Antitumour immune responses

# Antitumour immune responses

## Roshni Mitra, Sarvjeet Singh and Ashok Khar

The role of the immune system in combating tumour progression has been studied extensively. The two branches of the immune response – humoral and cell-mediated – act both independently and in concert to combat tumour progression, the success of which depends on the immunogenicity of the tumour cells. The immune system discriminates between transformed cells and normal cells by virtue of the presence of unique antigens on tumour cells. Despite this, the immune system is not always able to detect and kill cancerous cells because neoplasms have also evolved various strategies to escape immune surveillance. Attempts are being made to trigger the immune system into an early and efficient response against malignant cells, and various therapeutic modalities are being developed to enhance the strength of the immune response against tumours. This review aims to elucidate the tumouricidal role of various components of the immune system, including macrophages, lymphocytes, dendritic cells and complement.

Successful cancer immunotherapy is a dream of immunologists that, despite past decades of tremendous progress, has yet to be fully attained, mainly due to the complexity and heterogeneous nature of the disease. Nevertheless, the impressive progress made in immunology and basic tumour biology is likely to prove useful in the near future. Most cancer immunotherapies awaiting field trials have been principally aimed at activation of the host immune system to fight the growing neoplasm. One recent approach has been the use of monoclonal antibodies (mAbs), whose potential against tumours was envisaged as early as the 1970s (reviewed in Ref. 1). Considerable data are now available regarding the clinical trials of mAbs such as Rituximab against CD20 (Ref. 2), which is expressed by follicular lymphoma, and Herceptin against the human epidermal growth factor receptor 2 (HER-2), which is overexpressed on some breast cancers (Ref. 3). Other cancer immunotherapy approaches have focused on the various arms of the immune system that become activated in response to cancer and mediate its subsequent destruction; this particular area will be the subject of this review.

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1

In order to eliminate tumour cells, an immune reaction needs to be able to discriminate between the self-antigens produced by normal cells and antigens expressed particularly on tumour cells. This fact has led to a major breakthrough in the understanding of tumour immunology and the designing of cancer vaccines (Ref. 4). Several immunological mediators, including cytokines and chemokines, control host defence against tumours, and the cytokine profile near the tumour site is often an indicator of the subsequent success or failure of the immune response. The tumour environment thus plays an important role in tipping the scales either towards tumour growth or tumour destruction. Hence, it may be attractive to target the tumour environment rather than the tumour itself for immunotherapy. Despite the wide array of cytolytic molecules and tumouricidal cells present in the immune system, certain tumours have evolved various mechanisms to escape immune detection and attack, as will be discussed here. Finally, the article will focus on the various therapies involving immune participation that are currently under evaluation.

### The humoral immune response and tumour cells

Humoral immunity is mediated by Abs produced by B cells. Abs specifically recognise and neutralise antigens, and can be transferred to individuals by injecting either plasma or serum. Complement proteins present in serum also participate in the destruction of bacteria and other antigens.

Within the circulation, the complement system undergoes continuous low-level activation, thereby patrolling for potential invaders (Ref. 5). Whether this surveillance function also extends to tumour cells escaping into the blood from their original site has not been examined thoroughly; although, in principle, the complement system has the ability to destroy such nucleated cells. Activation of complement can lead to direct lysis of the target cells through complement fixation. This can occur either through the classical pathway, after binding of Abs to the target cells, or through the Ab-independent alternative pathway, whereby deposition of C3b, inactivated C3b (iC3b) and, in some cases, C1q or C4b on the target cell surface facilitates phagocytosis and other forms of complement-dependent cellular cytotoxicity.

The mechanisms underlying complement resistance in malignant cells have only recently been understood. Information on resistance mechanisms is important for immunotherapy of solid tumours with mAbs. IgG subclasses are activators of the complement system and efficient mediators of Ab-dependent cellular cytotoxicity (ADCC) and, hence, are important in tumour immunotherapy (Ref. 6). In addition, complement fixation can induce a strong inflammatory response, which might potentiate other antitumour immune mechanisms. However, despite these powerful mechanisms, tumour cells often escape elimination. Since the complement system has only a limited ability to discriminate between foreign and self, cells express one or more of a group of molecules known as membrane-bound complement-regulatory proteins (mCRPs) that regulate complement activation on the cell surface (e.g. in humans: CD35, CD46, CD55 and CD59) (Ref. 7). With the exception of CD35, these regulators are also expressed on most solid tumours. In addition, soluble complement inhibitors can also limit the complement attack against tumours. Blocking or downregulation of these inhibitors could be an important step in the advancement of immunotherapy.

Matthews et al. (Ref. 8) demonstrated that IgG2a Abs had a protective effect against mouse adenocarcinoma. Similarly, Seto et al. (Ref. 9), using mAbs against a C3H/He mammary tumour, found that IgG2a Abs suppressed tumour growth while IgM Abs were ineffective. However, overall passive immunisation protocols against different tumours have not been very successful. By contrast, the use of Abs as carriers of toxic molecules against cancer cells has considerably more promise.

### The cellular immune response and tumour cells

Cell-mediated immunity (CMI) is a term coined to describe any immune response in which Abs play a subordinate role. The effector cell types implicated in the tumour rejection process are cytotoxic T lymphocytes (CTLs), Ab-dependent killer cells, natural killer (NK) cells and macrophages, all of which act either alone or together with other cell types. Tumour destruction results from three major mechanisms: (1) destruction of the tumour by necrosis mediated by polymorphonuclear leukocytes; (2) indirect inhibition of angiogenesis by secondary interferon

γ (IFN-γ), tumour necrosis factor α (TNF-α) and chemokines; and (3) activation of leukocyte subsets capable of producing proinflammatory cytokines, CTLs and antitumour Abs (Ref. 10).
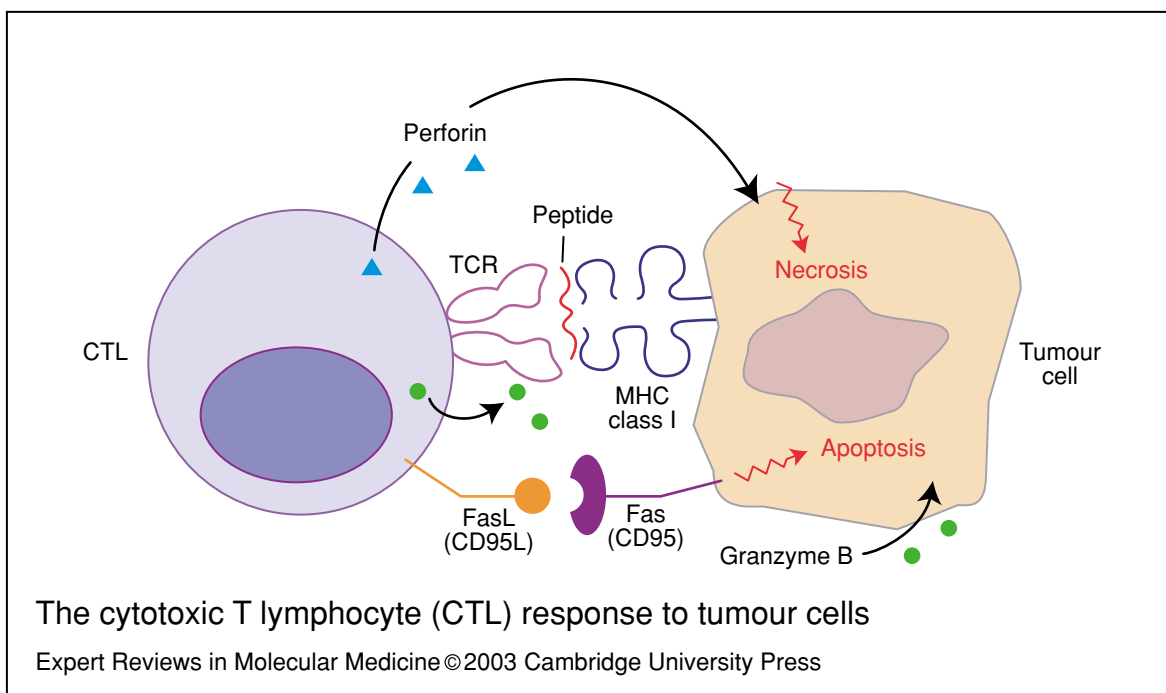
## Role of CTLs

Studies on T-cell activity in cancer patients with primary cancer lesions showed up to 70% positive reactions either in terms of proliferation or significant lysis (Ref. 11). Animal models clearly indicate that a certain level of expression of major histocompatibility complex (MHC) class I by the tumour is necessary to trigger a T-cell response through the T-cell receptor (TCR) complex, and in vitro studies have revealed a similar situation in human neoplasms. The use of tetrameric soluble peptide–MHC class I complexes to identify tumour-specific CD8+ T cells has allowed rapid and accurate analysis of human CD8+ T-cell responses in cancer patients (Ref. 12).

Tumour-specific MHC-restricted CTLs are defined as cytotoxic effectors that are able to lyse neoplastic cells but not normal autologous cells. Such CTLs, generally CD3+CD8+ cells, interact through their CD3/TCR complex with antigens associated with MHC class I molecules on the surface of tumour cells (Ref. 13). In addition, adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and its ligand on lymphocytes, leukocyte function-associated antigen 1 (LFA-1), which promote costimulatory signals for TCR-mediated activation of resting T cells (Ref. 14), play an essential role in modulating interactions between T cells and tumour targets. These cytotoxic effectors mediate tumour destruction by the release of perforins, (pore-forming proteins), serine esterases, and IFN-γ and/or TNF-α (Ref. 15) (Fig. 1).

The T-cell response is therefore highly relevant in the clinical control of cancer development and progression. This is supported by studies with animal tumours in which only tumour-specific T cells in combination with interleukin 2 (IL-2), when transferred to tumour-bearing animals, resulted in eradication of the malignancy (Ref. 16).



The cytotoxic T lymphocyte (CTL) response to tumour cells

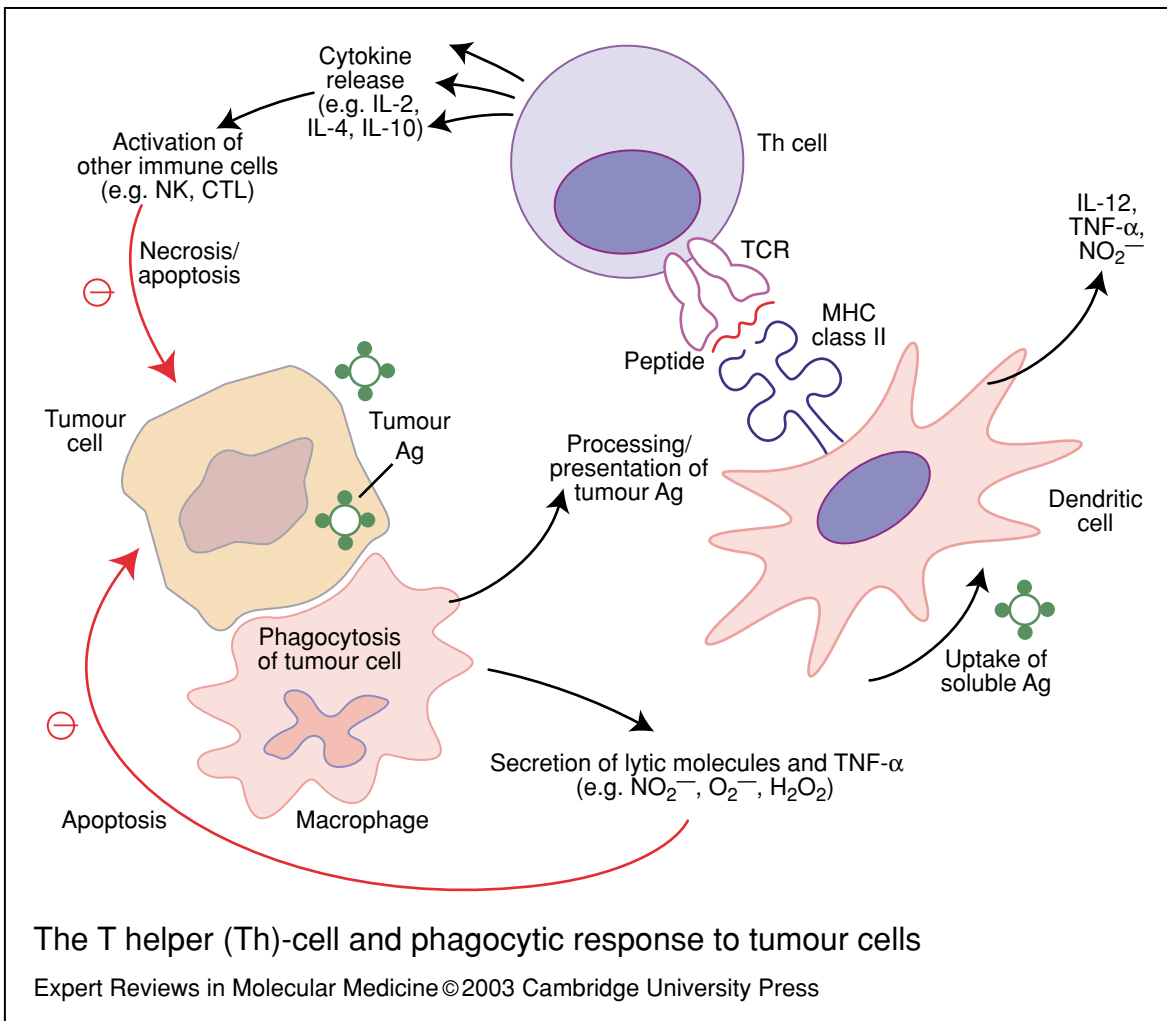Expert Reviews in Molecular Medicine © 2003 Cambridge University Press

**Figure 1. The cytotoxic T lymphocyte (CTL) response to tumour cells.** CTLs interact with tumour cells through binding of their T-cell receptor (TCR) to the major histocompatibility complex (MHC) class I molecules expressed on the tumour cell surface. The interaction also involves binding between adhesion molecules, such as intercellular cell adhesion molecule 1 (ICAM-1) with leukocyte function-associated antigen 1 (LFA-1) (not shown), and Fas (CD95) with Fas ligand (CD95L). The tumour cell destruction is affected by the release of perforins (pore-forming proteins), serine esterases, interferon γ (IFN-γ), granzyme B and/or tumour necrosis factor α (TNF-α) by CTLs **(fig001akh)**.

3

## Role of T helper (Th) cells

CD4$^+$ Th cells play a crucial role in the amplification and regulation of the cellular immune response (Fig. 2). Antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), engulf tumour cells and tumour cell products. Tumour cell antigens are processed and presented to Th cells via MHC class II molecules, which interact with the TCR on Th cells (Ref. 17). Th cells respond by secreting cytokines, which in turn activate other immune cells. Th cells are divided into different types according to

their cytokine profile (Ref. 18). Th1 cells are characterised by the production of IFN-γ and IL-2, whereas Th2 cells produce IL-4, IL-5 and IL-10. The discrimination of Th cells into additional types such as Th3 (Ref. 19) or so-called T regulatory 1 (Tr1)-type cells (Ref. 20) is characterised by the production of IL-10 and other inhibitory cytokines such as transforming growth factor β (TGF-β). Th cells producing a wide spectrum of cytokines are known as Th0 cells. All of these different subsets of Th cells can play an important regulatory role in



The T helper (Th)-cell and phagocytic response to tumour cells

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**Figure 2. The T helper (Th)-cell and phagocytic response to tumour cells.** Antigen-presenting cells (APCs), such as macrophages and dendritic cells, engulf tumour cells and tumour cell products. Tumour cell antigens are processed and presented to Th cells via major histocompatibility complex (MHC) class II molecules, which interact with the T-cell receptor (TCR) on Th cells. Th cells respond by secreting cytokines, which in turn activate other immune cells such as natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). Macrophages also secrete lytic molecules, such as nitric oxide (NO$_2^-$), superoxide anions (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), in addition to tumour necrosis factor α (TNF-α). Dendritic cells secrete interleukin12 (IL-12), tumour necrosis factor α (TNF-α) and NO$_2^-$ **(fig002akh)**.

4

controlling the initiation and downregulation of the immune response.

The main research focus on antitumour CTL responses, rather than Th-cell responses, has been because most tumours express MHC class I but lack MHC class II (Ref. 21). However, it has become evident that, for an efficient and long-lasting immunity against tumours, activation of tumour-specific Th cells is essential. Indeed, CD4+ T cells are crucial for elimination of foetal bovine lung (FBL) erythroleukaemia (Ref. 22). Furthermore, vaccination with a Moloney murine leukaemia virus (MuLV)-derived Th peptide was found to induce protection against a subsequent challenge with an MHC class II– lymphoma, the main effector cell type being CD8+ T cells. This, in turn, would imply that the role of CD4+ T cells in steering the immune response might be either beneficial or detrimental, depending on the antigenic and genetic factors involved in a specific tumour.
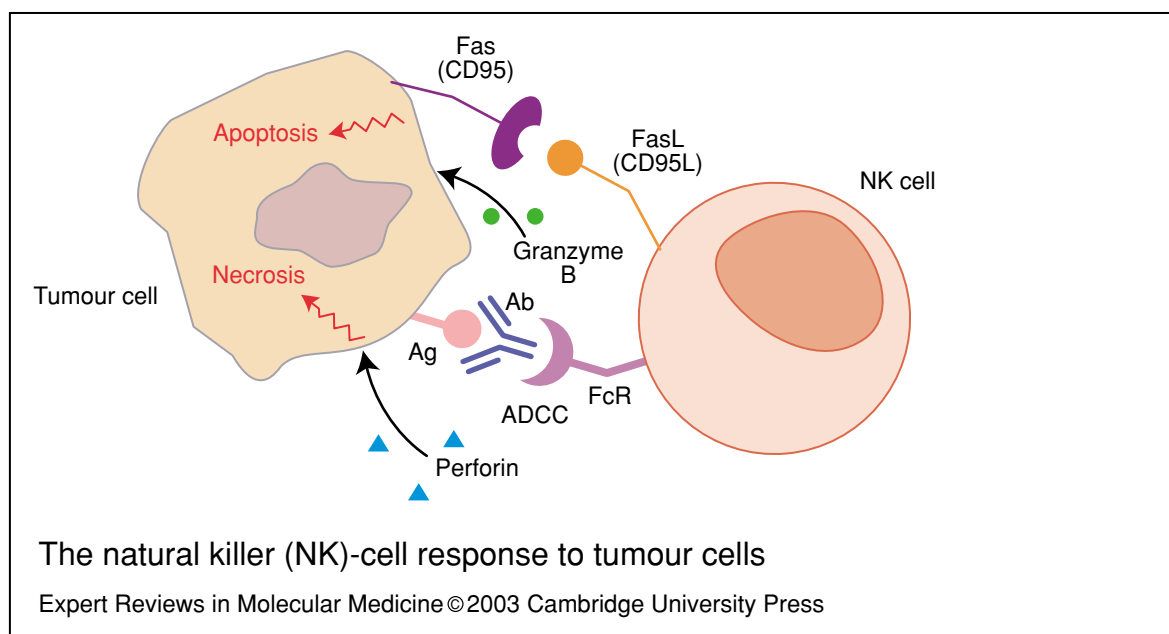
### Role of NK cells

The term 'natural killer' cell has been used to designate cells other than macrophages and polymorphonuclear leukocytes that are cytotoxic to neoplastic or non-neoplastic targets in the absence of specific TCR activation by the antigen. The mechanism of NK-mediated oncolysis involves recognition and conjugation of effector to target, delivery of death signals, and disintegration and death of the target cells (Ref. 23). Some tumours specifically stimulate NK cells by producing IFN, which activates NK-cell tumouricidal activity (Ref. 24).

There have been many studies demonstrating the sensitivity of tumours to lysis by NK cells in vitro and in vivo, as judged by tumour incidence and growth after subcutaneous injection of viable cells (Refs 25, 26). Indeed, mice inoculated intraperitoneally with an NK-sensitive variant of a methylcholanthrene-induced lymphoma survived longer than mice inoculated with an insensitive variant (Ref. 23). Thus, NK cells are well qualified to provide the first line of defence in both normal and T-cell-deficient hosts. This is because, unlike T cells, they do not require processing and presentation of antigen along with MHC molecules. In consequence, NK cells kill targets that have managed to escape T-cell-mediated killing because they do not express the appropriate MHC molecule (Fig. 3).

Patients with poor NK-cell activity possess lower resistance to infection and increased cancer



The natural killer (NK)-cell response to tumour cells

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**Figure 3. The natural killer (NK)-cell response to tumour cells.** NK cells kill tumour cells through mechanisms that involve: antibody (Ab)-dependent cellular cytotoxicity (ADCC), in which the Fc portion of an Ab bound to antigen (Ag) on the tumour cell surface binds to Fc receptor (FcR) on the NK cell; Fas (CD95)–Fas ligand (CD95L) interaction; and release of perforin and granzyme B molecules, which cause apoptosis/necrosis of the tumour cell **(fig003akh)**.

**Antitumour immune responses**

metastasis (Ref. 27). One mechanism by which NK cells lose their activity is if they fail to produce cytolytic factors following exposure to target cells. NK cells that have been inactivated by target cells exhibit downregulation and/or shedding of the FcγRIII CD16 receptor. These observations suggest that CD16 may have an active role in target-cell-induced NK-cell anergy and apoptosis. Target-cell-induced anergy in NK cells has been demonstrated following co-culture with fixed AK-5 tumour cells (a rat histiocytic tumour cell line) (Ref. 28). In addition, TNF-α secretion and Fas (CD95) upregulation on NK cells after co-culture may play an important role in signalling NK cells for functional anergy and apoptosis (Ref. 28).

Little information is available about the recognition structures on the surface of NK cells and target cells. However, Girardi et al. (Ref. 29) and Diefenbach et al. (Ref. 30) have identified the activating receptor for NK cells, and also T cells expressing the γδ or αβ TCR, as NKG2d on target cells. These findings unite innate and adaptive immunity within the realm of cancer immunology. The two best-characterised NKG2d ligands are MICA and MICB in human cells; these are nonclassical MHC molecules whose expression is induced by classical stress stimuli (e.g. heat shock, mechanical stress, etc.) (Refs 29, 30). Murine NKG2d binds to retinoic-acid-inducible gene family products such as Rae-1α–ε, as well as the H60 gene product (Refs 29, 30). Like MICA and MICB, Rae-1 and H60 appear to be upregulated in several tumours. Cerwenka et al. (Ref. 31) found that, upon induction of these ligands on mouse tumours, which normally do not express Rae-1β or H60, NK-cell-dependent tumour rejection took place. It was found that tumours that expressed these ligands and yet survived in the host were able to balance the expression of activating versus inhibitory ligands, resulting in the dominance of immunological tolerance to tumour cells.

## Role of B cells

B cells infiltrating cancer tissues produce IgG that recognises a common tumour antigen (Ref. 32). Screening of tumour-derived expression libraries for antigens that are detected by high-titre IgG Abs from the sera of cancer patients, using serological identification of antigens by recombinant expression cloning (SEREX), has allowed the systematic search for antigens of human cancers (Ref. 33). SEREX has led to the identification of a plenitude of new tumour antigens in many different tumours. In addition to tumour-associated Ab responses, circulating auto-Abs against several other self-antigens have also been described in cancer patients (Ref. 34).

## Role of macrophages

Macrophages mediate both ADCC and the nonspecific killing of tumour cells (Ref. 35). Macrophage-mediated cytotoxicity is most efficient when cell-to-cell contact is established, but several soluble factors that cause cytotoxicity have also been found in macrophage culture supernatants (Refs 36, 37) (Fig. 2). Activated macrophage cytotoxicity against tumour cells was dependent on the nitrite ion synthesis, and its inhibition lead to defective cytotoxic potential (Ref. 38). The cytocidal effect is primarily mediated by nitric oxide (NO) by virtue of its ability to inhibit mitochondrial respiration, prevent DNA replication and denature various essential iron–sulphur-containing enzymes present in target cells. Other antitumour products secreted by macrophages include TNF, hydrogen peroxide ($H_2O_2$) and FasL (CD95L), which are equally essential for macrophage cytotoxicity (Ref. 39).

Tumour-associated macrophages (TAMs) have received considerable attention. Their percentage in a tumour tissue may vary from 0–80%, the average being 20–30% (Ref. 40). The number of macrophages in an average-sized tumour amount to several fold more than the total number of macrophages in a normal mouse (Ref. 41). In addition, a tumour can cause marked qualitative changes in the subpopulations of macrophages present, these being distinguished by their distinctive, and sometimes mutually antagonistic, functions (Ref. 42). The local and systemic influence of tumour-derived factors (e.g. MHC class Ia expression and microenvironmental influences, such as $O_2$ tension) may be crucial in determining the nature and number of macrophages within tumours (Ref. 43). These could be the reasons why the nature of the tumour–macrophage relationship varies so much from tumour to tumour.

Macrophages are also a source of cytokines that can manipulate the emerging T-cell response. The definitive macrophage-activating molecule (IFN-γ) and its corresponding cytokine secreted by macrophages (IL-12) are discussed later. Given that monokines such as IL-12 synergise with costimulators B7.1 and B7.2 to enhance T-cell

proliferation (Ref. 44), macrophages are clearly a major factor in driving T-cell expansion as well as differentiation.

The role of host macrophages in AK-5 tumour remission has been elucidated by subcutaneous transplantation of AK-5 tumour cells, which led to the influx of macrophages into the peritoneal cavity where they were hyperactivated (Ref. 45). Subsequent depletion of activated macrophages from the peritoneal cavity and their migration to the tumour site coincided with tumour regression (Ref. 45). This could be because of the secretion of monocyte chemotactic substances by tumour cells or extracellular matrix proteins, which attract and activate macrophages directly (Ref. 46).

### Role of DCs

DCs function as APCs (Fig. 2). The presence of DCs in tumour infiltrates is a good prognostic factor, and interaction between the DC and tumour results in the release of antitumour cytokines (Ref. 47). In patients with stage III squamous cell carcinoma, a significantly better survival after radiation therapy was observed when infiltrating Langerhans cells were observed in tumour tissues, possibly because of stimulating T-cell-mediated antitumour activity (Ref. 48). Inhibition of metastatic cell appearance in a tumour might depend on the entry of DCs into the tumour, and the release of NO and other inhibitory cytokines by DCs. Conversely, a failure of DCs to enter tumours might be because of the ability of the tumour to produce cytokines that inhibit NO production (Ref. 49).

It was recently shown that antigen-pulsed DCs could directly sensitise T cells and stimulate the development of antigen-specific immune responses, including both protective and therapeutic antitumour responses. DC maturation and activation is mediated by the interaction between CD40 on DCs and CD40L on antigen-stimulated Th cells (Ref. 50). Further work is in progress to prove the therapeutic potential of activated DCs in cancer.

### Role of cytokines

Cytokines are powerful regulators of normal cell behaviour and play an important role in the host immune response against a wide variety of infections and cancers. The characteristics of the immune response to tumours are significantly influenced by the activity of Th-cell populations and their cytokine products. For example, Th1 cells preferentially secrete IFN-γ and IL-2, and are responsible for CMI, whereas Th2 cells produce IL-4, IL-5, IL-6 and IL-10, and enhance the humoral response. A Th3 subset, producing high levels of TGF-β with varying amounts of IL-4 and IL-10, has also been identified (Ref. 51). Th3 cells have an immunosuppressive phenotype in experimental models of oral tolerance and autoimmunity (Ref. 52). There is an antagonistic effect between Th1- and Th2-cell populations as the cytokines produced by one population nullify the proliferation and function of cells of the other type. These Th-cell populations and their cytokine products thus play a decisive role in the progression or regression of a tumour. The major cytokines and interleukins involved in immune responses are listed in Table 1 and Table 2.

The ability of cytokines to cause the regression of a tumour is exemplified by the AK-5 tumour model, where IL-2, IL-12 (Ref. 48), IFN-γ (Ref. 49) and TNF-α are found at high levels in tumour-regressing animals. Animals regressing the tumour show a predominant CD4/Th1-type of cytokine response and the tumour regression is primarily NK-cell-mediated (Ref. 50). NK cells are unable to kill AK-5 tumour cells until they are activated with IL-2, IL-12 and IFN-γ (Ref. 24). Cytokine levels at the tumour site were several-fold higher than in the circulation, suggesting their important role in the maintenance of tumour-infiltrating lymphocytes in an activated state against the tumour (Ref. 26).

### Tumour rejection antigens

#### TSAs and TAAs

Depending on the initial carcinogenic event and the type of tissue, tumour cells are known to express neo-antigens or oncofoetal antigens. These antigens determine the type of immune response evoked in the host (i.e. humoral or cell-mediated). Tumour-specific antigens (TSAs) are antigens expressed on tumour cells but not normal cells and are capable of eliciting an immune response in a syngeneic host. Antigens encoded by families of genes, such as MAGE, BAGE or GAGE, that are silent in all adult normal tissues except male germline cells are examples of TSAs. Some tumour antigens are also expressed concurrently on normal cells in the host, and expression may or may not be restricted to the type of tissue from which the tumour originated. These are called tumour-associated antigens (TAAs). Although some TAAs may induce an

**Antitumour immune responses**

7

**Antitumour immune responses**

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## Table 1. The major cytokines involved in immune responses[a] (tab001akh)

| Cytokine | Source | Target | Effect on target |
|----------|--------|--------|------------------|
| TNF-$\alpha$<br>TNF-$\beta$ | Macrophages<br>T cells | Macrophages<br>Granulocytes<br>Endothelium<br>Hypothalamus | Activation, increased adherence of leukocytes to endothelium, production of acute phase proteins[b], cellular catabolism |
| IFN-$\alpha$<br>IFN-$\beta$ | Leukocytes | All cells<br>Leukocytes | Antiviral effect, anti-proliferation, induction of MHC class I and II, activation |
| IFN-$\gamma$ | T cells<br>NK cells | Tissue cells<br>Endothelium | Antiviral effect, induction of MHC class I and II, activation |
| M-CSF | Macrophages<br>Endothelium<br>Fibroblasts | Committed progenitors | Stimulation of division and differentiation |
| G-CSF | Macrophages<br>Endothelium<br>Fibroblasts<br>T cells | Committed progenitors | Stimulation of division and differentiation |
| GM-CSF | T cells<br>Macrophages<br>Endothelium | Immature progenitors<br>Committed progenitors<br>Macrophages | Growth<br>Differentiation<br>Activation |
| MIF | T cells | Macrophages | Migration inhibition |
| Chemotactic factors | Lymphocytes<br>Macrophages<br>Granulocytes | Leukocytes | Attraction to site of infection or tissue damage |
| TGF-$\beta$ | T cells<br>Macrophages | T cells<br>Macrophages, others | Inhibits activation<br>Growth regulation |

[a] Modified table reproduced with permission from Elsevier Science ©1993 (Ref. 115).
[b] Production of acute phase proteins such as interleukin 1 and C-reactive protein act to limit the spread of the infection and stimulate the host response.
Abbreviations: G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; MIF, migration inhibition factor; NK, natural killer; TGF-$\beta$, transforming growth factor $\beta$; TNF, tumour necrosis factor.

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immune response in the host, they generally do not because of self-tolerance. TSAs can be expressed on the cell surface or in the cytoplasm or even the nucleus; however, surface antigens are more immunogenic (Ref. 53). Some TSAs exhibit great similarity with stress proteins, such as the gp96 class of proteins, which show sequence homology to heat shock protein 100 (Hsp100) and are inducible upon heat shock. It has been suggested that stress-induced proteins might not be tumour antigens per se but could be acting as carriers of immunogenic molecules (Ref. 54).

**Immune reactions to tumour antigens**

The quality and quantity of the immune response evoked is determined by the nature of the antigens expressed by the tumour. For example, carbohydrate antigens induce highly potent humoral immune responses, while protein antigens establish a CMI response (Ref. 55). Furthermore, the immunogenicity of a particular tumour depends on the processing of its antigens. In the absence of IFN-$\gamma$, the peptides presented to CTLs by MHC class I molecules result from the degradation of intracellular proteins by the

### Table 2. The major interleukins involved in immune responses[a] (tab002akh)

| Interleukin (IL) | Source | Target | Effect on target |
| --- | --- | --- | --- |
| IL-1a, -b | Monocytes<br>Dendritic cells<br>B cells<br>Endothelium<br>Fibroblasts<br>Astrocytes | T and B cells<br>Liver<br>Endothelium<br>Hypothalamus | Lymphocyte activation, inflammatory mediator, production of acute phase proteins[b], cellular catabolism |
| IL-2 | T cells<br>Natural killer (NK) cells | T and B cells<br>NK cells | Proliferation, activation, isotype switching |
| IL-3 | T cells | Stem-cell progenitors | Stimulation of cell production in bone marrow |
| IL-4 | T cells | T and B cells<br>Macrophages | Proliferation, differentiation, activation |
| IL-5 | T cells | B cells<br>Eosinophils | Proliferation, activation |
| IL-6 | T cells<br>Macrophages<br>Endothelium | T and B cells<br>Liver | Proliferation, production of acute phase proteins[b] |
| IL-7 | Stromal cells<br>Fibroblasts | Immature lymphoid cells | Proliferation, differentiation |
| IL-8 | Macrophages<br>Endothelium<br>Fibroblasts<br>T cells | Neutrophils<br>Eosinophils<br>T cells | Chemotactic factor |
| IL-9 | T cells | Progenitor cells | Haematopoiesis |
| IL-10 | T and B cells | Mast cells<br>T cells | Growth regulation, differentiation |

[a] Modified table reproduced with permission from Elsevier Science ©1993 (Ref. 115).
[b] Production of acute phase proteins such as interleukin 1 and C-reactive protein act to limit the spread of the infection and stimulate the host response.

proteosome. However, in the presence of IFN-γ, the three catalytic subunits of the proteosome are replaced by their homologues to form what is called the immunoproteosome (Ref. 56). This changes the cleavage pattern and the profile of antigenic peptides produced by the cell. Some peptides that are poorly processed by the standard proteosome are now processed more efficiently and act as better stimulators of CD8+ T cells. However, the reverse case, where the processing efficiency of the immunoproteosome for a peptide is lower than the standard proteosome, is also known (Ref. 57). Thus, the local concentration of IFN-γ has an effect on determining the immunogenicity of a tumour antigen. The initial carcinogenic event also affects the immunogenic profile of a tumour; for instance, ultraviolet (UV)-radiation-induced tumours have been shown to be highly immunogenic (Ref. 58). Furthermore, the spontaneous regression of AK-5 (Refs 59, 60) in syngeneic hosts is a consequence of the highly immunogenic nature of the tumour. The gene for the tumour rejection antigen in AK-5 has been cloned (Ref. 61) and a recombinant fusion protein has been found to impart immunogenicity to animals that resisted the growth of the AK-5 tumour on challenge (Ref. 61). The fact that

9

other tumours grew in the immunised animals suggests the specificity of antigenicity.

The capacity of a TSA to evoke an immune response is influenced by the interaction between the tumour and the host. In the classical immune response, soluble antigen is acquired and processed by specialised APCs and presented in the context of MHC class II molecules to CD4+ Th cells (Fig. 2). The resulting activated CD4+ T cells provide help to antigen-specific CD8+ T cells in the form of secreted cytokines (Ref. 62). These CD8+ T cells are also directly activated by target cells presenting endogenously synthesised antigenic peptide in the context of their MHC class I molecules (Fig. 1). This scheme functions effectively if the antigen is a soluble molecule or is released from the degraded and/or dying tumour cell. As tumour growth progresses, parts of the tumour can become necrotic and soluble tumour antigens are released. By this time, however, the tumour could have reached a threshold size against which any immune response is impotent. Thus, for an effective antitumour immune response, it is necessary for the tumour antigens to be accessible to the effector cells of the host at an early enough stage in tumour development.

It is now generally accepted that T cells mediate specific immune responses directed against tumour antigens. It has been possible to obtain highly specific CTLs from syngeneic animals and to use these to define tumour antigens (Ref. 63). Furthermore, MHC antigens on tumour cells have been shown to facilitate T-cell immune defence against cancer. Indeed, certain melanomas and lymphomas have higher expression of MHC class I antigens that seems to be associated with increased growth and metastasis and resistance to NK-cell-mediated killing of the target (Ref. 64).

### Immune evasion

The knowledge that syngeneic tumours possess tumour rejection antigens yet are still capable of escaping destruction by host immunity indicates that some form of immune evasion occurs. Some tumours have been shown to escape immune destruction using immunological ignorance mechanisms, others avoid confrontation with host effector cells by secreting anti-inflammatory and immunosuppressive factors, and others induce apoptosis of effector cells.

### Immunological ignorance

Silent tumour antigens are defined as all naturally presented MHC class I-associated peptides that are not immunogenic during tumour growth (Ref. 65). Studies using transgenic mice show that viral antigens expressed on pancreatic β-cells cannot delete or anergise peripheral responder CTLs, resulting in the peaceful coexistence of the antigens with CTLs that are fully competent at reacting to the antigens in vitro – a phenomenon called immunological ignorance (Ref. 65). This corroborates the observation that, in the majority of cancer patients, freshly isolated tumour-infiltrating T cells are usually inactive against autologous cancer cells, although these cells can be activated in vitro with lymphokines such as IL-2 (Ref. 66).

Many factors might contribute to antigen silencing in cancer cells, including: the nature of the TCR ligand (i.e. peptide–MHC); the threshold level of TCR occupation [there is a critical threshold of TCR molecules to be engaged with peptide–MHC in order to achieve a significant and detectable T-cell response (Ref. 65)]; the type of APC; and the status of the T cell (naive versus memory). One mechanism used by tumour cells, especially virally induced tumours, to evade the immune system is the downregulation of MHC class I expression (Ref. 67). This strategy prevents the presentation of processed tumour antigen peptides by MHC molecules, which is required for the CTL response. Another mechanism of immunological ignorance is imparted by the shedding of TAAs, which prevent the tumour cell from being recognised as different by immune mechanisms. This has been found in human bladder cancer cells (Ref. 68) and melanoma cells (Ref. 69). Some tumours have been shown to escape immune destruction by masking their surface antigens under immunological pressure by coating them in glycocalyx molecules (Ref. 70).

### Immunosuppression

Some tumours are not poorly immunogenic, but rather are immunosuppressive. Tumours produce cytokines, growth factors, chemotactic molecules and proteases that influence CMI (Ref. 71). Many tumour-derived molecules, such as IL-4, IL-6, IL-10, TGF-β1, prostaglandin $E_2$ ($PGE_2$) and macrophage colony-stimulating factor (M-CSF) deactivate or suppress immune cell cytotoxicity (Ref. 72). Other tumour cells avoid confrontation with host effector cells by

**Antitumour immune responses**

10

**Antitumour immune responses**

secreting anti-inflammatory factors that function to prevent host cell migration across vascular endothelium into the tumour mass (Ref. 73).

In addition to the direct secretion of immunosuppressive cytokines, cancer cells can induce host cells to release immune inhibitors (Ref. 74). Tumour-derived prostaglandins augment the production of inhibitory cytokines such as IL-10 (Ref. 75), while suppressing endogenous production of cytokines including IL-12 that are necessary for effective host-cell-mediated antitumour immune responses (Ref. 76). The enzyme cyclooxygenase 2 (COX-2) acts at the rate-limiting step of prostanoid production (Ref. 77), and COX-2 expression by tumour cells might be an important therapeutic target for pharmacological or gene therapy intervention.

One of the most potent immunosuppressive factors to be characterised is TGF-β (Ref. 78), which affects proliferation, activation and differentiation of the cells that participate in both innate and acquired immunity. TGF-β inhibits immunoregulatory cytokine production (Ref. 79), including IL-12 production by monocytes (Ref. 80). TGF-β has also been reported to be a potent inhibitor of CTL differentiation (Ref. 81), suggesting that this factor might affect the development of tumour-reactive CTLs in vivo. However, since TGF-β is also a potent inhibitor of the proliferation of neoplastic cells in vitro, this cytokine might exert dual effects on tumour growth in vivo (Ref. 82).

TAMs capable of suppressing lymphocyte responses have been known for some time (Ref. 83). Evans (Ref. 84) has shown that TAMs could enhance or suppress in vitro mitogen responses depending on the stage of tumour growth. TAMs from early or late tumours were suppressive, while TAMs in mid-stage tumours enhanced immune responses. Such changes in TAM function presumably reflect the self-regulatory potential of the macrophage system (Ref. 85). Macrophages release reactive nitrogen and oxygen intermediates (RNI and ROI), which directly suppress immune cells, and TNF-α, which suppresses indirectly by inducing macrophage production of inhibitory molecules such as $PGE_2$ (Ref. 86). Although the cytotoxic activity of RNI, ROI and TNF-α might benefit the tumour-bearing host, the suppressor activity of these molecules would prevent antitumour lymphocyte responses. However, no evidence regarding the existence of suppressor macrophages (Ref. 45) and their influence on immunosuppressive mechanisms of immune evasion are known.

## Apoptosis

Fas is a cell-surface member of the TNF receptor superfamily and, upon engagement by its ligand FasL, mediates programmed cell death, or 'apoptosis', of the Fas-expressing cell [see Ref. 87 for a review in this journal]. The Fas–FasL interaction is involved in the induction of apoptosis in immune cells, thereby maintaining selection and development, and limiting lymphoid expansion (Ref. 88). Some tumour cells have devised 'counterattack' mechanisms of immune escape by expressing FasL (Refs 89, 90, 91, 92), whereby the tumour cells resist Fas-mediated T-cell cytotoxicity but, by expressing functional FasL, mediate the peripheral deletion of tumour-reactive T-cell clones. The expression of FasL therefore provides a means of bestowing immune privilege on tumour cells. In addition, ascitic fluid has been found to contain high levels of soluble CD95L, which is shed by the tumour cells in the peritoneum and may have a role in immune evasion.

## Cancer immunotherapy

### Antitumour vaccines

Tumour cells are known to be poor immunogens and are consequently able to induce tolerance (see above). Thus, any vaccine would need to break this tolerance and activate populations of CTLs and Th cells. Tumour antigens themselves are primary agents in the development of vaccines (Ref. 93), although whole-cell-based cancer vaccines have also been successful (Ref. 94). Early studies used killed tumour cells or their lysates and more-recent attempts have used genetically engineered cells, such as tumour cells transduced with specific viral genes and allogenic MHC genes (Ref. 95). This has helped in the augmentation of MHC class I expression, a deficiency that is responsible for a weak CTL response. This has also been achieved by exposure of cells to IFN-γ or by activated DCs preincubated with 'tumour peptide preparations' (Ref. 96).

Tumour cells transfected with genes for different cytokines and costimulatory molecules are a recent addition to the repertoire of cancer vaccines. Among cytokines, granulocyte–macrophage colony-stimulating factor (GM-CSF) appears to be the most potent because of its ability to promote local DC differentiation at the

vaccination site, which in turn potentiates T-cell activation (Ref. 97). The recent pancreatic cancer vaccine GVAX is such an example that is already under Phase II clinical trials (Ref. 98). Also, increased local production of other cytokines, such as IL-2, IL-7 and IL-12 is mandatory for inducing a strong cytolytic response and this has been achieved by transfecting these genes into the tumour cell (Ref. 99).

One possible reason for an inefficient CTL response is a lack of costimulatory molecules. Cells transfected with genes that provide the costimulatory signal, such as B7-1 and/or B7-2, have been shown to be immunologically highly potent (Ref. 100). One important factor that needs to be considered when using tumour cells as vaccines is the production of active immunosuppressive molecules, such as TGF-β and PGE$_2$, by the tumour cells since these inhibit cytolytic immune responses. It is important to counteract any such inhibitor when tumour cells are being used for immunisation. One approach has used genetic modification to inhibit TGF-β expression with an antisense plasmid vector against the immunosuppressive molecule (Ref. 101).

Immunisation with antigens is another approach that is being tried and has shown some promise. Antigen-based vaccine development requires identification of the most potent tumour rejection antigens and the appropriate route by which it is delivered to the immune system. In the past few years, several MHC class I-restricted tumour antigens have been identified and clinical studies using antigenic peptide-pulsed DCs as vaccines have shown some therapeutic effects (Ref. 96). Since the majority of MHC class I-restricted antigens are non-mutated self-proteins, a potential threat of active immunisation with these proteins is the development of autoimmune disorders. Evidence is now accumulating that CD4+ Th cells are also actively involved in initiating and maintaining an antitumour immune response (Ref. 62). Thus, the search for MHC class II-restricted tumour antigens that can stimulate CD4+ T cells is underway.

Another candidate that has emerged as an antitumour vaccine is the DC, which as an APC plays a central role in the initiation of immune responses. DCs express 50-fold higher levels of MHC molecules than macrophages, thereby providing more peptide–MHC ligands for TCR engagement. In addition, they express high levels of adhesion and costimulatory molecules required for T-cell activation. On the basis of these facts, researchers have used isolated DCs or GM-CSF-induced DCs as antigen carriers for tumour vaccination and this strategy has now reached clinical trials (Ref. 94).

### Hsps in cancer therapy

Hsps are expressed as tumour antigens in many tumours. They are also known to serve as carriers for immunogenic peptides (Ref. 54). Hsp70 forms a complex with activated mutants of the tumour suppressor protein p53 in transformed cells and this particular interaction has been associated with immune responses against human breast cancers (Ref. 102).

Many mechanisms have been proposed by which Hsps might influence the immunogenicity of a tumour cell [see Ref. 103 for a review in this journal]. Hsps might constitute an immunological 'danger signal', thereby initiating immune responses, or the released Hsps from dying tumour cells might transfer antigenic peptides to APCs such as DCs and macrophages, which activate tumour-specific T cells. Furthermore, Hsps might increase the ability of tumour cells to process and present endogenous tumour antigens directly to specific T cells. The immunopotentiating effect of HSPs can be attributed to the function of HSPs as 'molecular adjuvants'. In many studies, administration of antigenic peptide with purified Hsps induced potent CD8+ T-cell responses. The use of Hsp–peptide complexes as therapeutic vaccines has been described by Srivastava et al. (Ref. 104).

Keeping in view the carrier function of Hsps, the chaperoning of tumour antigenic peptides into macrophages, via complexing with tumour-cell-derived Hsps, has been described as an effective measure against many tumours (Ref. 105). Thus, any treatment modality that aims at inducing the expression of Hsps specifically on tumour cells could prove to be an effective measure to fight cancer.

### Apoptosis in cancer therapy

Apoptosis might potentially be disrupted in tumour cells, conferring a survival advantage (see above). One of the major means to treat tumours is to induce their selective apoptosis. Emerging evidence suggests that, because of varying apoptosis thresholds (i.e. how soon or late apoptosis is induced in a cell), some treatments

might induce apoptosis in tumour cells (Ref. 106). This selection of 'apoptosis induction' is based on the fact that cell death processes are faster than cell proliferation process. Apoptosis is a major means of killing tumour cells by the immune system that involves T and NK cells. Thus, if the inherent property of tumour cell killing by apoptosis could somehow be accelerated, it would provide great therapeutic potential. Chemotherapy and radiation therapy also work towards this end of inducing apoptosis in tumour cells.

However, tumour cell killing by apoptosis has a darker side that compromises its promise as the most effective therapy for cancer. It has been observed that tumour immunogenicity is determined by the mechanism of cell death that can be apoptotic or necrotic (Ref. 107). When tumour cells are killed predominantly by apoptosis, the immunogenicity of the remaining tumour cells decreases, whereas tumour cell killing by necrosis leads to an elevated immunogenic response. The change in the immunogenicity brought about by an apoptotic or a non-apoptotic process is mediated by the expression of Hsps. It is suggested that the mechanism of tumour cell death in vivo could also provide such an immunological signal (Ref. 108).

### Angiogenesis in cancer therapy

The growth of any tumour inside the body is critically dependent on how efficiently the blood vasculature is formed and maintained around it. There is a constant requirement for vascular supply in solid tumours and this tumour-associated neovascularisation gives the tumour its critical growth advantage (Ref. 109). Angiogenesis and the vascular density of tumours have been shown to be associated with tumour metastasis, the most dreadful part of neoplastic growth. A higher rate of vasculature has been shown to be associated with higher rates of mortality (Ref. 110).

Since the discovery of the role of angiogenesis in the pathogenesis of tumour growth and metastasis, new cancer treatment strategies that exploit selective inhibition of tumour neovascularisation have been explored. In this context, a class of proteins that requires special mention is the thrombospondin (TSP) family, which are secreted proteins widely distributed in the extracellular matrix of numerous tissues. TSP1 and TSP2 have anti-angiogenic properties

(Ref. 111). Transfection of glioblastoma, breast carcinoma and other tumour cells with a plasmid expressing TSP1 protein resulted in reduced tumour growth in animal models (Ref. 112). In all of these models, inhibition of tumour growth was generally accompanied by a decrease in tumour vascularisation, confirming the hypothesis that angiostatic factors could be used to inhibit tumour growth. Systemic release of high levels of TSP1 by overexpression also reduced tumour growth and metastasis. However, there are other animal models where TSP1 augmented tumour growth, thereby casting doubts on the antitumourigenic potential of TSP1 (Ref. 113). With over 20 anti-angiogenic drugs in Phase I, II and III clinical trials, the search for better substitutes is underway. These drugs have many advantages when compared with other treatment modalities because the drug targets (endothelial cells) are readily accessible from the blood circulation and are genetically stable cells, and hence are unlikely to develop resistance to cytostatic therapy.

### Conclusion

Strategies to elicit an immune response against tumours hold much promise. Indeed, more than five decades of work have provided leads in several directions that could finally be developed to achieve tumour regression. As discussed here, such strategies work only when the tumour itself is highly immunogenic or is made immunogenic by different procedures. The considerable amount of effort and experimentation invested particularly over the past decade in establishing an effective means to fight cancer has provided a better understanding of the immune system and the immune responses involved in tumour regression. Out of this, the host's own immune system has emerged as a powerful and promising tool to fight cancer. Various approaches to immune therapy have been implemented, including the development of vaccines and gene therapy, and the use of genetically engineered cells and other therapeutic modalities that involve heat shock proteins and cancer genomics (Ref. 114). Some of these have shown promising results during early trials, but a more elaborate analysis and the overall effect on the host have yet to be established. Nevertheless, a common theme that has arisen from all of these approaches is the finding that stimulation of the host's T- and B-cell activity can be used for the specific eliminatination of tumour cells.

**Antitumour immune responses**

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**Antitumour immune responses**

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**Antitumour immune responses**

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## Features associated with this article

### Figures
Figure 1. The cytotoxic T lymphocyte (CTL) response to tumour cells
Figure 2. The T helper (Th)-cell and phagocytic response to tumour cells
Figure 3. The natural killer (NK)-cell response to tumour cells

### Tables
Table 1. The major cytokines involved in immune responses
Table 2. The major interleukins involved in immune responses

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