly and very specifically, whilst ignoring healthy cells. The technology, called ImmTAC is based on the "T-cell receptor", the protein that sticks out of the surface of the T-cell and binds to its enemy target. ImmTACs are bispecific proteins that bind strongly to cancer cells at one end, and T-cells at the other – so introducing cancer cells to destruction.

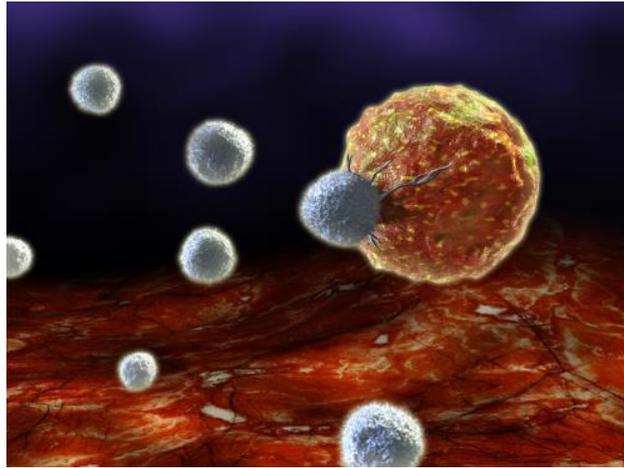


Image courtesy of Immunocore: T cell (grey) killing a tumour cell (yellow)

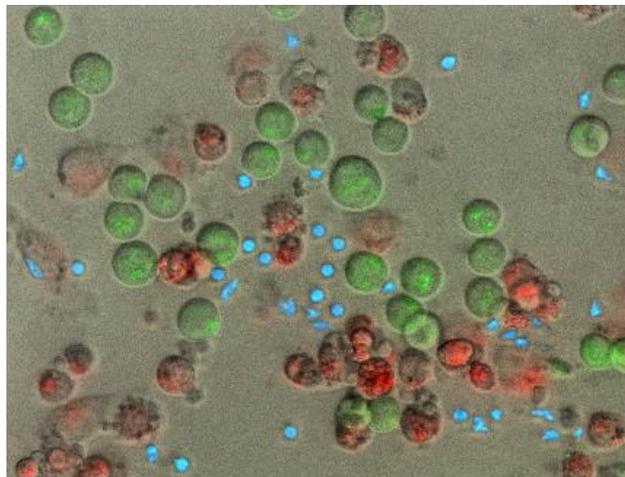


Image courtesy of Immunocore: Melanoma cancer cells (red) are targeted and killed by T cells (blue) when activated by Immunocore's drug, IMCgp100 (a melanoma specific ImmTAC). Healthy cells (green) are ignored and left undamaged.

This process uses the scaffold of the T-cell receptor to make something that is very good at recognizing cancer even if it doesn't exist naturally. Although T-cells are naturally constructed to recognise cancer cells they can be forced to do so. The potential you have if you can engineer T-cell receptors is quite enormous. You can find any type of cell and any kind of target. This means the approach can in theory be used against any cancer, whether it is tumours of the prostate, breast, liver or the pancreas.

The key to the success of the technique is being able to distinguish between a cancer cell and a normal, healthy cell. Immunocore's drugs do this by recognising small proteins or

peptides that stick out from the surface membrane of cancer cells. All cells extrude peptides on their membranes and these peptides act like a shop window, informing practitioners of what is going on within the cell, and whether it is cancerous or not.

As T Cell Receptors naturally recognise diseased cells engineering high affinity T Cell Receptors and linking them to an antibody fragment, anti-CD3 can activate the immune system to kill the targeted cancer or viral cells. These bi-specific proteins, called ImmTACS, have the potential to be extremely potent anti-cancer or anti-viral agents.

The most advanced ImmTAC drug, IMCgp100, is currently in clinical trials in melanoma patients in both the US and UK. This research has the potential to have a profound effect upon the way cancer is treated.

Conclusion

Using the body's immune system to fight cancer is one of the most promising areas of therapy, and could prove particularly helpful in the treatment of metastatic disease, when the cancer has spread from its original site. The immune system is complex and is composed of many kinds of cells, proteins and chemical messengers that modulate how it works. The challenge is to build a database of peptide targets on cancer cells in order to design T-cell receptors that can target them, leaving healthy cells alone and so minimising possible side effects – or that is the hope.

The first phase clinical trial of IMCgp100 in 31 patients with late stage malignant melanoma was designed to evaluate the safety of IMCgp100 and to establish a tolerable dose. Dose dependent toxicity has been demonstrated and the Maximum Tolerated Dose established as 600 ng/kg. Data from the Phase I trial indicated promising early signs of efficacy and Immunocore has initiated a Phase IIa study to optimise the dosing regimen and maximise the efficacy of IMCgp100. A danger with deploying T-cells against cancer is their potency. Yet it is this very potency that it is so exciting because it could lead to a cure for metastatic disease that has spread around the body. It is not possible currently to make a single-mechanism drug that would come anywhere near to a T-cell in terms of its potency. To make an impact on cancer, a potent treatment is required. Exploiting the immune defences to recognise and eliminate cells that have become cancerous has this potency and immunotherapy may hold one of the keys to the treatment of cancer.

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