Review

Cancer immunoediting and “spontaneous” tumor regression

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The combination of host protective and tumor-promoting actions of the immune system throughout tumor development is termed cancer immunoediting. This review briefly summarizes the currently vast evidence supporting the immune system’s role in not only protecting against developing cancer, but also sculpting tumor immunogenicity and immune escape. We also briefly summarize the history of immunotherapy and discuss the immunoediting process in the context of spontaneous tumor regression and whether this observation can be utilized in future treatment regimens.

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Contents

Introduction .......................................................... 1
The evidence for cancer immunoediting in humans ........................................... 2
Tumor-intrinsic factors that suppress the immune system .................................... 3
The role of the immune system in immunosurveillance ....................................... 5
Immunotherapy: is induction of “spontaneous” regression the answer? .............. 6
Conclusions .................................................................. 7
Acknowledgments .................................................................. 7
References ........................................................................... 7

Introduction

In 1957 Burnet and Thomas put forward the hypothesis that the immune system could recognize and destroy nascent transformed cells. Their theory was termed cancer immunosurveillance and it was thought that this process was an important host protection mechanism to inhibit carcinogenesis and maintain regular cellular homeostasis. It was also suggested that immunosurveillance primarily functions as part of a more general process of immunoediting [16–18].

Cancer immunoediting is the ability of the immune system to control and shape cancer, and is the result of three phases – elimination, equilibrium and escape – that function either independently or in sequence. Elimination is a four-stage process beginning with the anti-tumor immune response. Here, the cells of the innate immune system recognize the presence of a growing tumor as a consequence of local tissue damage caused by stromal remodeling, which induces inflammatory signals essential for the recruitment of cells belonging to the innate immune system to the tumor site. During this process, the infiltrating lymphocytes such as the natural killer cells and natural killer T cells are stimulated to produce IFN-gamma, which induces tumor cell death. Chemokines are also produced, which play an important role in promoting tumor cell death by preventing angiogenesis. In the third part of the elimination phase, natural killer cells and macrophages transactivate one another via the reciprocal production of IFN-gamma and IL-12. Tumor cell death occurs via apoptosis and the production of reactive oxygen and nitrogen intermediates. Finally, elimination sees tumor-specific CD8+ T cells home to the tumor site, and cytolytic T-lymphocytes destroying the antigen-bearing tumor cells that remain at the site [16–18].

Tumor cell variants that have survived the elimination phase enter the equilibrium phase, where lymphocytes and IFN-gamma exert a selection pressure on those tumor cells that are genetically unstable and mutating. Tumor cell variants that have acquired...
resistance to elimination then enter the escape phase. Here, tumor cells continue to grow and expand in an uncontrolled manner, a process that may eventually lead to malignancy.

It is an evolutionary necessity for mammals to suppress the emergence of dysregulated abnormally growing cells. Cancer cells – and the tumors they form – grow in a multi-step process that, during the final stages of disease, results in full escape from the actions of the host immune system. There have been a number of excellent reviews that have covered this topic in great detail [8,16–18,63]. The aim of this review is to place the observation of spontaneous tumor regression into the context of recently described concepts of tumor host immune system interactions, and explore potential mechanisms by which the immune system might be manipulated to induce tumor regression.

The evidence for cancer immunoediting in humans

The concept put forward by Burnet and Thomas half a century ago was largely neglected due to a lack of supporting experimental evidence. However, it has now emerged from numerous studies involving murine tumor models and human cancers that the immune system can not only protect the host against the development of primary tumors but, under certain conditions, also promote tumor growth leading to more aggressive disease.

It has long been accepted that patients with congenital or acquired immunodeficiencies have an increased incidence of viral-induced malignancies [5]. By contrast, such an association with non-viral cancers has been difficult to prove, which has hampered attempts to establish the idea of immunoediting as a significant factor in tumor progression. Nonetheless, the main lines of evidence to support the concept of immunoediting in humans include:

- patients receiving antigen-specific immunotherapies for progressive tumors demonstrate downregulation of tumor-associated antigen (TAA) expression [31,32];
- transplant recipients on immunosuppressive drug regimes have a higher incidence of non-viral cancers when compared with an immunocompetent control population [4,34];
- cancer patients may develop spontaneous immune responses to their tumors [18]; the presence of lymphocytes within the tumor is a good prognostic indicator of patient survival [21,53].

Individuals who have an otherwise normal immune system, but are immunosuppressed to prevent rejection of transplanted organs, have an increased probability of developing a variety of sporadic non-viral cancers [34], implying that immunosuppression allowed the transplant patient to either develop a tumor de novo or allowed the outgrowth of occult tumors that were being controlled by the donor’s unsuppressed immune system. A recent report described the occurrence of melanoma 1–2 years post-transplant in allograft recipients each of whom received kidneys from the same cadavric donor who had been treated for primary melanoma some 16 years previously and was considered tumor free at the time of organ donation [43]. This can be considered as evidence of the existence of an equilibrium phase of immunoediting and suggests a protective effect of the immune system against tumor development.

Evidence now exists that demonstrates the elimination phase of the immunoediting process before clinical symptoms are evident [73] and it is possible that a large number of potential tumors are eradicated in this way. If this eradication does not occur, there is thought to be a variable period of “latency” – the equilibrium phase – before the emergence of clinically detectable malignant disease. It has been estimated that for many solid tumors there may be up to a 20-year gap between the induction of mutation and the clinical detection of the tumor [41]. A dynamic interaction exists between the immune system and cancer cells in which new variants of tumor cells arise over time that have an increased resistance to immune attack and an enhanced potential for survival in the immunocompetent host (Fig. 1). However, much is still unknown about the mechanism of this phase of tumor–immune system interaction. A recent study reported that the type, density and location of immune cells within tumors could predict clinical outcome. It was claimed that the presence, location and density of T cells and their products within the tumor is a much better predictor of survival than the currently used tumor staging criteria based on size and spread of a tumor. However, it was the
presence of high CD3 cell populations in both central and peripheral tumor zones compared to low CD3 cell populations in both central and peripheral tumor zones that defined a population in which multivariate analyses of UICC-TNM stages were no longer significantly associated with overall survival [21]. Furthermore, two additional study groups yielded no extra information regarding pathologic stage with respect to survival. Clearly, this research needs to be repeated in larger groups of colorectal cancer patients and in those with other cancers. There have also been suggestions that melanoma T cells can hinder that particular cancer’s progression, whilst in breast cancer a small number of cases suggest that the opposite may be true.

There is also extensive experimental support for the elimination and escape processes based on murine studies [37]. Using a mouse model of primary chemical carcinogenesis, it was shown that immunodeficient mice develop more carcinogen-induced and spontaneous cancers than wild-type mice, and that tumor cells from immunodeficient mice are more immunogenic than those from immunocompetent mice. This study went on to further elucidate the process of equilibrium, revealing that tumor cells in equilibrium are highly immunogenic and those spontaneously exiting equilibrium became growing tumors with attenuated immunogenicity. Moreover, it was shown that elimination and equilibrium can be mechanistically distinguished because elimination requires the actions of both innate and adaptive immunity, whilst equilibrium is maintained solely by adaptive immunity. Similarly, equilibrium and escape can be differentiated as equilibrium represents a time of tumor cell persistence without expansion, and escape is characterized by progressive tumor growth [16–18,63].

Perhaps the strongest evidence for tumor immunoediting comes from studies of tumor-associated antigens (TAA) [39]. The ability to overcome intrinsic tolerance to strict “self” TAAAs was demonstrated using an immunotherapeutic strategy. Here, an alphavirus-based, virus-like replicon particle (VRP) was used to elicit immune responses to a non-mutated TAA rat neu in an aggressive rat mammary tumor model. Using this VRP-based strategy an effective anti-tumor immunity was generated in the setting of a pre-existing tumor and resulted in nearly half the rats being cured. Downregulation of rat neu expression was also observed in those tumors that showed initial responses followed by tumor escape with resumption of rapid tumor growth.

Despite the existence of tumor-specific immune cells, most tumors have devised strategies to avoid immune attack. Galectin-1 (Gal-1) is a negative regulator of T cell activation, and survival plays a pivotal role in promoting escape from T cell-dependent immunity, awarding immune privilege to tumor cells. Gal-1 expression is correlated with the aggressiveness of tumors, the acquisition of metastatic phenotypes, and has been identified as a major immunosuppressive factor secreted by human and murine melanoma cells. Blocking Gal-1 production by melanoma cells was shown to both restore the ability of the host immune system to initiate a tumor-specific primary Tn1 response and provide resistance to subsequent challenge with Gal-1-expressing tumor cells [58].

A number of studies point towards the existence of an equilibrium state and indicate that maintaining cancer in this state may represent a relevant goal of cancer immunotherapy in which enhanced adaptive tumor immunity could result in improved immune control. Some observations contradict the idea that the immune system spontaneously mounts lethal attacks against tumor cells. If a cycle of immune pressure followed by tumor escape operates during tumor development, then progressive tumor growth interspersed with periods of reduction would be expected, but solid tumors generally grow larger and larger. However, it is possible that selection is taking place at a cellular level during early tumor development. Selection of tumor variants would not necessarily result in gross or macroscopic changes on a microscopic or cellular level. Likewise, a lack of inflammation at the site of solid tumors may cast doubt on the idea of spontaneous immune attack and immune escape. Although immune cells can be observed in or around tumors, spontaneous local inflammation is often not seen clinically or histologically, which may represent these tumors to be at the escape stage. This raises the possibility that some of the tumor-promoting actions of inflammation may be a result of interference with the ability of the adaptive immune system to hold cancers in equilibrium [16–18,63].

A major goal of cancer immunotherapy is to generate a large number of tumor-specific T cells that can last in vivo for a long time and resist tolerization [57]. Existing methods for treatment focus on reshaping the normal T cell repertoire and fall into two categories – those relying on massive expansion of the few antigen-specific T cells and creating autologous T cells of a desired specificity with T cell receptor gene transfer. Immunotherapy may reduce tumor mass to a handful of cells; but, if the functional activity of the immune system has been altered slightly, tumor regrowth is likely. A combination of immunotherapy and chemotherapy may provide a more practical method of tumor management. Guidoboni et al. [29] reported that the improved survival of patients with MSI-H was associated with the higher frequency of activated tumor infiltrating lymphocytes in these cancers. Consequently, it has been suggested that such lymphocytes may represent a host immune response that contributes to improved survival and could be harnessed in the treatment of cancers. The hypothesis that colorectal cancers with MSI-H are more immunogenic than microsatellite stable cancers is very attractive, as study of these cancers may provide crucial insights into cancer immunology. The further elucidation of immune responses to CRC in vivo is crucial to the continuing development of immunotherapy.

Tumor-intrinsic factors that suppress the immune system

There are a multitude of tumor-intrinsic factors that suppress the immune system. The following section will concentrate on tumor MHC/peptide expression and its importance in malignancy. Tumor cells can lose HLA class I molecules through a variety of mechanisms, an event associated with invasive and metastatic lesions [51] (Fig. 2). Total loss of HLA class I expression is common in tumors such as melanoma, colorectal and prostate carcinoma [710]. Mechanisms that underlie total loss of HLA class I can include mutations in one copy of the β2-microglobulin gene in association with LOH involving the second allele – the loss of β2-microglobulin has been observed in patients experiencing objective partial responses after T cell-based immunotherapy. Defects in MHC genes and in the antigen processing and transport pathway can also cause total loss of HLA class I [19,56]. Downregulation of the proteasome multi-catalytic complex subunits LMP-2 and LMP-7 and of peptide transporters TAP-1 and TAP-2 have been reported in tumor histologies that include small cell lung carcinoma, non-small cell lung cancer, prostate adenocarcinoma and renal cell carcinoma [14,60]. HLA class I can often be upregulated by treatment with IFNγ in these situations.

Selective HLA haplotype loss can be due to LOH on chromosome 6, and there are several mechanisms involved in locus downregulation, more frequently with HLA-B than HLA-A antigens [9,44]. For example, in melanomas c-Myc oncogene overexpression correlates with selective HLA-B locus downregulation, and loss of transcription factor binding to locus-specific regulatory elements can induce HLA-B locus downregulation in colorectal carcinoma cells [27]. In melanoma, the gene products of the
HLA-C locus are often expressed poorly or not at all [2]. The defects underlying HLA class I allele-specific loss include mutations in the genes encoding HLA class I heavy chain.

There are a number of reasons why tumor cells that have lost HLA class I are not destroyed by NK cells, including: a loss of, or downregulation of, NKG2D ligand expression; a lack of stimulatory cytokines such as IL-2, IL-12, IL-15 or type I IFNs; or a lack of expression of costimulatory molecules such as CD80/B7-1, CD86/B7-2/B7.2, CD40 and CD70. The lack of expression of costimulatory molecules by tumor cells may lead to T cell deficiency and suboptimal activation of NK cells [63]. In an experimental setting, insertion of genes encoding CD80/B7-1, CD86/B7-2/B7.2, or both into tumors generally increases the immunogenicity of those tumors, but does not necessarily lead to regression [22].

Loss of HLA class I as a mechanism for immune escape fails to account for cells being susceptible to NK cell lysis. NK cells express activation receptors such as NKG2D, which bind to ligands such as MICA/B (MHC class I chain-related proteins A and B) expressed on stressed epithelial cells [70]. Activation of NK cells through this signaling pathway can overcome the inhibitory effect of HLA class I binding receptors. However, tumors may shed MICA/B from their surface, providing a systemic supply of soluble NKG2D ligand which can downregulate NKG2D expression on γδ and NK cells [52]. These cells are generally activated by stress ligands and are not affected by MHC-I inhibition.

Loss of surface antigen expression can also occur independently of the dysregulation of HLA class I expression. Tumor antigen-associated HLA expression will inevitably be heterogeneous within a tumor. As this expression is related to the efficiency of cytotoxic T-lymphocyte clearance, there will be a progressive selection for tumor cells with lower levels of tumor antigen-associated HLA. Decreased presentation of tumor-associated antigens (TAAs) is associated with disease progression, although the exact mechanism of their downregulation is not clear in most cases [7]. For example, decreased expression of melanoma–melanocyte differentiation antigens (MDAs) is associated with disease progression. Decreased antigen expression has also been found in residual tumors after peptide vaccination. In addition, the amount of tumor antigen expressed may also be important for recognition. The exact mechanisms that control the downregulation of tumor antigens are not known in most cases, but it is likely that epitope immunodominance plays a part. The theory of immunodominance, as it relates to tumor escape, predicts that one of the ways that antigen-loss variants within a tumor are shielded from immune pressure. In contrast, parental tumor cells that carry the immunodominant epitope serve as a red flag for immune attack, thereby diverting attention from the tumor variants. Once the parental cells are eliminated, a new hierarchy is established among the variant subpopulations, and formerly immunorecessive epitopes become dominant. A tumor variant that has lost the restricting HLA class I allele while retaining the immunodominant antigen could cross-present this antigen to CD8+ CTLs by DCs and maintain an immunodominant response to a “phantom” target at the expense of more appropriate and effective responses to other antigens.

T cells recognize tumors through T cell receptor (TCR) interactions with major histocompatibility complexes (MHC) that may be displaying tumor-associated peptides or lipids [62]. Interferon gamma (IFNγ) secreted at the tumor site by a variety of cells, such as cytotoxic T cells or NK cells, augments MHC expression on tumor cells, increasing immunogenicity.

The presence of immature DCs carrying tumor-associated antigens to the draining lymph nodes close to the tumor site is

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**Fig. 2.** Mechanisms of tumor escape: (a) tumor-specific antigen can mutate away from cytotoxic T cell recognition or MHC-1/tumor antigen complexes can be internalized, (b) immature dendritic cells induce naive CD4 cells to become regulatory T cells that inhibit effector T cell responses, (c) tumor cells release inhibitory cytokines such as TGFβ that prevent immune cell function, (d) pro-inflammatory mediators drive tumor cell proliferation and prevent apoptosis.
critical to the outcome of the tumor-specific adaptive immune response. Immature DCs lack high expression of costimulatory molecules such as B7.1, B7.2 and CD40. These surface molecules engage CD28 and CD40L on naïve T cells, providing signals that, in addition to those received through the TCR and certain cytokine receptors, leads to massive T cell clonal expansion and differentiation into potent tumor-specific effector T cells. Naïve T cells primed in the absence of sufficient co-stimulation can become anergic, or may even develop into CD4+ regulatory T cells (Tr1 cells). These Tr1 cells have the capacity to protect the tumor by inhibiting effector T cell responses through the action of inhibitory cytokines such as IL-10.

The role of the immune system in immunosurveillance

The initiation of anti-tumor immune responses occurs when the cells of the innate immune system are alerted to the presence of a growing tumor. Dendritic cells (DCs) act as sentinels for monitoring tissue damage, infection and possibly transformation [65]. DCs pick up antigen at these sites and become activated by “danger” signals, for example, microbial products and pro-inflammatory cytokines. These signals promote DC maturation, permitting them to efficiently activate naïve T cells in lymphoid tissue. Activated T-lymphocytes can develop as cytotoxic T cells and helper T cells, promoting cell-mediated and humoral immunity to the tumor, respectively. Activated DCs are also known to influence the function of other elements of both the innate and the adaptive immune system, for example, natural killer (NK), macrophages, γδ cells and NKT cells.

Effective anti-tumor immunity is also suppressed by the microenvironment of the solid tumor. The accumulation of CD4+CD25+Foxp3+ regulatory T cells (T-reg) in both the peripheral blood and the tumor may maintain the immunosuppressive environment, which may be exacerbated by the large amounts of TGF-β that some tumors can produce. Indeed, the administration of a CD25-targeted toxin to deplete nT-reg cells in human cancer patients has been shown to result in an increase in effector T cell function [13].

Within the epithelia there is a considerable portion of constitutively resident T cells, known as intraepithelial lymphocytes (IELs). These IELs display limited T cell receptor (TCR) diversity and may recognize autologous proteins expressed on epithelial cells after infection or malignant transformation [33]. Consistent with this, human colorectal carcinomas show upregulated expression of two major histocompatibility complex (MHC) class I related molecules, MICA and MICB, and are targets for cytolyis by intestinal TCR/γδ IELs expressing NKG2d, a receptor for MICA and MICB [28]. The capacity of either γδ or MICA to regulate malignancy in vivo was uncertain until it was shown that mice lacking γδ cells were highly susceptible to multiple regimens of cutaneous carcinogenesis. It was shown that after exposure to carcinogens the skin cells expressed Rae-1 and H60, MHC-related molecules that structurally resemble human MICA [25]. Furthermore, a recent paper demonstrated that γδ T cells may also suppress the activity of conventional effector T cells and DCs through unidentified soluble factors [55].

Unfortunately, chemokines alone seem to show limited anti-tumor efficacy, so there are now new approaches being developed that combine a chemoattractant with cytokines that are known for their stimulating properties on T cell, NK cells and DCs. This strategy first proved successful when the T- and NK-cell-activating cytokine, IL-2, was combined with the T- and NK-cell-attracting chemokine XCL1 in gene transfer studies [15]. The delivery of XCL1 alone by transfection fibroblast into A30 myelomas caused tumor infiltration by CD4+ lymphocytes, but failed to induce tumor regression. The introduction of IL-2 alone into the tumor site also showed little effect on tumor development. By contrast, co-injection of fibroblast that express XCL1 or IL-2 greatly increased T-lymphocyte infiltration into myeloma tumors and provided protection from tumor growth in a CD4+ and CD8+ T cell-dependent manner [15]. This work, and other recent findings, has provided experimental evidence that the introduction of chemokines into the tumor environment results in the recruitment of relevant leukocytes subsets in vivo and decreases tumorigenicity of malignant cells. In addition, the combination of chemokines with other immunostimulatory cytokines provides enhanced and long-term tumor immunity.

Although the development of immune-based therapies has focussed primarily on vaccines and cytokines, increased understanding of the function of costimulatory molecules has provided a new approach for immune therapy. Such molecules possess properties involved in both lymphocyte activation and immune-inhibitory function. The costimulatory molecule with the greatest translation into the clinic so far is CTL-associated antigen 4 (CTLA-4) [31,32]. There are two principle mechanisms by which engagement with CTLA-4 leads to T cell inhibition, the first involving competitive binding with CD28 for B7 on the APC, and the second being direct intracellular inhibitory signals mediated by the CTLA-4 cytoplasmic tail. To date, there have been numerous clinical trials using human monoclonal antibodies to test the blockade of CTLA-4 signaling on a variety of cancers. Most experience has been with the treatment of metastatic melanoma where significant anti-tumor activity and potential autoimmune-related toxicities were observed [32]. Clearly, further clinical investigation using this type of therapy is needed, and, as our understanding of costimulatory pathways improves, this approach may represent a viable alternative to other forms of therapy.

Tumors often possess regions of hypoxia due to rapid cell division and aberrant blood vessel formation, a situation that attracts immune cell infiltration, which in turn contributes to an inflammatory tumor microenvironment. This inflammation is characterized by the abundant presence of growth factors and chemokines, an environment that promotes tumor cell proliferation and survival. The chemokine network is now regarded as an essential part of an intricate system of immunosurveillance, affecting and regulating either directly or indirectly, the growth and metastasis of malignant cells. Progression in developing effective immunotherapeutics against tumors has been furthered by features of the chemokine/chemokine receptor network. Findings in experimental tumor models have shown that the introduction of a single chemokine is enough to induce tumor regression and immunity to subsequent tumor challenge [6,11,20,24,30,36,38,42,47,49,59,69]. For example, a mouse model of mammary adenocarcinoma expressing CCL16 tumor cells induced the recruitment of antigen presenting cells (APCs), T cells and granulocytes. This response resulted in the CD8+ T cell rejection of tumor cells and the development of anti-tumor immunity, thereby implicating CCL16 in the initiation of specific cytotoxic activity against tumor cells and tumor-specific lymphocyte activation [24].

Hypoxia also causes a temporary shutdown in tumor growth until neovascularization occurs. Macrophages accumulate at ischemic and hypoxic sites and respond rapidly by upregulating hypoxia-inducible factors (HIFs) 1 and 2, which are key regulators controlling the transcriptional response to low oxygen tension [61]. As a consequence, TAMs upregulate VEGF in hypoxic areas suggesting “co-operation” with tumor cells in the phenomenon of neovascularization. TAMs express matrix metalloproteinase 7 (MMP-7) which promote the invasion of tumor cells through the basement membrane and also stimulates endothelial cell migration during tumor angiogenesis [48]. Therefore, it appears that the concept of cross talk between the epithelium, stroma and
Immunotherapy: is induction of “spontaneous” regression the answer?

Advances in immunology over the past 50 years have improved our understanding of the interaction of the host with the developing tumor and have allowed the development of anti-tumor immunotherapies. The identification of TAAs has allowed the tailoring of therapies to specific targets on tumor cells. Progress in recombinant technology has allowed the development of cancer vaccines for active immunization and cellular therapy for adoptive-transfer treatments. Immunotherapy aims to restore the activity of the host immune system to combat cancer. Active immunotherapy aims to induce a long-lasting preventative or therapeutic immune response to a defined TAA. Passive immunotherapy provides a tumor antigen-specific immune response via the administration of high quantities of effector molecules, such as antibodies, or cytotoxic T cells. However, these regimes are short-lived and usually require multiple dosing.

There are over 150 clinical trials currently in progress using a variety of immune-based molecular treatments. Unfortunately, to date, there has been a disappointing lack of success in these trials. The identification of the first human melanoma TAA in the early 1990s stimulated attempts to boost the numbers of tumor-specific cytotoxic T cells in melanoma patients, but with limited success [68]. Immunotherapy studies in human cancer patients using single strategies have been unable to show appreciable clinical efficacy. Indeed, a recent promising attempt to combine the cancer treatments of vaccination, genetic engineering, cytokine therapy and adoptive cell transfer in order to treat end stage metastatic melanoma had a success rate of only 13% [46].

Recognition that some cancer patients who develop severe concurrent bacterial infections undergo concomitant remission of their malignant disease dates back hundreds, if not thousands of years. Cases of spontaneous tumor regression have been published over the last few centuries with each recording regression associated with varied pyogenic and non-pyogenic infections such as diphtheria, influenza, malaria, syphilis and erysipelas. In 1813, Vautier reported the regression of tumors in patients suffering from gas gangrene, which is commonly caused by Clostridial infection [45]. The first description of successful cancer immunotherapy came in 1890 by the New York surgeon William Coley. He inoculated the Gram-positive Streptococcus pyogenes in patients with inoperable tumors with limited success. Once he incorporated the Gram-negative Serratia marcescens to improve the toxicity, he successfully treated sarcoma patients by “vaccination” with this mixture composed of attenuated heat-killed S. pyogenes and S. marcescens, which became known as “Coley’s Toxins” [12]. Despite the adverse side effects of this treatment, 51.9% of patients with inoperable soft tissue sarcomas demonstrated complete tumor regression and more than 5-year survival, and 21.2% were rendered free of clinical evidence of the disease for at least 20 years post-treatment [64]. Taken at face value, this response rate has remained unsurpassed by subsequent immunotherapy trials, and was widely used in the treatment of sarcomas until 1963. It is now recognized that the anti-tumor effects of Coley’s toxins can be attributed to interleukin-12 (IL-12) [67]. IL-12 is thought to augment the function of pre-existing tumor-specific T cells to induce tumor rejection [40]. This is due to the fact that IL-12 receptors are preferentially expressed on activated T cells, which explains why IL-12 therapy is effective against established tumors.

This empirical observation of the regression of tumors in patients with concomitant bacterial infections as a result of immune stimulation has led to the most successful of all immunotherapies: the use via instillation of Bacille Calmette-Guérin (BCG) for the treatment of superficial bladder tumors [54]. BCG induces anti-tumor immunity via the production of cytokines such as IL-2, TNFα and interferon-γ. Unfortunately, this treatment is also characterized by significant toxicity and ineffectiveness in approximately 30–50% of cases [1].

The solid tumor microenvironment is the perfect breeding ground for anaerobic bacteria. Therefore, there has been a lot of interest in using anaerobic bacteria in attempting to induce the “spontaneous regression” that was originally observed by Vautier, Coley and others. The three main classes of bacteria that have subsequently been tested in the immunotherapy of solid tumors are: (1) lactic acid Gram-positive anaerobic bacteria, (2) intracellular Gram-negative facultative anaerobes and (3) the strictly anaerobic Gram-positive Clostridia. Bifidobacteria represents the...
first category, which were shown to be highly selectively and localized primarily within the tumor cells. However, no oncolytic effect was observed [35]. The main disadvantage of using bifidocacteria for the oncolytic treatment of cancer is that they are non-spore forming and thus difficult to store and handle. *Salmonella* represent the second category and are common causes of intestinal infections [23], and were also found, 50 years ago, to colonize human tumors [26]. The pathogenicity of salmonellae causing septic shock means that all strains used as tumor-targeting agents are attenuated. However, two clinical trials reported in 2002 and 2003 using attenuated salmonellae failed to demonstrate significant clinical efficacy, as a result of insufficient colonization of the tumor [50,66]. The final and most promising group is represented by *Clostridium*, which is one of the largest prokaryotic genus, composed of a variety of obligate anaerobic rod-shaped sporulating bacteria. Even though it represents pathogens producing potent toxins such as *Clostridium tetani*, *C. botulinum* and *C. difficile*, the majority of these species are non-pathogenic organisms occupying the ecosystem of the soil.

An important characteristic of *Clostridium* is its ability to sporulate, which contributes to the virulence and pathogenicity of this organism leading to outbreaks of food poisoning in the community and life threatening colitis in hospitals. However, this same capacity to form spores in combination with its strictly anaerobic nature serves as an ideal vector for the treatment of cancer, as these spores, administered intravenously, will preferentially germinate in the hypoxic and necrotic areas of solid tumors [3]. Clostridial spores show a distinct advantage over Bifidobacterium and Salmonella with regard to ease of production, convenient storage and better oncolytic effects [71,72]. A potential way to harness the phenomenon of “spontaneous” regression to control and potentially eradicate tumors may be to use a Coley’s toxin-based treatment in association with many of the techniques currently being used in immunotherapy trials (Fig. 3). The induction of an anti-tumor immune response by the injection of a Coley’s toxin-like substance or bacterial vector such as clostridial spores may be supplemented with the use of adoptive cell transfer of fresh immature DCs, naïve CTLs, or both. This may replenish the pool of tumor-antigen-specific effector cells that are capable of responding to the tumor with the added help from the “toxins”. Likewise, it may prove beneficial to remove tumor-specific regulatory T cells and γδ T cells, which contribute to the immunosuppressive tumor microenvironment [55].

**Conclusions**

The fact that both the innate and adaptive immune systems can recognize and respond to developing tumors is beyond doubt. However, the tumor microenvironment often suppresses these responses and inflammation may even promote tumor growth and metastasis. In this light, it may pay dividends to return to observations made many decades before modern medicine established the current treatment regimes for cancer. The human immune system is at its best when facing its age-old adversary, microbial infection. If the immune system can be tricked into treating an established tumor as a grumbling infection, the prospects for survival may greatly improve. Indeed, perhaps a Coley’s toxin-based strategy is not as old-fashioned and barbaric as it once may have appeared.

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**References**
