Discuss how chronic inflammation and the immune system both promote and protect against cancer and how this may be therapeutically modulated

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Introduction

Widely acknowledged as the “father of modern pathology,” Rudolf Virchow was one of the first to suggest a link between inflammation and cancer back in the nineteenth century, when he noticed an infiltrate of leucocytes within tumours.[1] Recent evidence has now demonstrated that virtually every tumour has an inflammatory cell infiltrate as part of a complex ‘tumour microenvironment.’[2] Tumour-associated macrophages (TAMs) are the most abundant, but other innate immune cells (namely neutrophils, dendritic cells and natural killer (NK) cells) and adaptive immune cells (B and T lymphocytes) are also found.[3]

From an epidemiological perspective, it is clear that inflammation and the immune system can have differing effects on cancer. Many chronic inflammatory diseases increase the risk of developing certain cancers (see table 1), but conversely, a deficient immune system may predispose to a different subset of cancers. The classic example here is HIV/AIDS and the increased risk of Kaposi sarcoma as well as a variety of non-Hodgkin lymphomas.[4] Overall, this highlights that part of the immune system could promote cancer and part of it could protect against it. This essay will explore the evidence supporting this hypothesis and suggest mechanisms for these two opposing effects, before discussing how inflammation and the immune system could be targeted in cancer therapeutics and what lies ahead in this ever-growing area of active research.

The cancer-promoting effects of inflammation

The inflammatory diseases that predispose to cancer are of different aetiologies, suggesting that inflammation itself rather than some other factor is positively associated with cancer. It is thought that immune cells recruited to the tumour microenvironment secrete bioactive

molecules, such as growth factors, cytokines and prostaglandins, that have the potential to act at various points in carcinogenesis from initiation to metastasis (see figure 1).[2,3]

Table 1: Chronic inflammatory diseases that predispose to cancer. Adapted from Ref[5].

Aetiology	Underlying Inflammatory Condition	Associated Cancer(s)
Infectious	Viral hepatitis	Hepatocellular carcinoma
	Helicobacter pylori infection	Gastric lymphoma, adenocarcinoma
	HPV infection	Cervical squamous cell carcinoma
	Infectious mononucleosis	Various lymphomas
Auto-immune	Ulcerative colitis/Crohn's disease	Colorectal adenocarcinoma
	Coeliac disease	T-cell lymphoma
	Sjögren syndrome/Hashimoto's thyroiditis	MALT lymphoma
Environmental/ other	Asbestos-related disease	Mesothelioma, bronchial carcinoma
	Smoking-associated bronchitis	Bronchial carcinoma
	Pancreatitis	Pancreatic carcinoma
	Primary sclerosing cholangitis	Cholangiocarcinoma
	Reflux oesophagitis/Barret oesophagus	Oesophageal adenocarcinoma

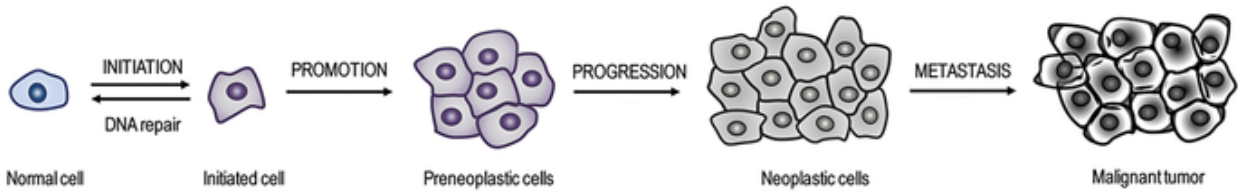

Figure 1: The stages of carcinogenesis. *Initiation* involves the accumulation of genetic mutations that influence cell proliferation and survival. Continued proliferation through *promotion* and *progression* ultimately gives rise to a malignant neoplasm which has the ability to *invade* into surrounding tissues and spread to distant sites (*metastasis*). Adapted from Ref[6].

I. Initiation: inflammation-induced mutagenesis & genomic instability

Cancer results from a stepwise accumulation of several driver mutations that either activate oncogenes or inactivate tumour-suppressor genes.[2,7] Genetic analysis from surgical resections of colitis-associated colorectal cancer shows that *TP53* mutations occur earlier than in sporadic colorectal cancer and often even in non-dysplastic but inflammed mucosa.[8] This suggests that long-standing inflammation, such as inflammatory bowel disease, can induce the initial mutations needed to drive tumourigenesis.

We have known for a while that inflammatory cells produce reactive oxygen species which could be one potential source of mutagens,[3,9] but evidence now exists to suggest that inflammation contributes to generalised genomic instability within tumour cells.[10] For example, inflammation

upregulates HIF-1α, a transcription factor which is shown to silence the mismatch repair genes, *MSH2* and *MSH6,* in human colon cancer cells.[11] This leads to microsatellite instability, which increases the frequency of DNA replication errors throughout the genome, and is linked with many sporadic human cancers. Furthermore, pro-inflammatory cytokines can induce expression of activation-induced cytidine deaminase.[12] This mutagenic enzyme converts cytosine to uracil and 5-methylcytosine to thymine, and thus has the potential to change C:G base pairs to T:A. This process occurs in B-lymphocytes to create variation in immunoglobulins, but is also linked to the development of B-cell lymphoma and solid tumours such as gastric and liver cancers.[12]

II. Promotion & progression: tumours as wounds that do not heal

In an essay in *The New England Journal of Medicine*, Dvorak first postulated in 1986 that 'tumours are wounds that do not heal,'[13] suggesting that tumours activate and exploit the host wound-healing response for their own growth. Supporting such parallels, cellular imaging in a transgenic zebrafish cancer model demonstrates that pre-neoplastic cells are able to recruit neutrophils and macrophages in similar fashion to wound healing.[14,15] Depletion of macrophages and neutrophils by genetic knockdown results in reduced proliferation of individual neoplastic clones, suggesting that inflammatory cells provide essential trophic support for neoplastic cells.[14,15] To assess *how* inflammatory cells provide this trophic support, the aurhors block PGE_2 synthesis which also leads to reduced proliferation of transformed cells. This suggests that PGE_2 production, either by immune cells (paracrine signalling) or neoplastic cells themselves (autocrine signalling) provides essential trophic support for tumours.[16]

In humans, PGE_2 is synthesised by cycloxygenase-2 (COX-2), and interestingly regular use of aspirin (which is a COX inhibitor) is protective in many cancers.[17] COX-2 expression is driven by NF-κB, which has emerged as a central pro-tumourigenic transcription factor. Indeed, genetic disruption of the NF-κB pathway in a mouse colitis-associated carcinoma model inhibits the proliferation of cancer cells and promotes their apoptosis.[18] NF-κB signalling is upregulated by cytokine signalling networks facilitated by recruited immune cells. For example, Pikarsky and colleagues show that TNFα production by adjacent inflammatory cells increases NF-κB signalling

in transformed hepatocytes in a mouse hepatitis model and that this promotes progression to hepatocellular carcinoma.[19]

In addition to COX-2, NF-κB acts on a variety of other downstream target genes to elicit its pro-tumourigenic effects, including anti-apoptotic genes (e.g. Bcl-2 and Bcl-xL),[20,21] cell cycle genes (e.g. cyclin D1)[22] and pro-angiogenic genes (e.g. IL-8).[23] Furthermore, NF-κB can increase the expression of several chemokines and cytokines that help recruit inflammatory cells to create a positive-feedback loop of chemokine production and inflammatory cell recruitment.[24] The TNFα/NF-κB pathway represents a prototypical signalling axis in inflammation-driven cancer (figure 2), but is by no means the only pathway involved. A whole host of pro-inflammatory cytokines acting through various transcription factors (e.g. AP1 and STAT3) also have demonstrated pro-tumourigenic effects.[3]

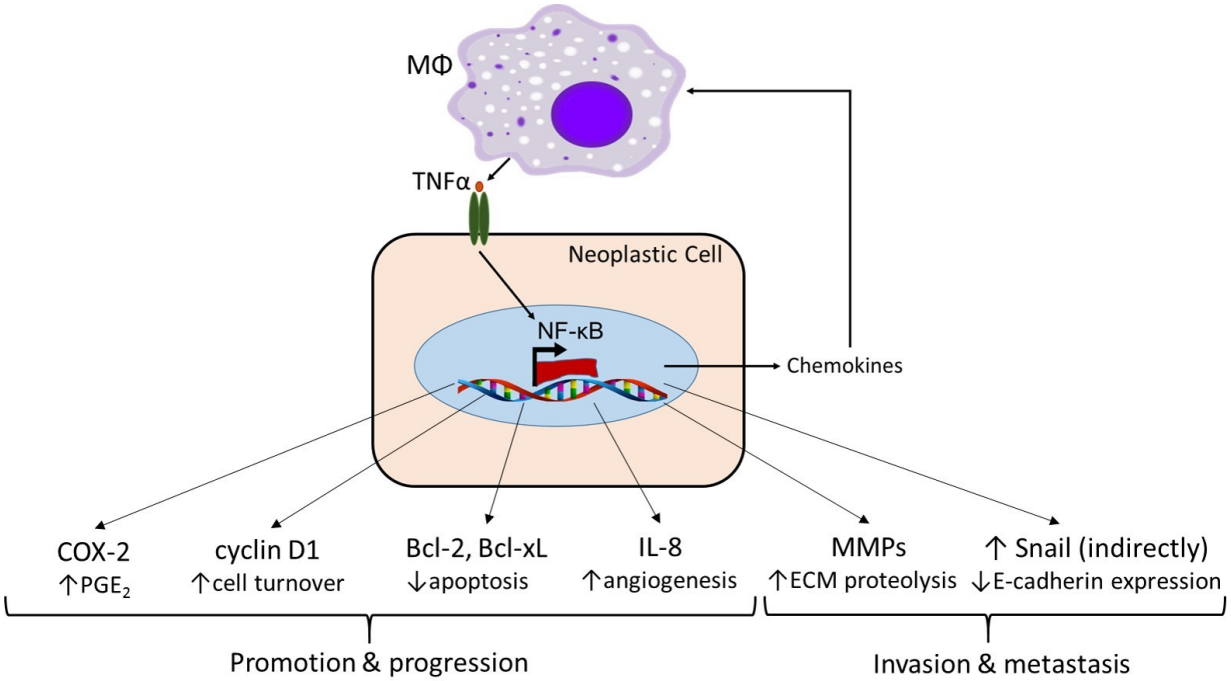

Figure 2: Schematic of the pro-tumourigenic effects of the TNFα/NF-κB signalling pathway. Inflammatory cells, such as tumour-associated macrophages, secrete TNFα which upregulates NF-κB in neoplastic cells, increasing the transcription of a number of pro-tumourigenic target genes.

III. Invasion & metastasis: induction of epithelial-mesenchymal transition

The ability to invade and metastasise is key to our understanding of cancer as this is what separates a malignant process from a benign one. In a murine breast cancer model, CSF-1 knockout (and hence macrophage depletion) delays the development of lung metastases,[25]

suggesting macrophage-derived factors can drive invasion and metastasis. The first step of the metastasis is widely regarded as 'epithelial-mesenchymal transition' (EMT), whereby epithelial cells transdifferentiate into mesenchymal stem cells.[2,26] For neoplastic cells, this means they lose cell-cell adhesion and gain motility, allowing invasion through the basal membrane and eventually into the blood stream or lymphatics.[2]

In EMT, a crucial event is reduced expression of the cell adhesion molecule, E-cadherin.[26,27] Here again, the TNFα/NF-κB pathway is critical. Work in human cancer cells shows that TNFα production by macrophages, acting through NF-κB, upregulates Snail, a known transcriptional repressor for E-cadherin.[27] The production of matrix metalloproteinases (MMPs) and subsequent proteolysis of the extracellular matrix is also necessary for invasion and metastasis. Evidence from a mouse colorectal cancer model, suggests that recruited inflammatory cells secrete MMPs.[28] Other studies demonstrate that MMP expression is driven by TNFα via the NF-κB pathway,[29,30] again highlighting the importance of this central signalling axis in cancer.

Immunosurveillance and anti-tumour immunity

The immune system is thought to protect against cancer through 'immunosurveillance,' a mechanism by which immune cells constantly survey host tissue to recognise and eliminate transformed cells.[31] Mice with homozygous null mutations in recombinase activating gene 2 (*RAG-2*$^{-/-}$), an essential factor for V(D)J recombination, are functionally deplete of both B- and T-lymphocytes.[32] Compared to wild-type controls, they show a greater incidence of both chemically induced sarcomas and spontaneous epithelial tumours, hence suggesting a role for lymphocytes in immunosurveillance.[32] Similar experiments have also identified NK cells as having an important role in immunosurveillance.[33]

The mechanism for immunosurveillance can be conceptualised as two distinct parts. Immune cells must first be able to recognise neoplastic cells as distinct from self and then must selectively eliminate them. The mechanisms for both these parts are summarised in figure 3.

I. Immune recognition of neoplastic cells

When lymphocytes derived from melanoma patients are incubated *in vitro* with tumour cells from the same patient, they proliferate to form populations of cytotoxic T-lymphocytes (CTLs)

specific for that tumour.[34] This suggests that, whilst tumours are composed of 'self-derived cells,' they can express antigens that are recognised by the immune system as foreign.

Such tumour antigens could be presented on MHC surface molecules, and it is likely that T-lymphocytes recognise tumour antigens in this way. However, we know that NK cells also play a key role in tumour immunosurveillance, so tumours must express other molecular patterns that the innate immune system can recognise. The Natural Killer Group 2D receptor (NKG2D), found on NK cells and some CTLs, is one possible recognition receptor for molecular patterns of cellular transformation.[35] Indeed, immunohistochemistry and flow cytometry have revealed that NKG2D ligands are frequently expressed in a variety of human cancers.[36,37] Moreover, NKG2D-knockout mice show accelerated progression of myc-induced B-cell lymphoma, thus highlighting the *in vivo* importance of NKG2D in immunosurveillance.[38]

II. Immunosurveillance host effector mechanisms

Interferon-γ (IFNγ) release is one proposed mechanism by which CTLs and NK cells can eliminate tumour cells. Indeed, $IFN\gamma^{-/-}$ mice develop spontaneous lymphomas and lung adenocarcinomas, and show a greater incidence of chemically-induced sarcoma compared to wild-type controls.[39] Moreover, this susceptibility can be reversed after complete bone marrow irradiation and reconstitution, but not when the donor is deficient of γδ T-cells (a type of CTL), suggesting that γδ T-cells are a major source of IFNγ in cancer immunosurveillance.[39] Similar experiments have also identified the production of cytolytic molecules, such as perforin and granzyme, as other important effector mechanisms.[40]

NK cells express TNF-related apoptosis-inducing ligand (TRAIL), which is able to selectively induce apoptosis in transformed cells via a death-receptor pathway. $TRAIL^{-/-}$ mice have a greater incidence of chemically-induced sarcomas compared to controls,[41] and conversely, administration of recombinant TRAIL to mice with solid tumours suppresses progression and improves survival.[42] This highlights the importance of TRAIL-induced apoptosis in cancer immunosurveillance and as a potential therapeutic target.

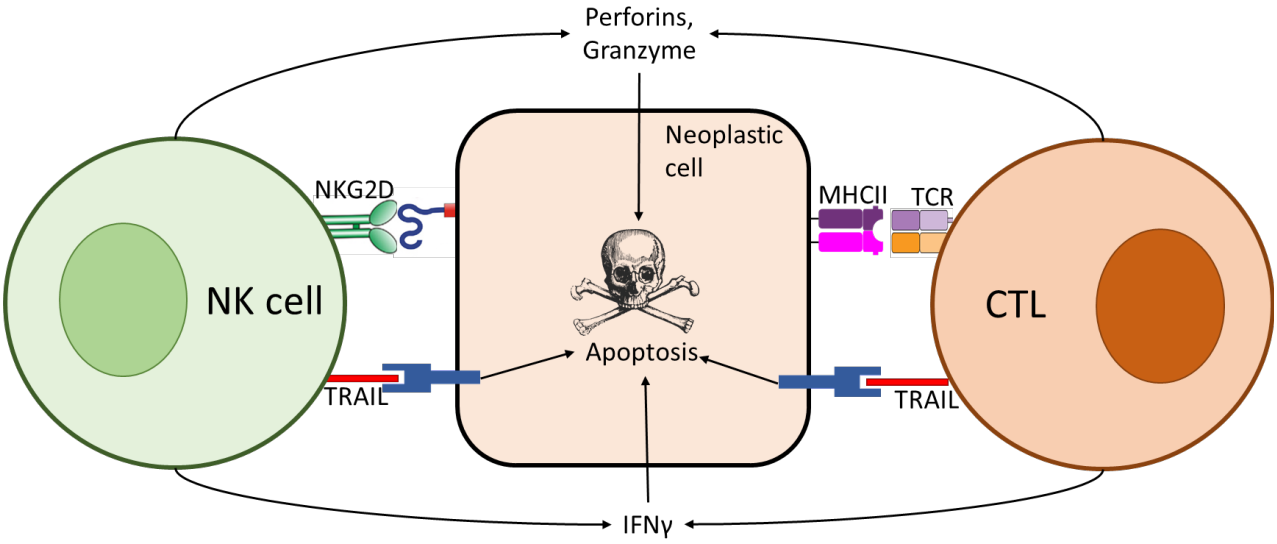

Figure 3: A schematic overview of the mechanisms of immunosurveillance. Cytotoxic T-lymphocytes (CTLs) recognise tumour antigens displayed on MHC class II through their T-cell receptor (TCR), whereas NK cells use other mechanisms such as the NKG2D receptor. NK cells and CTLs then kill neoplastic cells through expressing TNF-related apoptosis inducing ligand (TRAIL), and releasing interferon-gamma (IFNγ), perforins and granzyme. Collectively these mechanisms induce apoptosis.

The balance between promotion and protection

As we have seen, the immune system can have very different effects on cancer. Experimental work has shown that mice depleted in macrophages are protected from cancer, whereas mice depleted in lymphocytes are more susceptible, suggesting that different immune system components could have opposing effects.

CTLs and NK cells play a key role in immunosurveillance, but the role of helper T-cells is less clear. They may adopt different phenotypes, of which Th1 cells tend to promote cell-mediated immunity, and Th2 cells tend to support humoral immunity and wound-healing.[43] Thus, it is likely that Th1 cells are important for immunosurveillance, and indeed clinical evidence suggests that a higher Th2/Th1 ratio amongst tumour-infiltrating lymphocytes is associated with a poor prognosis.[44] Both clinical and experimental data suggest that TAMs are associated with tumour promotion, but macrophages also show different phenotypes. M1 (“activated”) macrophages express MHC class II, are involved in antigen-presentation to Th1 cells, and have the ability to kill pathogens and cells. In contrast, M2 (“alternatively-activated”) macrophages are involved in Th2-type responses such as humoral immunity and wound healing.[45,46] Gene expression analysis of TAMs in murine mammary

tumours suggests their phenotype closely resembles the M2 phenotype, highlighting that the M2 macrophage phenotype is pro-tumourigenic.[47]

Increasing evidence now supports the theory that tumours are constantly evolving in Darwinian fashion.[2] The successful tumour will acquire mutations that helps it trigger cancer-promoting inflammation and evolve mechanisms to resist immune attack and escape destruction (figure 4). For example, expression of the rearranged oncogene *RET* (implicated in papillary thyroid carcinoma) in thyrocytes induces the expression of a number of pro-inflammatory cytokines and chemokines.[48] Conversely, a number of cancers overexpress programmed death-ligand 1 (PD-L1), which binds the PD-1 receptor on T-cells to dampen down the T-cell response.[49]

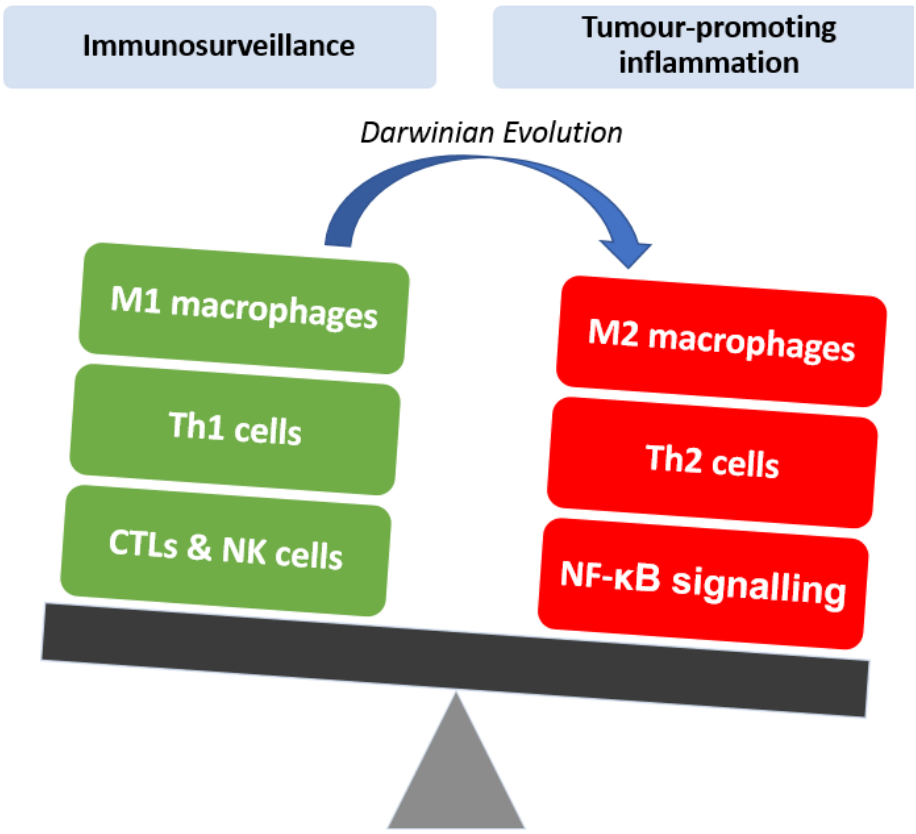

Figure 4: The balance between immunosurveillance and tumour-promoting inflammation. The immune system components that promote and protect against cancer act together in a fine balance. Natural selection allows tumours to evolve mechanisms to tilt the balance in favour of promotion.

Targeting inflammation in anti-cancer therapy

Cancer therapeutics is probably one the biggest areas of active research in medicine, yet one major bottleneck is the evolution of drug resistance. Targeting recruited immune cells reduces this problem because, unlike neoplastic cells, immune cells have a stable genome. To target cancer-related inflammation, we can either limit tumour-promoting inflammation or enhance anti-tumour immunity.

Limiting tumour-promoting inflammation

Anti-inflammatory drugs have the potential to be used in combination with traditional cancer chemotherapy. As previously mentioned, COX-2-mediated PGE_2 production is one of the main trophic mechanisms by which inflammation can enhance neoplastic cell proliferation. Accordingly, COX inhibitors would make an obvious candidate anti-inflammatory drug in cancer treatment. Indeed a meta-analysis of case-control and cohort studies shows that regular aspirin therapy significantly reduces the risk of developing colorectal cancer amongst other solid tumours.[17] Such encouraging results have prompted researchers to undertake a phase III clinical trial testing whether aspirin is an effective adjunct.[50] Given aspirin is a very low-cost drug easily available worldwide, the results of this trial are of great importance.

NF-κB signalling represents the point of convergence of many mechanisms of tumour-promoting inflammation, and thus is an ideal potential target. A number of small molecule inhibitors of the NF-κB pathway are in the pipeline, but the challenge is balancing the benefits of NF-κB inhibition with the potential side effects resulting from global suppression.[51] Whilst some NF-κB inhibitors have demonstrated efficacy in pre-clinical models, none have entered clinical trials and recent research has instead focussed on targeting molecules downstream of NF-κB for a more selective anti-cancer effect.[51,52]

Enhancing anti-tumour immunity

The other option is to upregulate immunosurveillance by enabling the host immune system to better respond to cancer. The most established of such ‘immunotherapy’ is probably the use of monoclonal antibodies against tumours expressing a particular antigen or receptor. Starting with

rituximab for B-cell lymphoma, the list of monoclonals used in cancer treatment has grown and grown and is now at the cornerstone of modern oncology.[53]

As previously mentioned, a number of cancers overexpress PD-L1, which inhibits surrounding T-cells. Antibodies blocking the PD-1 receptor (pembrolizumab and nivolumab) have now been developed and are licensed for use in metastatic melanoma and non-small cell lung cancer.[54] Rather than acting directly on cancer cells, these 'immune checkpoint inhibitors' instead upregulate the T-cell immunosurveillance response. Therefore, these could theoretically be used against any cancer regardless of antigenic signature, but at the same time could have off-target effects related to global T-cell overactivity such as autoimmune diseases and graft-versus-host disease.[54]

To circumvent such potential off-target effects, another avenue of research has focussed on infusing T-cells that are specific to the tumour, an approach known as adoptive T-cell transfer (ACT).[55] The earliest example of this used a protocol of extracting tumour-infiltrating lymphocytes from surgical resections, expanding them by culture *in vitro*, and infusing them back into the patient.[56] Whilst this demonstrated both safety and efficacy in mediating cancer regression, extracting lymphocytes from tumour resections poses significant technical challenges.[55] With rapid advances in genetic technology, recent work has focussed on using normal peripheral blood T-cells and introducing genes to encode receptors against specific tumour antigens.[55] Clinical trials using such engineered T-cells have demonstrated a good response amongst a number of antigens across various tumour types.[55,57–59]

Moving forward, advances in stem cell technology and gene editing are set to revolutionise cell-based therapy in all areas of medicine.[60] For more general purposes, it has been proposed to create HLA-matched banks of induced pluripotent stem cells and it is estimated that around 150 donors will be needed to cover the majority of the UK population.[61] Future work could focus on creating 'à la carte' T-cells against any tumour antigen through directed differentiation of iPS cells from such banks. This would be much quicker than having to culture and engineer T-cells on-demand and would be a more selective therapy than the immune checkpoint inhibitors described above (figure 5). Such an approach holds great potential and one can envisage whole 'armies' of

immune system components, or even cells with novel functions, being engineered from stem cells as a cancer therapy of the future.

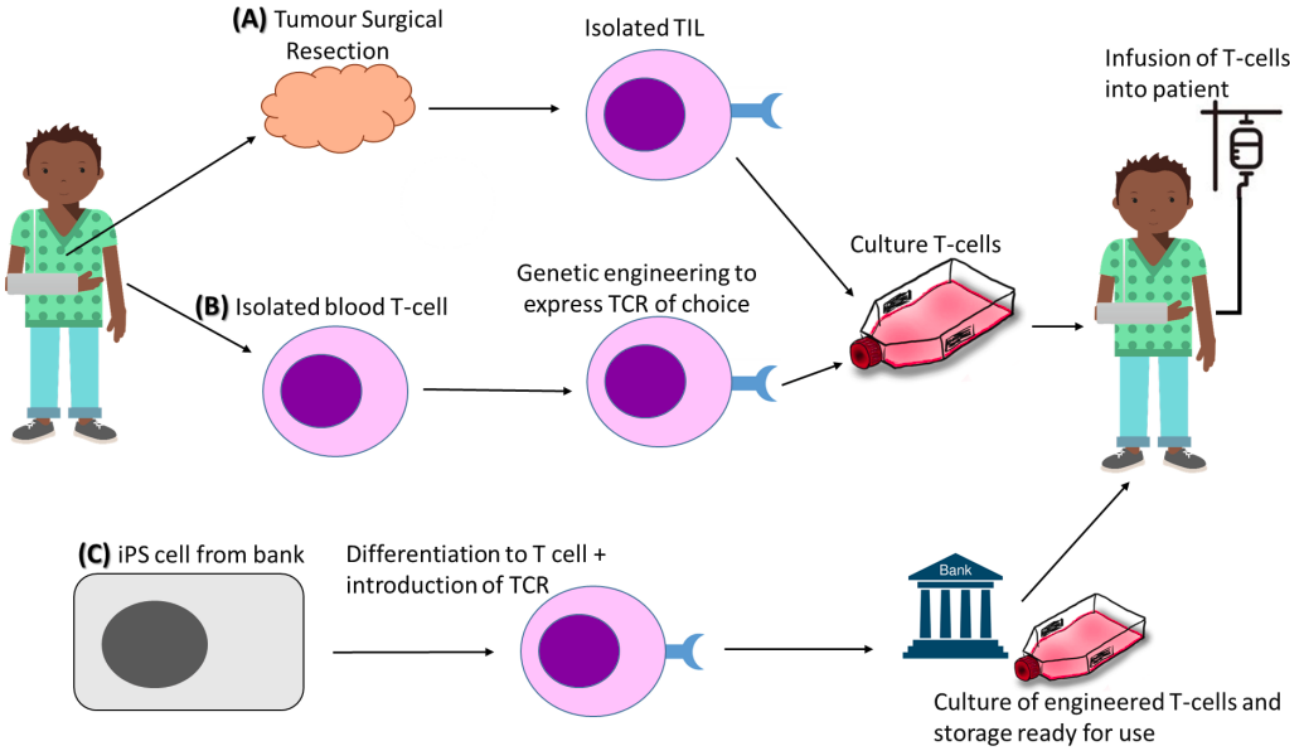

Figure 5: Use of T-cells in cancer immunotherapy. T-cells with a TCR specific to tumour antigens can be sourced from either: (A) a surgical resection of the primary tumour, (B) engineered from peripheral blood T-cells or (C) differentiated from iPS cells. The latter method, whilst still only theoretical, is a more universal approach.

Conclusion

The immune system both promotes and protects against cancer through distinct mechanisms. The control of these two opposing immune system 'modes' is largely dependent on the phenotypes of immune cells, with M1 macrophages and Th1 cells being protective, whilst M2 macrophages and Th2 cells are pro-tumourigenic. This in turn is controlled by complex cytokine signalling networks such as the TNFα/NF-κB signalling axis.

Most tumours trigger an inflammatory response to some degree or another, highlighting an ideal drug target. Anti-inflammatory drugs such as NSAIDs have shown promise as a potential adjuvant therapy and we await further evaluation from clinical trials. Immunotherapy, which aims to help the immune system fight off cancer, has become a huge area of active research and is set to revolutionise practice in modern oncology. The use of monoclonal antibodies is already very well established and research looking into T-cell based therapy, whilst in its infancy, is promising.

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