Autoimmunity associated with immunotherapy of cancer

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Short title: Autoimmunity from immunotherapy
Abstract

In this age of promise of new therapies for cancer, immunotherapy is emerging as an exciting treatment option for patients. Vaccines and cytokines are being tested extensively in clinical trials and strategies using monoclonal antibodies and cell transfer are mediating dramatic regression of tumors in patients with certain malignancies. However, although initially advocated as being more specific for cancer and having fewer side effects than conventional therapies, it is becoming increasingly clear that many immunotherapies can lead to immune reactions against normal tissues. Immunotoxicities resulting from treatment can range from relatively minor conditions, such as skin depigmentation, to severe toxicities against crucial organ systems, such as liver, bowel and lung. Treatment-related toxicity has correlated with better responses in some cases, and it is likely that serious adverse events from immune-mediated reactions will increase in frequency and severity as immunotherapeutic approaches become more effective. This review introduces immunotherapeutic approaches to cancer treatment, provides details of toxicities arising from therapy and discusses future potential ways to avoid or circumvent these side effects.
Introduction

Immunotherapy holds much promise for the treatment of cancer. The immune system is capable of dramatic and decisive responses against infectious disease, which is accomplished with exquisite specificity against antigen. Components of immunity are seen as potentially more specific weapons to direct against tumors than chemotherapy or radiation. With our expanding knowledge of tumor-associated antigens (TAA), there are many different approaches being developed to direct immunity against transformed cells.

Immunotherapies may involve the active generation of immunity to TAA, via vaccination with peptides or peptide-pulsed dendritic cells. In addition, administration of immune modulators such as cytokines can boost existing anti-tumor immunity and target immune effector cells to sites of tumor growth. Monoclonal antibodies harness both innate and adaptive immune mechanisms and direct them against tumor cells. Additionally, the effector functions of cytotoxic T lymphocytes (CTL) have proven them to be particularly useful in targeting TAA in adoptive immunotherapeutic protocols.

Some TAA are tumor specific, whose expression is entirely limited to tumors, examples of which include viral antigens expressed on cells in which viral oncogenes have contributed to cellular transformation. In these cases, immunotherapy can be employed with fine specificity and very little toxicity against normal tissues. However, most TAA are expressed by some cells of normal tissues and the potential exists for on-target toxicity against these tissues. These on-target toxicities can be assigned to two broad categories. Firstly, they can comprise “true” autoimmunity, involving a fundamental induction of endogenous immunity against self antigens, and we will refer to this type as “autoimmunity”. Secondly, they can be more “drug-like” in nature, where damage is mediated directly by the immunomodulatory agent, and these toxicities
will be referred to as “immune-mediated”. Toxicities have been described in a proportion of patients using a range of immunotherapeutic approaches. As immune-based cancer therapies become more potent, it is likely that autoimmune and immune-mediated toxicities will become more severe. Indeed, these toxicities can be associated with better anti-tumor responses resulting from immunotherapy.

In this review, we will introduce various immunotherapeutic approaches and provide details of toxicity to normal tissues resulting from these approaches, as well as describe potential ways to overcome these toxicities.

**Autoimmunity associated with adoptive immunotherapy**

Adoptive immunotherapy is a very promising approach to treating cancer that involves the isolation of leukocytes and their activation and expansion *in vitro* followed by infusion into patients. Advantages of this type of approach include the opportunity to manipulate and activate lymphocytes away from the *in vivo* immunosuppressive environment, and their expansion to vast numbers, thereby circumventing many regulatory checkpoints and delivering “instant” immunity. Adoptive immunotherapy has been demonstrated to induce regression of established tumors, often complete, in both mouse models of disease and in patients. However, apart from EBV-associated malignancies, this form of therapy targets antigens expressed on some normal tissues besides tumor cells, and immune-mediated toxicity has been observed in the treatment of both mice and humans (*Table 1, Figure 1*).
Tumor-infiltrating lymphocytes and melanoma

Tumor inhibition has been described using adoptive immunotherapy in various animal models over the past 50 years, but antigen specificity and its expression on normal tissue was largely undefined in earlier models. More recently, mouse tumor models with known tumor antigens also expressed on self tissues have become available and effective anti-tumor responses following adoptive immunotherapy have been observed. However, immune-mediated toxicity has been observed in these mouse models targeting normal tissues including skin, eye, colon and the B cell compartment as summarized in Table 1. In the following discussion, we will focus on these toxicities following adoptive immunotherapy in humans.

One of the most promising applications of adoptive immunotherapy in the clinic involves the use of tumor-infiltrating lymphocytes (TIL) to treat melanoma. Lymphocytes derived from tumors can be expanded to yield many billions of cells that are reactive with a range of melanoma antigens including gp100, MART-1 and tyrosinase.

Following demonstrations that prior lymphodepletion could lead to enhanced persistence and activity of transferred T cells, adoptive immunotherapy protocols were modified to include preconditioning regimens to deplete cells of the hematopoietic system. Depletion regimens ranged from non-myeloablative, using cyclophosphamide and fludarabine, to fully ablative regimens using chemotherapy and whole body irradiation, together with stem cell support. Objective response rates of adoptive immunotherapy reached 70% with these modifications, but immune-mediated toxicities were also observed, with reports of vitiligo and occasional reports of ocular toxicity, involving responses against melanin-containing cells important in retina function.
In cases where endogenous TIL are difficult to obtain, tumor-specific T cells can be generated from peripheral blood lymphocytes by genetic modification with genes encoding specific TCR α and β chains. In a 2009 trial, melanoma patients treated with gene-modified T cells reactive with melanoma/melanocyte antigens, experienced destruction of normal melanocytes in the skin (27 of 36 patients), as well as responses against normal cells of the eye and ear, which occurred in approximately 50% of 20 patients receiving T cells modified with a highly reactive TCR. These patients were administered steroids to inhibit T cell activity, which led to resolution of both uveitis and hearing loss.

Interestingly, in addition to the above on-target immune toxicities, the potential for immune-mediated off-target toxicity using adoptive transfer of TCR gene-modified T cells has been demonstrated in mice. In a study using mouse T cells genetically engineered to express TCR alpha and beta chains specific for ovalbumin, severe toxicity was observed including cachexia, anemia, pancreatitis and colitis. Reactivity against these normal tissues was thought to be due to mispairing of introduced and endogenous TCR chains leading to T cells with neo-specificities. These findings were also extended to include T cells modified to express other TCRs including those specific for gp100, SV40 large T antigen, TRP-2 and influenza virus nucleoprotein, although the incidence of lethal toxicity varied depending on the TCR. The propensity of TCR mispairing to induce immune-mediated toxicity in humans has yet to be determined fully but it has not been observed to date in clinical trial.

Genetically redirected T cells in adoptive immunotherapy

By far the greatest application of adoptive immunotherapy has been in the melanoma setting as described above. This is largely due to the availability of specific T cells, and
extension to other common cancers is restricted by a lack of availability of endogenous T cells of appropriate specificity. However, T cells reactive with a range of common cancers can be generated by genetic modification of peripheral blood lymphocytes with chimeric antigen receptors (CARs), whose specificity is derived from monoclonal antibodies specific for cell surface TAA. CAR-modified T cells have been used in clinical trials for a range of cancers including ovarian cancer, neuroblastoma, colon cancer and lymphoma. The use of CAR-modified T cells is in its infancy and only limited anti-tumor effects have been described to date. Nevertheless, in a phase I study for renal cell carcinoma (RCC) targeting the TAA carbonic anhydrase IX, adoptive transfer of gene-modified T cells led to grade 3-4 liver toxicity in three of seven patients treated. Toxicity was thought to be due to the T cells targeting the CAIX antigen also present on bile ducts. Toxicity was resolved in this study after cessation of adoptive T cell transfer or administration of steroids.

CAR-modified T cell activity against normal cells was also observed in a clinical study targeting CD19 for the treatment of follicular lymphoma. The CAR was composed of a single-chain anti-CD19 antibody linked to CD28 and the zeta chain of the CD3-TCR complex. In this study, dramatic regression of malignant cells was observed, but a prolonged depletion of normal B cells was also observed leading to greatly decreased levels of serum immunoglobulin. While low levels of serum antibody is concerning, administration of exogenous immunoglobulin can correct for this deficiency thereby providing protection against infection. In another study targeting CD19 with CAR-modified T cells, this time for chronic lymphocytic leukemia, treatment was well tolerated in 3 patients receiving T cell transfer in the absence of prior lymphodepletion, with only transient fevers experienced. However, a patient receiving T cells following lymphodepletion developed hypotension, dyspnea and renal failure and died four days
after treatment.\textsuperscript{16} Death in this case was not thought to be due to treatment and was attributed to sepsis due to infection.

More recently, adoptive transfer of CAR-modified T cells, specific for the TAA Her-2, led to death of a patient receiving treatment for colon cancer.\textsuperscript{17} Toxicity involved respiratory distress and may have been due to CAR-expressing T cell activity against normal Her-2-expressing cells of the lung. Increased levels of serum cytokines including IFN-\(\gamma\), GM-CSF, IL-6 and TNF-\(\alpha\) were observed, together with hypotension, brachycardia and gastrointestinal bleeding, which led to cardiac arrest. In another study targeting Her-2 no toxicity was observed,\textsuperscript{18} although comparisons between this study using autologous CTL clones and the CAR-expressing study are difficult due to significant differences in the studies, including the use of lymphodepleting conditioning and greater numbers of T cells in the CAR study. Thus, many different organ systems can become targets of immune-mediated damage following adoptive immunotherapy, a recent example of which involved severe, yet transient, colitis in 3 of 3 patients receiving T cells specific for the colon cancer-associated antigen, CEA.\textsuperscript{19}

In the above examples, the target tumor antigens were also expressed by a range of normal tissues. However, antigens with greater tumor specificity are emerging as more desirable targets. The cancer/testes antigen, NY-ESO-1, represents such a target that is expressed on a range of tumors, but whose normal tissue expression is limited to testes.\textsuperscript{20} In a phase I study, over 50\% of patients with either melanoma or synovial cell sarcoma experienced objective clinical responses in the absence of any immune-mediated toxicity.\textsuperscript{21} Similarly, in a phase I study targeting the carbohydrate antigen, GD2, with genetically redirected T cells, no T cell-related adverse events were observed, despite apparent anti-tumor activity demonstrated in 4 of 8
evaluable patients. GD2 is an attractive target since it is expressed on neuroblastoma and many melanomas but normal tissue expression is largely limited to brain and peripheral nerves.

**Autoimmunity associated with antibody therapy**

There is much promise and excitement in the use of monoclonal antibodies for immunotherapy, with 10 approved by the FDA for the treatment of cancer (reviewed by Weiner et al). These antibodies are specific for a variety of molecular targets expressed on a range of cancers including lymphomas, leukemias, breast cancer and colorectal cancer. A variety of effector mechanisms are employed by antibodies against tumor cells, which include antagonizing growth factors and their receptors, or inducing their degradation. Alternatively, antibodies may activate antibody-dependent cell-mediated cytotoxicity (ADCC) or the complement pathway. Finally, antibodies may also be used to antagonize receptors such as cytotoxic T lymphocyte antigen-4 (CTLA-4), which normally downregulate immune responses.

Clinical trials are currently under way to test the potential of antibodies to mediate tumor regression. However, just as spontaneously arising tumor-specific antibodies have been shown to induce autoimmune pathologies in paraneoplastic neurologic disorders, toxicities against normal tissues have also been observed in a proportion of patients receiving exogenous antibody (Table 2).

Toxicities arising from antibody administration can occur in various ways. Firstly, toxicity can follow the induction of potent endogenous autoimmunity against both tumor antigens and other self antigens, resulting in both on- and off-target toxicities. Secondly, toxicities can involve on-target depletion of normal cell subsets, compromising normal tissue
function. In this case, cell depletion and resulting toxicity is generally transient, persisting only during antibody administration after which normal cells are replaced from precursors. Nevertheless, even transient depletion can have severe consequences.

Some therapeutic antibodies mediate their anti-tumor activity by blocking growth factor receptors. Antibodies included in this category include bevacizumab, cetuximab and trastuzumab, which target vascular endothelial growth factor receptor (VEGF-R), epidermal growth factor receptor (EGF-R) and human epidermal growth factor receptor-2 (Her-2) respectively. Toxicities against a range of normal tissues including gastrointestinal tract, skin and lung have been observed using these antibodies for cancer therapy. However, since these antibodies mediate their effects largely by blocking the signaling ability of growth factor receptors, which is reminiscent of the action of drugs rather than immunity, we have not discussed these antibodies here, but cite references as information for the reader.25-27

Autoimmunity involving on- and off-target toxicities.

An immune response is a complex process that is subject to many controls and balances, including the involvement of inhibitory molecules on lymphocytes that downregulate responses (upon elimination of antigen) or prevent inappropriate activity against self antigens. CTLA-4 is one such regulatory molecule expressed by T cells that transmits an inhibitory signal to T cells upon binding to CD80 and CD86 on antigen presenting cells. The targeting of this inhibitory receptor in immunotherapy has been used to break immune tolerance of T cells to TAA, resulting in the expansion of T cells that elicit anti-tumor effects.28 However, in addition to tumor regression, anti-CTLA-4 antibodies, such as ipilimumab and tremelimumab, have been associated with autoimmunity affecting tissues including the thyroid, lung, joints, gastric mucosa
and liver (Table 2). In a clinical study using ipilimumab to treat hematopoietic malignancy after recurrence following allogeneic hematopoietic cell transplantation, complete remissions were observed in two Hodgkin’s disease patients (2 of 29) and a partial remission in a patient with mantle cell lymphoma. However, grade 2 to 4 autoimmune events thought to be treatment-related observed in 4 patients in this study, included arthritis, hyperthyroidism and recurring pneumonitis.29

Toxicities can be common and sometimes severe, as seen in a study using ipilimumab alone or together with vaccine for the treatment of melanoma or renal cell carcinoma (RCC). In this study, 21% of 198 patients developed grade 3/4 enterocolitis, with 4 patients suffering colonic perforation leading to 2 deaths and one colectomy.30 Enterocolitis resolved in most patients following administration of corticosteroids, providing further evidence of immune involvement. However, it was not clear if lymphocyte responses were directed against endogenous antigens from normal bowel or antigens derived from intestinal microflora. Gastrointestinal toxicity was also observed in another study, where 11 of 71 patients receiving ipilimumab developed grade 3-4 diarrhea, and gastrointestinal bleeding required colectomy in one patient.31

Of significant interest is that autoimmunity has been demonstrated to be associated with clinical response, suggesting that the greater the immune dysregulation mediated by anti-CTLA-4, the greater the anti-tumor effect. In some of these cases specific T cells have been identified that were associated with both anti-tumor activity and autoimmunity, where Melan-A specific cytotoxic T cells were observed infiltrating tumors and skin rashes together with a 30-fold increase in circulating Melan-A specific cytotoxic T cells following treatment with ipilimumab.32
Further randomized clinical studies continue in order to determine if the benefits of anti-CTLA-4 therapy justify the risk of autoimmune side effects. In a recent phase III study involving 676 melanoma patients, ipilimumab (with or without gp100 vaccine) was demonstrated to significantly improve survival of patients compared to patients receiving vaccine alone (10.1 months median survival compared with 6.4 months). Complete or partial tumor responses were observed in 9.4% of patients receiving treatment that included ipilimumab compared with 1.5% responses in patients not receiving the antibody. Immune-related adverse events occurred in 60% of ipilimumab treated patients, with approximately 15% of patients experiencing grade 3 or 4 immune-related adverse events. Toxicity was largely reversed with appropriate treatment, although 7 deaths occurred that were thought to be immune-related due to conditions including colitis, bowel perforation, liver failure and one patient with Guillain-Barré syndrome.

A range of measures can be taken to manage immune-mediated toxicities, including the use of corticosteroids, which can decrease the severity of toxicity. Interestingly, anti-tumor responses have been observed to be maintained in the presence of steroid treatment, suggesting that anti-tumor activity can be separated from auto-reactivity, at least in some circumstances.

Anti-CTLA-4 antibodies have induced anti-tumor responses most markedly against RCC and melanoma, which suggests that these two cancers are exceptionally immunogenic. Induction of autoimmunity by anti-CTLA-4 may occur through dysregulation of a pre-existing immune response to self antigens, which was held in check by CTLA-4, or through a de novo expansion of self-reactive T cells from naïve precursors in the absence of the CTLA-4 checkpoint.

Immune checkpoints are attractive targets for immunotherapies and much interest is being expressed in targeting immune inhibitory pathways other than CTLA-4. Programmed
death-1 (PD-1) is expressed by activated T cells and expression of its ligand PD-1-L is expressed by a range of cell types including antigen presenting cells and sometimes by tumor cells themselves. Interaction of PD-1 with its ligand inhibits T cell responses and downregulates immunity.

In a clinical study MDX-1106, a PD-1-blocking antibody, was used for the treatment of a range of malignancies including melanoma, renal cell carcinoma, non-small cell lung carcinoma and prostate cancer. Results from the first 39 patients indicate that 3 patients had objective tumor responses. Treatment was well tolerated with a single case of colitis the only grade 3/4 adverse event reported potentially related to antibody administration. A more detailed knowledge of immune checkpoints may lead to effective and safe immunotherapies.

**On-target toxicities from depletion of normal cell subsets**

In order to apply effector mechanisms of ADCC and complement against malignant cells, the identification of cell surface markers is necessary. However, most molecular targets of antibodies against hematologic cancers are lineage-specific rather than tumor-specific, and consequently immune-mediated toxicity is induced against normal blood cells, which can compromise immunity or hematopoiesis. Fortunately, target markers can sometimes be selected that are not expressed on stem cells or lineage precursors, therefore normal cells can often be reconstituted after cessation of therapy. Nevertheless, toxicities can occur due to lowered immunity following immune-mediated depletion of normal leukocytes, as seen when using antibodies for the treatment of some hematologic malignancies.

Gemtuzumab Ozogamicin (Mylotarg ®) is an antibody-calicheamicin conjugate that targets the CD33 surface antigen. Gemtuzumab treatment has been successful in the treatment of
CD33 positive AML, following internalization of the conjugate. However, the expression of CD33 on normal cells (myeloid progenitor cells and monocytes) has resulted in a large proportion of patients experiencing neutropenia (>90%) resulting in pneumonia and infections (~20%) that can be life threatening. Another serious effect of Gemtuzumab is the development of hepatotoxicity such as vascular obstructive disease (VOD), which damages the hepatic sinusoidal epithelium. This is thought to occur via receptor-mediated uptake of the antibody–calicheamicin complex through CD33 expression on liver cell populations such as Kupffer cells (up to 48% of patients in some studies). Although CD33 is still a target of interest for treating AML and some patients can benefit from treatment with gemtuzumab, Mylotarg was voluntarily withdrawn from the market in June 2010 because no significant benefit of the antibody conjugate in combination with chemotherapy was demonstrated above chemotherapy alone.

Alemtuzumab (MabCampath ®) provides another example where subsets of normal leukocytes are depleted following treatment of hematologic malignancy. Alemtuzumab is used in the treatment of chronic lymphocytic leukemia (CLL) and binds to CD52, which is present on both normal and malignant B and T lymphocytes, as well as monocytes, macrophages and eosinophils. The use of alemtuzumab has yielded promising results with objective response rates of up to 90% in CLL and encouraging results against other malignancies such as T cell prolymphocytic leukemia and peripheral T cell lymphoma. Due to expression of CD52 on normal leukocytes, alemtuzumab also depletes normal immune cell populations, which can result in severe infections that can be fatal.

On-target depletion of normal leukocytes has also been observed using Rituximab, which is specific for CD20 expressed on a variety of non-Hodgkin’s lymphomas and normal mature B
Rituximab has been used in the treatment of malignancies including follicular and diffuse large B cell lymphoma.\textsuperscript{46,47} It has been shown to mediate lysis of lymphoma cells through a complement-dependent mechanism, and it has substantially increased the survival rates of patients with B cell lymphomas since its approval by the FDA in 1997. However, the use of this antibody has been associated with toxicities including tumor lysis syndrome, infections and reactivation of viruses such as Hepatitis B and Herpes Simplex virus.\textsuperscript{48,49} Although antibody-producing plasma cells are not depleted with Rituximab, several other cell subsets including mature B cells are depleted, which is thought to be responsible for increased rates of infection. Interestingly, depletion of neutrophils has also been observed following the use of rituximab.\textsuperscript{50} The extent of neutropenia did not generally have clinical significance in these cases, and although the mechanism of neutrophil depletion were not clear, the production of anti-neutrophil antibodies and disruption of neutrophil homeostasis resulting from B cell depletion have been proposed as potential contributing causes.

**Autoimmunity from cytokine administration**

A range of cytokines are being tested in the clinic for their ability to recruit immunity against tumor cells by inducing the activation, proliferation and survival of tumor-specific lymphocyte populations.\textsuperscript{2} The complexity of cytokine interactions with the immune system means that often the outcomes of their use cannot be fully predicted. It is therefore not entirely unexpected that autoimmune pathologies have been encountered in a number of cytokine trials to date (Table 3).
Interferon-α

IFN-α is a prominent and sometimes first-line therapy against many cancers, including melanoma, RCC, cutaneous T cell lymphoma, chronic myeloid leukemia, bladder and ovarian cancer. The receptor for IFN-α is almost ubiquitously expressed, and can signal to leukocytes and tumors in a variety of ways. It can have pro- or anti-proliferative effects, pro- or anti-apoptotic effects, modulate cell immunogenicity, promote immune responses and inhibit angiogenesis.

An extensive range of autoimmune complications have been reported following IFN-α therapy for cancer (Table 3). Adverse events include diabetes and vitiligo, as well as the aggravation of pre-existing autoimmune diseases. Prospective studies have indicated that autoimmunity in patients correlates with prolonged relapse-free and overall survival. This provides evidence that in humans IFN-α is working against cancer at least in part by boosting the immune response. The mechanisms by which IFN-α induces cancer regression remain the subject of ongoing investigation. It has been shown to act both on host leukocytes and directly on tumor cells.

Both B and T cell-mediated autoimmunities have been reported in response to IFN-α, which is consistent with known activities of type-I interferons. Type-I interferons can promote skewing of T cells to a cytotoxic phenotype by stimulating their expression of the IL-12R. They complement this by also inducing genes coding for MHC-I and TAAs on tumor and non-tumor populations. IFN-α may indirectly promote the proliferation of memory T cell populations and also promotes B-cell switching to the IgG2a isotype. IgG2a is capable of targeting cells for phagocytosis and triggering complement-mediated cytotoxicity.
In summary, type-I IFNs are powerful modulators of anti-tumor immunity. The information available regarding their effects on leukocytes, host stroma and tumor cells serves as a further example of the pleiotropic effects that cytokines can mediate.

**Interleukin-2**

IL-2 has been widely applied in the clinic in the treatment of melanoma, RCC and AML and is one of only a few cytokines that has progressed to FDA approval in the treatment of cancer. Used alone in the treatment of metastatic melanoma it has produced objective response rates of up to 16%. The anti-tumor effect of IL-2 stems at least in part from its ability to act as a growth factor for T cells and to enhance the cytolytic activity of NK cells.

Non-immune systemic toxicities associated with high-dose IL-2 therapy are well appreciated. IL-2 therapy induces systemic inflammation, the exact mechanism of which is poorly understood, and the main symptomatic outcomes are hypotension and capillary leak syndrome, accompanied by flu-like symptoms, vomiting and diarrhea. Despite its toxicity, advances in managing symptoms, and the resolution of toxicity following cessation of administration, have led to its continued use in the cancer setting.

In addition to these systemic toxicities, IL-2 therapy is known to both exacerbate autoimmunity and trigger it *de novo* ([Table 3](#)). In a notable case study, both of these outcomes were detected within the same patient following high-dose IL-2 therapy for RCC, with worsening of diabetes and induction of myasthenia gravis and polymyositis. There is evidence that IL-2 can augment humoral responses specific for autoantigens and drive expansion of NK and T cells. Thus, both humoral and cell-mediated immune mechanisms may contribute to autoimmunity resulting from cytokine therapy. Interestingly, similar to observations using
antibodies or adoptive immunotherapy for cancer treatment as discussed above, anti-tumor responses in patients receiving cytokines can correlate with autoimmunity.\textsuperscript{63,64}

The best evidence that high-dose IL-2 can induce on-target (tumor antigen) autoimmunity stems from a prospective study of patients treated for metastatic melanoma and RCC \textsuperscript{67}. Here, vitiligo (autoimmune destruction of melanocytes) was observed in 11/74 of patients treated for melanoma, but 0/104 treated for RCC. Development of vitiligo in melanoma patients correlated with objective responses to therapy, indicating that immune targeting of melanoma differentiation antigens was underpinning both outcomes. Other autoimmune toxicities reported in conjunction with IL-2 therapy include diabetes mellitus and hypothyroidism (Table 3). These are presumed to represent examples of off-target autoimmunity in the absence of reported antigen sharing between tumors and the affected tissues.

**Autoimmunity associated with cancer vaccines**

Vaccines are a major focus of cancer immunotherapy, and lessons learned from successful vaccines against infectious disease are being applied to the treatment of malignant disease. Antigen formulations used in cancer vaccines include peptides and whole proteins in combination with adjuvants to improve immunogenicity or pulsed directly onto dendritic cells to facilitate antigen presentation. In addition, antigen has been encoded in recombinant viruses in attempts to generate robust immune responses to TAA in parallel to viral antigens.

The vaccination approach has shown considerable promise in preclinical murine models, although breaking of tolerance towards TAA expressed by both the tumor and healthy peripheral tissues of mice has been observed to result in autoimmune disease in some cases (summarized in Table 4). In humans, cancer vaccines have been studied intensively, particularly in the
melanoma setting. Increased frequencies of antigen-specific circulating T cells have often been observed following vaccine administration, although overall clinical responses rates of only 2.6% have been achieved.\(^6^8\) Perhaps in keeping with the low response rate, there have been few reports of autoimmunity arising in patients following vaccination with TAA. An evaluation of autoimmunity in melanoma patients treated with IL-2 and vaccines reported the occurrence of autoimmune thyroiditis and autoimmune insulitis, but these events were infrequent.\(^6^9\) Perhaps as cancer vaccines become more potent as they can be in animal models, we might see a concomitant increase in autoimmune sequelae.

**Concluding remarks and future perspective**

In summary, while immunotherapy holds great promise for the treatment of a range of malignancies, many of these therapies can be associated with autoimmunity against normal self tissues (Figure 1). Mechanisms involved in immune-mediated damage of normal tissues are varied, and a greater understanding of these mechanisms will enable enhanced and more specific forms of immunotherapy for cancer.

The most obvious means of toxicity results from expression of TAA on normal tissue (Figure 2). Thus, antibodies raised against these antigens can react against normal cells either by inducing complement-mediated lysis or facilitating ADCC by innate leukocytes. Adoptively transferred T cells specific for TAA can likewise react directly against normal tissues expressing those antigens as part of their normal proteome. However, toxicity can also result from de novo induction of immune responses against other antigens on normal tissues. For example, inhibition of CTLA-4 can dysregulate normal lymphocyte homeostasis, leading to expansion of self-reactive T cells able to respond against normal tissues that do not express TAA.\(^7^0\) Similarly,
immunotherapy can lead to epitope spreading in which immune responses can be raised against additional molecular targets, including those expressed on normal tissues.\textsuperscript{71}

As our knowledge of the human proteome and the cancer genome increases, opportunities are arising to refine the choice of antigens for immunotherapy. Next-generation genome sequencing allows cataloging of all the mutations within any given tumor cell.\textsuperscript{72} This could allow the rapid identification of truly tumor-unique antigens and greater personalization of vaccine and adoptive immunotherapies in the near-future. Already anti-idiotype vaccines are showing promise for the specific targeting of malignant B cell lymphomas.\textsuperscript{73,74}

Methods to reduce immune-mediated toxicity in adoptive immunotherapy may involve identifying multiple antigens that together constitute a tumor signature or “barcode”.\textsuperscript{75} Gene constructs encoding several antigen receptors can be developed where the threshold for cytotoxic activity is only reached if the complete “barcode” is recognized. Alternatively, constructs can be designed which shut down T cell activity if a barcode indicative of normal healthy tissue is encountered.

In the case of cytokine therapy, systemic toxicities and the deregulation of immune responses may also be reduced through more targeted delivery of cytokines to the tumor site, rather than systemically.\textsuperscript{76}

In antibody therapy, it is useful to consider that not all antibodies for a specific target molecule are equal in their immune side effects, as seen using TGN1412, a monoclonal antibody specific for the T cell costimulatory molecule CD28. TGN1412 was thought to hold promise as a treatment for cancer and rheumatoid arthritis, but a safety trial performed in 6 healthy volunteers demonstrated severe toxicity including hypotension and respiratory distress.\textsuperscript{77} Toxicity was thought to be due to high levels of serum cytokines in response to CD28 ligation occurring
within hours of antibody administration. TGN1412 differed from other anti-CD28 antibodies in that it had superagonist qualities, not possessed by all anti-CD28 antibodies. Similarly, individual anti-CTLA-4 antibodies can differ in their capacity to induce autoimmunity, as demonstrated in mice engineered to express human CTLA-4. In this mouse model, several antibodies varied in their relative abilities to induce autoimmunity and protection against tumor. An antibody was identified that produced the strongest anti-tumor activity and the least autoimmunity. Thus, it may be possible to separate anti-tumor activity from autoimmunity by antibody selection.

Selective downregulation of autoimmunity may also be possible using immunosuppressants. The use of corticosteroids to counteract severe immune-mediated toxicity has surprisingly indicated that steroid treatment may not always signal the end of an immunotherapy’s beneficial impact, with tumor responses being maintained after resolution of autoimmunity using steroids. A further consideration is whether it is possible to kinetically separate anti-tumor immunity from autoimmunity. Regulatory mechanisms on healthy tissues may allow them to withstand immune toxicity for a period of time.

Immunotherapy is an exciting and increasingly effective treatment option for cancer. However, it is becoming increasingly clear that cancer immunotherapy is a balancing act between anti-tumor immunity and immune toxicity. The association between immune toxicity and increased anti-tumor effects following immunotherapy highlights the need for strategies that can mitigate the risk of these toxicities during immunotherapy, while preserving activity against malignancy.
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References


Table 1: Immune-mediated toxicities associated with adoptive immunotherapy of cancer

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<td>CAR-modified T cells</td>
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<td>CD19</td>
<td>Lymphoma</td>
<td>Depletion of normal B cells</td>
<td>87, 88</td>
</tr>
<tr>
<td>CAR-modified T cells</td>
<td></td>
<td>VEGF-R2</td>
<td>Various</td>
<td>Various organs, likely due to cytokine-induced hypotension</td>
<td>89</td>
</tr>
<tr>
<td>Treatment</td>
<td>Tumor Type</td>
<td>Effect</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------------</td>
<td>---------------------------------------------</td>
<td>-----------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIL &amp; IL-2</td>
<td>various Melanoma</td>
<td>Autoimmune thyroiditis, systemic and ocular autoimmunity</td>
<td>90-92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIL with lymphodepletion and IL-2</td>
<td>various Melanoma</td>
<td>Vitiligo, uveitis</td>
<td>9,93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>MART-1 Melanoma</td>
<td>Melanocyte destruction</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCR gene modified T cells</td>
<td>MART-1, gp100 Melanoma</td>
<td>Melanocyte destruction</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR modified T cells</td>
<td>CAIX Renal cell carcinoma</td>
<td>Liver toxicity (grade 2-4) 3/7 patients; lower grade in 4/7 with reduced dosing</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR-modified T cells</td>
<td>CD19 Lymphoma</td>
<td>B cell depletion</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR-modified T cells</td>
<td>Her-2 Colorectal cancer</td>
<td>Lung toxicity</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR-modified T cells</td>
<td>CEA Colorectal cancer</td>
<td>Colitis</td>
<td>19</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. Immune-mediated toxicities associated with antibody therapy of cancer

<table>
<thead>
<tr>
<th>Antibody regimen</th>
<th>Target antigen</th>
<th>Cancer type</th>
<th>Toxicities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipilimumab</td>
<td>CTLA-4</td>
<td>Hodgkin’s disease, myeloma, AML, CML, CLL, NHL</td>
<td>Arthritis, hyperthyroidism, pneumonitis</td>
<td>29</td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>CTLA-4</td>
<td>Melanoma</td>
<td>Colitis, bowel perforation, vitiligo, hypophysitis</td>
<td>33</td>
</tr>
<tr>
<td>Tremelimimub or ipilimumab</td>
<td>CTLA-4</td>
<td>Melanoma</td>
<td>Colitis, dermatitis</td>
<td>30,32,95</td>
</tr>
<tr>
<td>Anti-CTLA-4 + TAA peptides</td>
<td>CTLA-4</td>
<td>Melanoma</td>
<td>Colitis, dermatitis, uveitis, enterocolitis, hepatitis, hypophysitis, vitiligo, pulmonary leukocyte infiltration</td>
<td>28,96-99</td>
</tr>
<tr>
<td>Anti-CTLA-4 (with vaccines and cytokines)</td>
<td>CTLA-4</td>
<td>Melanoma (mouse and human)</td>
<td>Melanocyte destruction, enteritis</td>
<td>31,100-102</td>
</tr>
<tr>
<td>Anti-CTLA-4, irradiated tumor cells, GM-CSF</td>
<td>CTLA-4</td>
<td>C2 prostate cancer (mouse)</td>
<td>Prostatitis, destruction of prostate epithelium</td>
<td>103</td>
</tr>
<tr>
<td>MDX-1106</td>
<td>PD-1</td>
<td>Melanoma, RCC, NSCLC, prostate</td>
<td>Colitis</td>
<td>34</td>
</tr>
<tr>
<td>Alemtuzumab</td>
<td>CD52</td>
<td>Chronic lymphocytic leukemia</td>
<td>Destruction of normal leukocytes, leading to susceptibility to infections</td>
<td>42,44,104</td>
</tr>
<tr>
<td>Gemtuzumab</td>
<td>CD33</td>
<td>Acute myeloid leukemia</td>
<td>Infection, sepsis, pneumonia</td>
<td>36-38</td>
</tr>
<tr>
<td>Rituxumab</td>
<td>CD20</td>
<td>Lymphoma</td>
<td>Suppression of B cells leading to deficiency in immunoglobulin and infections</td>
<td>49,105-107</td>
</tr>
<tr>
<td>Cytokine administered</td>
<td>Cancer Targeted</td>
<td>Autoimmune events</td>
<td>References</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>IFN-α</td>
<td>Chronic myeloid leukemia</td>
<td>Thyroditis, sarcoidosis, systemic lupus erythmatosis associated with auto-antibodies, autoimmune thrombocytopenic purpura, acute pancreatitis, hyperthyroidism, aggravation of psoriasis</td>
<td>51,108-111</td>
<td></td>
</tr>
<tr>
<td>IFN-α</td>
<td>Cutaneous T cell lymphoma</td>
<td>Psoriasis</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>IFN-α</td>
<td>Mid-gut carcinoid tumors</td>
<td>Systemic lupus erythmatosis, pernicious anaemia, thyroid disease</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>IFN-α2b</td>
<td>Melanoma</td>
<td>Hypothyroidism</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>IFN-α2b and piroxicam</td>
<td>Melanoma</td>
<td>Vitiligo, pulmonary interstitial fibrosis</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>GM-CSF with gp100 and tyrosinase peptides</td>
<td>Melanoma</td>
<td>Vitiligo</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>Renal cell carcinoma</td>
<td>Hypothyroidism, myasthenia gravis, diabetes mellitus (insulin dependent) and myositis</td>
<td>62-64,117</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>Melanoma</td>
<td>Vitiligo</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>Colorectal cancer</td>
<td>Diabetes mellitus</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>IL-2 and IFN-γ</td>
<td>Renal cell carcinoma</td>
<td>Auto-antibodies against erythrocytes</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>IL-2 and IFN-α2b</td>
<td>Renal cell carcinoma, melanoma</td>
<td>Anti-thyroid antibodies, hypothyroidism, autoimmune thyroiditis</td>
<td>63,120,121</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Autoimmunity associated with cancer vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Malignancy (species)</th>
<th>Autoimmune events</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRP-1 encoded by virus</td>
<td>Melanoma (mouse)</td>
<td>Vitiligo</td>
<td>122,123</td>
</tr>
<tr>
<td>CD20 + adjuvant</td>
<td>Lymphoma (mouse)</td>
<td>Depletion of normal B cells</td>
<td>124</td>
</tr>
<tr>
<td>Her-2 encoded by plasmids</td>
<td>Breast cancer expressing Her-2 (mouse)</td>
<td>Impaired lactation, accelerated involution of mammary gland</td>
<td>125,126</td>
</tr>
<tr>
<td>DC (peptide pulsed) CD40 &amp; IL-2 (fibroblasts)</td>
<td>Lymphoblastic leukaemia (mouse)</td>
<td>Systemic autoimmunity, immunity against fibroblasts, hepatomegaly, splenomegaly fur loss, cachexia,</td>
<td>127</td>
</tr>
<tr>
<td>DC (peptide-pulsed) β-gal and LCMV model antigens</td>
<td>EL-4 thymoma (mouse)</td>
<td>Diabetes, myocarditis</td>
<td>128</td>
</tr>
<tr>
<td>Pulsed DCs or tumor cells and either GM-CSF and adjuvant or GM-CSF, adjuvant and peptides</td>
<td>Melanoma (human)</td>
<td>Vitiligo, diabetes, thyroiditis, ocular toxicity</td>
<td>69</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Immune toxicity associated with immunotherapy of cancer. Therapeutic strategies including vaccines, adoptive immunotherapy, cytokines and antibodies can induce immunity against tumor antigens. However, these immune responses can also cause damage to a variety of organ systems as shown.

Figure 2. Mechanisms of immune toxicity associated with immunotherapy. (1) The delivery of exogenous antibody specific for TAA expressed on both tumor and normal tissue can result in damage to normal tissue mediated by complement or ADCC mediated by innate immune cells such as macrophages. (2) Similarly, transfer of TAA-specific T cells can lead to destruction of normal tissue if the TAA is also expressed on cells of normal tissue. (3) Alternate ways of inducing immune reactivity towards normal tissue include dysregulation of normal immune homeostasis using anti-CTLA-4, resulting in expansion of self-reactive T cells. (4) It is also possible that induction of immunity to tumor cells could lead to epitope spreading whereby T cells reactive with self antigens are generated.
Figure 1
Figure 2

1. Antibodies
2. T cells
3. Dysregulated immune homeostasis
4. Epitope spreading

Normal tissue
Macrophage
Adoptive immunotherapy
Vaccines/adjuvants

Tumor
Autoimmunity associated with immunotherapy of cancer

Sally M. Amos, Connie P.M. Duong, Jennifer A. Westwood, David S. Ritchie, Richard P. Junghans, Phillip K. Darcy and Michael H. Kershaw