1.1 INTRODUCTION

The increasingly sophisticated understanding that we have of how tumors and the immune system coevolve in the host is leading to a steady stream of new ways to apply this knowledge through the design of cancer vaccines. The rapidity with which these vaccine approaches can in some cases be translated into a clinical trial is a testimony to the years of fundamental medical research that has given rise to the current fields of bone marrow transplantation, adoptive immunotherapy, antibody therapy, and experimental vaccine development. As the science of cancer vaccines has advanced, so has a panoply of regulatory and institutional hurdles that seems to threaten the use of these therapeutic advances, especially in the traditional setting of an academic medical center. In this introductory chapter we will outline the field of cancer vaccines as a whole by highlighting the contributions that follow in subsequent chapters. Our hope is that this entire volume will serve as a reminder as to how far the science of cancer vaccines has come, and also as an inspiration to keep pushing forward with experimental vaccine trials because of the pressing
Background

From the dawn of modern oncology, two fundamental observations were made about interactions between the immune system and cancer: (1) in some cases, if the tumor mass or the draining lymph nodes were heavily infiltrated with lymphocytes, the patient did better [1,2], and (2) it was recognized that certain immunodeficiencies were associated with the development of cancer [3]. This lead to two related issues: how tumors arise in the face of an otherwise healthy immune system, and whether tumor formation is a relatively rare or common occurrence. In light of these issues a paradigm arose in the midtwentieth century that held sway for considerable time, namely, that of tumor immunosurveillance. Tumor immunosurveillance theories proposed that tumor formation, or at least the cellular mutations that lead to cancer, occur with a regular frequency and that the immune system is able to recognize and for the most part eliminate aberrant cells [4].

Current Perspectives

Today the immune system and cancer are not thought to interact in this way, and the shaping of both the tumor phenotype and the immune response to it is recognized as a mutual evolutionary process [5]. Also, our ideas about tumor formation have changed. Rather than viewing cancer as the expression of a single mutational event or oncogene, tumor formation is now clearly recognized as a stepwise process that may take considerable time to develop into clinical disease [6]. Nevertheless, studies seeking to demonstrate immune surveillance gave us the tools to study how the immune system recognizes and eliminates cellular substrates (i.e., tumors). The term cancer vaccine is used because we are emphasizing the goal of immunotherapy, namely, to recognize and eliminate malignant cells. For effective vaccination, appropriate protein/peptide targets must be identified and immune regulatory T cell responses that have evolved with the tumor must be countered. How we study and implement these two components is a central theme of this book.

1.2 ADJUVANTS: ENHANCING INTERACTIONS BETWEEN INNATE AND ADAPTIVE IMMUNITY

Adjuvants are compounds known to stimulate the immune system through deposition effects and the inclusion of compounds now known to stimulate the innate immune system [7]. While heat-killed mycobacterium suspended in mineral oil has been the standard adjuvant in animal studies, the severity of delayed-type hypersensitivity (DTH) reactions to this formulation has prohibited its use in the clinic [8]. The administration of another well-characterized bacterial strain is
perhaps the best-known bacterium-based adjuvant system in humans. The attenuated mycobacterium known as bacillus Calmette–Guérin (BCG) was originally developed as a tuberculosis vaccine. In some instances, where activation of the innate immune system in situ is sufficient, adjuvant administration alone may be therapeutic. The anticancer effects of BCG instillation, specifically in bladder carcinoma, are clearly documented in Chapter 3. Although BCG has clear and powerful effects, it must be given in repeated or maintenance doses demonstrating that while adjuvant-like effects alone may be able to control cancer in situ, expansion of more specific cancer stem cell targeting or a longlived immune memory response has not occurred. There is still more we can learn from BCG therapy as part of its effect may by attributable to the uptake of BCG by specific integrins on the tumor cell surface, leading to antigen-specific immune responses as well. Thus, what was once considered primarily an adjuvant-like effect may convert tumor cells that preferentially bind and internalize BCG into targets of the adaptive immune response.

In the study of adjuvants, many have used lipopolysaccharide-like structures of the bacterial cell wall to stimulate immunity. However, the immunostimulatory effects of complex saccharides may be far broader than simple adjuvant effects. In Chapter 2 Wilson and Danishefsky present a compelling new argument that appropriately constructed glycoconjugates have the ability to evoke strong antitumor antibody responses on their own. And as with other vaccine responses, a strong antitumor glycoprotein IgG response correlates with a strong antitumor T cell response. Unlike oligopeptide vaccines, glycoconjugates present a number of challenges in their synthesis. Nevertheless, because of their heterogeneity and structural complexity, we are only beginning to scratch the surface in terms of discovering appropriate immune targets. Immune responses to glycoconjugate vaccines such as Globo-H-KLH in adjuvant (a hexasaccharide found expressed on breast cancer, conjugated to keyhole limpet hemocyanin (KLH) and administered in adjuvant QS21) are harbingers of renewed interest in targeting tumor-associated oligosaccharide antigens. Currently, both multimolecular and multivalent antigen strategies are being developed and evaluated in clinical trials.

The most direct means to initiate an adjuvant effect is to stimulate the innate immune system with known toll-like receptor ligands. The family of toll-like receptors (TLRs) appears to play a pivotal role in the generation of different classes of innate immune responses by differential detection of conserved pathogen-expressed molecules. Bacterial CpG DNA and synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG are potent inducers of the innate immune system, including dendritic cells (DCs), macrophages, and natural killer (NK) and NK T cells. In Chapter 4 Speiser and Krieg examine methods of enhancing vaccine-induced immune responses by activation of selected TLR. In particular, the targeting of TLR9 has emerged as a powerful tool in the generation of Th1 adaptive immunity, and has shown promise for enhancing the efficacy of cancer vaccination. The studies performed to date show great promise for the clinical application of TLR9 activation with CpG ODN for enhancing the clinical outcomes from cancer vaccination. The safety of TLR9 activation with CpG ODN appears good, and its
selective and strong biological effects hold promise for further development and application in larger numbers of patients.

1.3 ANTIGEN-SPECIFIC THERAPY: NOVEL PRESENTATION OF PEPTIDE AND PROTEIN ANTIGENS

Many of the advances made in cancer vaccine therapy have featured novel means to present cancer-associated peptide or protein antigens to the immune system with a goal to both break immune tolerance and expand antigen-specific effector cells. The most direct means is to directly use a recombinant peptide, protein, or DNA plasmid vector as a vaccine. The use of polyepitope vaccines, synthesized and injected directly as peptides, or as expressed by DNA plasmid or adenoviral vectors, is highlighted in Chapter 5. Rather than focusing on a single cancer-associated epitope, polyepitope vaccines allow for either multiple-peptide antigen targets, or multiple epitopes from the same antigen that are presented by different MHC alleles, to be expressed in a single engineered peptide product. Polyepitope vaccines for melanoma and Her2/neu+ breast cancer are currently in phase I trials. In these trials, plasmid-based DNA vectors are used to express the polyepitope. Intriguing studies are also under way with plasmid DNA vectors in subjects who are papilloma virus–seropositive, with the intent to induce protective immunity. The use of DNA vaccines is further explored in the context of cervical cancer in Chapter 6. Transfection of antigen-presenting cells with a DNA vaccine can be used to engineer the intracellular routing of the protein antigen of interest and thus enhance the vaccine effect. For example, inclusion of a lysosomal targeting motif can preferentially target the class II MHC pathway and lead to better CD4 T cell responses. Peptide motifs that can target antigens to either class I MHC or proteasomal processing compartments have also been described. In this model of cancer vaccination, vaccine constructs can include, in addition to the tumor-specific antigen, proteins that promote interaction between antigen-presenting cells (APCs) and responding T cells, or genes that prevent apoptosis of transfected APCs and thereby allow for a better vaccine effect. As the science of defining what an ideal APC actually is advances, so will our ability to engineer these responses with plasmid DNA vectors.

The advantage of peptide or DNA constructs is that they can be directly synthesized in the laboratory, and therefore they are simpler to use than biological vectors (i.e., modified pathogens) for presenting cancer-associated antigens to the immune system. In Chapter 7 Wansley explores the evolution of recombinant poxviruses as cancer vaccines, and discusses potential new directions for combination therapies. These recombinant viruses have been made to elicit tumor-specific immune responses by modifying the virus to express a variety of tumor-associated antigens, including carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), and MUC1. In order to augment the immune response to these tumor-associated antigens, a vaccinia vector expressing three costimulatory molecules—B7-1, ICAM-1, and LFA-3 (TRICOM)—has been developed, and has exhibited some efficacy in the clinic. More recent studies have examined combining TRICOM vectors
with various cytokines [such as interleukin 2 (IL-2) and granulocyte–macrophage colony-stimulating factor (GM-CSF)], and with standard-of-care radiation therapy. Both of these approaches have shown promise in humans, and are being tested further. Since poxviruses have been shown to be safe in humans both alone and combined with other standard-of-care therapies, the use of these vectors in combination with other anticancer therapies is very promising.

Biologic vectors, including whole bacteria, have also been used as a vaccine platform, and we highlight this field by focusing on the work of Paterson and colleagues in Chapter 8 with *Listeria monocytogenes*. *Listeria* is an intracellular pathogen, and as such, when used as a bacterial vaccine vector, it is relatively free of the effects of neutralizing antibody. Up to four repeated doses have been given in experimental animals with minimal antivector responses. A bacterial vector can be regarded as the ultimate adjuvant, as a myriad of innate immune-stimulating structures are present. *Listeria* is of special interest because once it is internalized by a professional APC such as a dendritic cell, it escapes the phagolysosome, enters the cytosol, and is able to load both class I and class II major histocompatibility complex (MHC) with engineered antigen. The safety profile of administering a live (albeit attenuated) strain of bacteria in cancer patients, especially those who may have a congenital immunodeficiency, is a concern. This concern can be countered with either clearance of the bacterial vector with antibiotic chemotherapy or the use of psoralen and UV-treated or irradiated *Listeria*. As with other DNA vectors, DNA expression vectors transfected into *Listeria* allows for generation of fusion proteins that route the antigenic protein to the appropriate intracellular compartment or appropriate degradation machinery, which appears to greatly enhance the immune response to those sequences. The use of bacterial vaccines as vaccine platforms is still at an early stage with respect to clinical translation, and phase I studies are just getting under way.

In addition to viral and bacterial vectors, exciting new work described by Munson and colleagues (see Chapter 9) focuses on recombinant nonpathogenic brewer’s yeast, *Saccharomyces cerevisiae*, as novel vectors for cancer immunotherapy. This vector platform triggers both innate and adaptive immune responses, delivers polypeptide antigens that are effectively processed into a full complement of appropriate-sized peptides competent for presentation by MHC class I and class II pathways, and elicits potent T cell immune responses against tumor cells expressing target antigens. This chapter discusses methods for engineering yeast to express tumor antigens and the unique properties of recombinant yeast in the activation of innate and adaptive immune responses. The broad applicability of the yeast-based immunotherapy to elicit protective T cell immune responses has been demonstrated in preclinical studies with numerous foreign, mutated, and overexpressed antigens, and several recombinant yeast-based vaccines are currently being evaluated clinically.

### 1.4 CELL-BASED CANCER VACCINES

Cancer immunotherapy can also be approached through the use of tumor cells as a platform to initiate a therapeutic immune response. Tumor cells by themselves are
typically not immunogenic. However, as the science of determining how an effective APC initiates an immune response advance, these strategies are being used to alter tumor cells and render them as loci of immune stimulation. The most general method for cell-based immunotherapy is to use a single representative cancer cell as a universal vaccine for all patients with that same type of cancer. Some investigators consider this approach as suboptimal as it is allogeneic, where the MHC type of the vaccine and the patient do not match, and the immune system may be distracted from generating a tumor–antigen-specific to an allospecific response. Others argue that an allogeneic vaccine will be effective for just this reason, and that the alloimmune response will serve to amplify the cancer–antigen-specific response. This issue will be addressed in Chapter 10, where Copier and Dalgleish discuss the use of whole tumor cells as vaccines in this unique approach to cancer therapy, as tumor-specific antigens do not have to be known a priori for the vaccine to be effective. These vaccines rely on the idea that there are multiple tumor antigens within the cells themselves against which the immune response can be activated. Highlighted are clinical trials where allogeneic cell lines were used as vaccines for prostate cancer and melanoma, and showed extended survival as compared to the control arms. The modification of whole-cell vaccines to elicit a more robust immune response has been addressed in several ways, by the addition of BCG to the vaccine, or the modification of the cells to express costimulatory molecules or secrete cytokines. While autologous whole-cell vaccines are now being tested in combination with chemotherapy, future work is expected to address the efficacy of combination therapy with allogeneic whole-cell vaccines.

Research on the use of an allogeneic vaccine in the context of existing chemotherapeutic treatment is highlighted in Chapter 11. Chemotherapy for cancer can enhance or modulate immune-based approaches. In mouse models of breast cancer where a GM-CSF-producing cell-based vaccine is combined with chemotherapy in neu transgenic mice, low doses of cytoxan (cyclophosphamide, CY), and paclitaxel (PTX) were found to augment vaccine activity if given prior to vaccination, but not if given after. In contrast, low doses of doxorubicin (DOX) were found to augment vaccine activity if given after vaccination, but inhibited vaccine activity when given prior to immunization. These effects are attributable to a combination of inhibiting T-regulatory (Treg) activity, altering immunologic skewing toward a Th2 response and activation of CD8 cells. Combinations of cell-based vaccines with antibody (HER2/neu-specific) therapy also augment antitumor immunity, and antibody therapy alone is also enhanced by CY. A human allogeneic, GM-CSF-secreting, breast tumor vaccine is now being evaluated clinically. It is composed of two cell lines, SKBR3 and T47D, both of which have been genetically modified to secrete human GM-CSF by plasmid DNA transfection. These have been tested both in sequence with standard breast cancer therapeutics and more recently in combination with tumor-specific antibody.

The idea of using a cell-based vaccine to induce antitumor immunity brings a renewed focus to the type of effector cells vaccines are able to expand, and to the Treg cell system that coevolves with the tumor in the host [9]. In Chapter 12
the latest findings on manipulating the Treg cell networks are presented. Growing evidence has demonstrated that cancers utilize active mechanisms to block host antitumor immunity. Significant evidence implicates CD4⁺CD25⁺ Treg cells (Tregs) as important mediators of active immune evasion in cancer. Four strategies to inhibit Treg numbers or activity are highlighted: removal by depletion, interference of trafficking, inhibition of differentiation, or blocking of Treg function. The FDA-approved fusion protein denileukin diftitox (Ontak) has received attention recently as a potential agent to deplete functional Treg. As Ontak theoretically depletes any T cell bearing IL-2 receptors (including effector T cells), its utility may be limited in some settings. Ontak has been shown to reduce Treg numbers in the blood of some patients with cancer. In Chapter 12 Rütter discusses strategies to block Treg activity and presents preliminary data in this regard.

The mechanisms by which bone marrow transplantation induces antitumor immunity are numerous, and yet to be fully defined. They include direct cytotoxic effects and antigen release initiated by the preparative regimen, graft-versus-tumor effects, and the expansion of antigen-specific antitumor effector cells. High-dose chemotherapy or radiotherapy for solid tumors followed by hematopoietic stem cell transplantation (HSCT) reduces tumor burden, but many patients still relapse with disease following this intensive treatment as a result of incomplete elimination of tumor cells, inadequate graft-versus-tumor effects, and delayed immune reconstitution after HSCT. In Chapter 13 Jing and Johnson summarize recent work in their lab, and the work of other investigators, utilizing experimental mouse models of human autologous HSCT to determine the optimal parameters for inducing effective vaccine-induced antitumor immunity early after HSCT. Their work highlights the effectiveness of using a cell-based vaccine as a means to induce antineuroblastoma immunity. The animal data indicate that immune status early after HSCT is crucial to the success of early posttransplant vaccination, and that transfer of immunocompetent lymphocytes may be required if autologous HSCT is to be effectively used as a platform for tumor vaccination.

In Chapter 14 Gress and Sportes discusses the clinical use of HSCT (both autologous and allogeneic) as a platform for tumor vaccination. As shown by their research group, high-dose therapy followed by hematopoietic stem cell rescue can provide a time window for vaccination against residual tumor cells before the patient relapses with disease. Similar to the preclinical data, the clinical data also suggest that the ability to effectively administer tumor vaccines early after HSCT may be dependent on the adoptive transfer of “naive” or preactivated lymphocytes collected prior to transplant. Furthermore, in pediatric patients or young adults, thymus-mediated T cell reconstitution at later timepoints posttransplantation provides rationale for prolonged tumor vaccine administration to prevent late-disease relapses. The use of T-cell-depleting nonmyeloablative chemotherapy as an alternative vaccine platform to HSCT is also discussed.

Cell-based vaccines, administered directly or in the context of HSCT are designed to introduce a locus of immune activation that can generate antitumor effector cells that can then circulate throughout the body to sites of distant disease. Conventional treatment such as surgical removal of tumor followed by radiation
and chemotherapy may prevent effective immune recognition of cancers due to the loss of a major source of antigens, and damage to preexisting cytotoxic T lymphocytes (CTL) by radiation and chemotherapy. In Chapter 15 Drs. Yu and Fu discuss an alternative strategy, the use of the primary tumor as the site of CTL priming prior to surgical resection. It has been demonstrated that the creation of lymphoid-like structures within a tumor can lead to the rapid recruitment of naive lymphocytes and expansion of CD8+ T cells. Strategies utilized for the generation of these tertiary lymphoid structures include the use of lymphotoxin α and β, and another member of the TNF family, LIGHT (see Section 15.5, for a definition of this acronym). LIGHT expression within the tumor environment recruits naive T cells and generates tumor-specific CTLs that can survive and exit the microenvironment to patrol peripheral tissues and eradicate disseminated metastases. These strategies could prove a potent strategy for enhancing antitumor immunity and permitting a clinically desirable outcome for cancer patients.

The identification of immune costimulatory molecules was a key advance in the engineering of cancer vaccines. With a view toward the future application of more recently described immune costimulatory molecules, in Chapter 16 Dr. Leiping Chen’s group describes new molecules that may be included in cell-based vaccines or as targets of immunomodulation in their own right. The molecules that are currently being brought to phase I clinical trials for therapeutic applicability are members of both the PD-1/B7-H1/B7-DC pathway and the CD137/CD137L pathway. Exciting advances can still be made in learning how both positive and negative signals mediated by immune costimulatory molecules orchestrate antitumor immune responses.

1.5 DEFINING EFFECTIVE CLINICAL RESPONSES

The development and use of cancer vaccines has a long history in experimental medicine. However, to gauge the effectiveness of a cancer vaccine, standard ways to evaluate the immune response must be formulated. Chapter 17, from Dr. Kaufman’s laboratory, introduces the various immune assays that are currently being used to monitor responses in clinical cancer vaccine trials. In Chapter 18, the final contribution in this volume, Dr. Whiteside discusses in detail the laboratory services necessary to support a cancer vaccine trial, the assays and limitations of immune monitoring for the detection of tumor-specific T cells after cancer vaccine administration, and the advantages of having a central laboratory operated as a good laboratory practice (GLP) facility to produce the vaccine and perform the immune monitoring assays.

In this volume we have attempted to present some of the best current research on cancer vaccines. We did not include sections devoted to adoptive immunotherapy or dendritic cell strategies for vaccination, as these could serve as volumes in their own right. We also did not concentrate on the identification and testing of new cancer antigens, as this field is also vast and exploding with new targets, due in part to the revolution in gene expression profiling and completion of the
human genome project. Our aim was to present examples of how, once identified, antigens can be translated into effective loci of immune stimulation. Cancer vaccine development is still evolving as it changes from a small-scale academic enterprise into an applied science requiring a large clinical infrastructure. Our hope is that we have stimulated further interest in all of these fields, and that the mechanisms for cancer vaccine development presented in this volume will continue to develop. We could think of no better goal than to offer patients a whole new palette of therapeutic options on the basis of our ability to induce tumor immunity.

REFERENCES
