TUMOR IMMUNOLOGY
AND CANCER VACCINES
CONTENTS

Preface vii

I. BASIC TUMOR IMMUNOLOGY

1. Antigen Processing and Presentation 3
   Laurence C. Eisenlohr and Jay L. Rothstein

2. Antigen Recognition and T-Cell Biology 37
   Michael I. Nishimura, Jeffrey J. Roszkowski, Tamson V. Moore,
   Natasha Brasic, Mark D. Mckee, and Timothy M. Clay

3. Mechanisms of Tumor Evasion 61
   Michael Campoli, Soldano Ferrone, Arnold H. Zea,
   Paulo C. Rodriguez, and Augusto C. Ochoa

4. Tumor Antigens and Tumor Antigen Discovery 89
   Daniel F. Graziano and Olivera J. Finn

II. CANCER VACCINE DEVELOPMENT

5. Peptide Vaccines against Cancer 115
   Jay A. Berzofsky, Sangkon Oh, and Masaki Terabe

6. DNA Vaccination in Immunotherapy of Cancer 137
   Andrew Y. Choo, Daniel K. Choo, J. Joseph Kim, and David B. Weiner

7. Antibody Inducing Polyvalent Cancer Vaccines 157
   Govind Ragupathi, John Gathuru, and Philip Livingston

8. Dendritic Cell-based Vaccines for Cancer Therapy 181
   A. Groleau, A. Sloan, and J.J. Mule

9. Undefined-antigen Vaccines 207
   Hong-Ming Hu, Yiwei Chu, and Walter J. Urba

10. Cancer Vaccines in Combination with Multimodality Therapy 227
    Leisla A. Emens, R. Todd Reilly, and Elizabeth M. Jaffee
III. VACCINE-ENHANCING STRATEGIES
11. Cytokine Therapy for Cancer: Antigen Presentation 249
   SAMEEK ROYCHOWDHURY AND MICHAEL A. CALIGIURI
12. Tinkering with Nature: The Tale of Optimizing Peptide Based Cancer Vaccines 267
   OLIVIER MICHELIN, JEAN-SEBASTIEN BLANCHET, THERES FAGERBERG, DANILA VALMORI, VERENA RUBIO-GODOY, DANIEL SPEISER, MAHA AYYOUB, PEDRO ALVES, IMMANUEL LUESCHER, JEAN-EDOUARD GAIRIN, JEAN-CHARLES CEROTTINI, AND PEDRO ROMERO
13. Adoptive Cellular Immunotherapy of Cancer: A three-signal paradigm for translating recent developments into improved treatment strategies 293
   SHAWN M. JENSEN AND BERNARD A. FOX

IV. CLINICAL TRIALS DESIGN
14. Clinical Trial Designs for Therapeutic Cancer Vaccines 339
   RICHARD SIMON
15. Clinical Trial Design and Regulatory Issues for Therapeutic Cancer Vaccines 351
   JAN CASADEI, HOWARD Z. STREICHER, AND JAY J. GREENBLATT
16. Immune Monitoring 369
   PAUL J. MOSCA, TIMOTHY M. CLAY, MICHAEL A. MORSE, AND H. KIM LYERLY

Index 389
It all started with an observation. Edward Jenner, an English physician, observed that milkmaids who contracted cowpox were rarely victims of smallpox epidemic, a disease that inflicted a heavy toll on humankind with an estimate of 500 million victims worldwide. In 1796, Jenner inoculated the extracted fluid from blisters on the hand of a milkmaid who was infected with cowpox into the arm an 8 year old peasant boy. After the boy recovered from a mild illness caused by this inoculation, Jenner exposed him to smallpox and to his delight the boy did not develop the disease. He published his work in 1798 in three publications titled “Vaccination Against smallpox”, where the term vaccination is derived from the Latin word “vacca” meaning cow. Jenner was recognized to be the father of modern immunology, and his work marked the commencement of a new dawn in medicine that led to the 1979 declaration by the World Health Organization (WHO) of the global eradication of smallpox. By the beginning of the 20th century, vaccines for typhoid fever, rabies, polio, plaque and diphtherias were in use, and nowadays we are equipped with effective vaccines against more than 20 infectious diseases such as meningitis, rubella, whooping cough, rabies, and hepatitis B among others.

It is indisputable that the immune system plays a role in the natural history of cancer. This theory is supported in animal models by the fact that tumors develop earlier and more frequently in nude mice than in mice with normal immune systems. In humans, the principal evidence comes from many facts including that many ‘immunocompromized’ cancer patients have higher incidences of a number of tumor types, including those of the lung, colon, kidney and pancreas, as well as malignant
melanoma; immune response modifiers have been shown to be effective in treating
tumors and in some anecdotes; tumors are known to regress spontaneously; and
increased patient survival correlates with the presence of T cells (or tumor infiltrat-
ing lymphocytes, TIL) in a variety of tumors such as melanoma, neuroblastoma, and
breast, bladder, colon, prostate, ovary, and rectal cancers. This indicates that tumors
are amenable for immune recognition, and hence, are able to present antigens that are
recognized by the immune cells. These antigens are called tumor antigens. Therefore,
it is concluded that tumors develop due to the failure of the immune system to
recognize and reject cancer, this is called “Tumor immune escape”; we now under-
stand some of the factors that lead to tumor immune escape which will be discussed
along with the principle of tumor antigens in the chapters of this book.

Advances in both immunology and molecular biology in the past decade have
led to the identification and characterization of these tumor antigens. That in turn
led to the revival of immunotherapy as the fourth modality of treatment of cancer.
This treatment can be highly specific and an effective therapy based on the ability to
develop tumor-specific antigen directed vaccines. The concept of Immunotherapy
for cancer is over one hundred years old. The first reported “Cancer Vaccine” trial
was by W.B. Coley in 1894. Coley’s toxin’s, as it was called, was not so much a
vaccine as a non-specific immuno-stimulant. He used thirteen different preparations
of bacterial extracts, between 1892 and 1936, to treat patients with a variety of
malignancies with surprising success. He and others, including investigators at Mayo
Clinic, reported over 50% durable responses in patient populations where 10-15%
survival was historically expected. About the same time, in the early 1900’s, Paul
Ehrlich proposed the concept of “Immune Surveillance”. Ehrlich suggested that
tumors present unique antigens that could be recognized by the immune system,
leading to continuous identification and removal of transformed cells. It was another
fifty years before his theory could be proven. In the 1950’s, when inbred mouse
strains became available, Ehrlich’s theory was tested and proved the immunogenicity
of tumors. The tumor antigens were subsequently identified.

The new era of biotechnology is helping us rapidly progress in our efforts to
identify tumor antigens, compare their immunogenecity, and then design effective
delivery system to present the most powerful antigens to the immune system. With
the completion of the human genome project, new technologies such as microarray
analysis and proteomics have been added to our repertoire and have proved useful
in identifying antigens that produce the best immune response; a pivotal requisite
to the success of a cancer vaccine. Such a success is also dependent on how the
antigen is delivered to the patient, the vehicle used along with the choice of adjuvant
and cytokines. This wealthy “vaccine basket” provides researchers with tremendous
choices when planning clinical trials and emphasizes the need to compare different
strategies of vaccine design and delivery according to its efficacy in combating cancer
in clinical trials.

In lieu of the tremendous amount of knowledge in areas of tumor immunology and
cancer vaccines, we recognized the need to provide researchers and clinicians alike
with a comprehensive up-to-date book on tumor immunology and cancer vaccines.
The first section of the book includes in depth analysis of basic tumor immunology, both cellular and humoral. This section explains mechanisms of antigen presentation, as well as the molecular reasons why tumors evade the immune system. The second section includes six chapters encompassing different vaccine strategies with emphasis on their preclinical development and current clinical data. How to enhance the immune response to cancer vaccines is the question tackled by the third section of this book. It documents preclinical and clinical developments in cytokine therapy, peptide vaccines and adoptive cellular immunotherapy. Finally, the last section of the book emphasizes the different issues regarding clinical trials design and application in addition to the latest advances in immune monitoring.

*Tumor Immunology and Cancer Vaccines* is the fruit of tremendous cooperation between our knowledgeable and devoted authors and the commitment and foresight of our publisher. We worked hard to make this book an effective resource, which we hope will translate to discoveries in the field of tumor immunology and more effective treatments of patients with cancer.
I. BASIC TUMOR IMMUNOLOGY
1. ANTIGEN PROCESSING AND PRESENTATION

LAURENCE C. EISENLOHR AND JAY L. ROTHSTEIN

Thomas Jefferson University

In the ongoing search for effective and reliable immune-based approaches to cancer therapy, much of the work is focused on T lymphocytes as effectors. CD8\(^+\) T lymphocytes \((T_{CD8^+})\) are of particular interest as they combine specificity and lethality at a level that no current chemotherapeutic or radiation regimen can match. One can only marvel at the effectiveness with which these cells are able to clear an acute respiratory tract infection, leaving the involved tissues intact—the precise goal of cancer therapy. CD4\(^+\) T lymphocytes \((T_{CD4^+})\), relatively specific, but generally less cytotoxic than \(T_{CD8^+}\), can also mediate potent anti-tumor effects in certain settings. While a great deal has been learned about how \(T_{CD4^+}\) and \(T_{CD8^+}\) responses are induced and sustained, further exploration will be necessary if the full potential of these populations is to be harnessed. One aspect worthy of closer inspection is that of antigen processing and presentation—the various intracellular steps that prepare antigen for T cell recognition. It is intuitive that greater understanding and controlled manipulation of these events, which usher in the adaptive response, could have profound influence on the final character of the anti-tumor immunity that is engendered.

1. INTRODUCTION

This chapter will review fundamental aspects of antigen processing and presentation with special emphasis on how they pertain to tumor-specific immunity. Three points must be made at the outset. First, there is no intent to evaluate the relative efficacy of various therapeutic strategies that have been based on principles of antigen processing and presentation. Only a handful of possible permutations have been tested at this
point and, in any event, outcomes will certainly be different depending upon the experimental model or clinical situation. Second, there is minimal segregation of findings in animal models (usually mouse) and humans. Most of the fundamental cell biology is similar even though decades of experimentation and practical application have made it clear that success in mouse models does not ensure success in patients. Finally, the topic of tumor antigen processing and presentation is now sufficiently large that a comprehensive review in a single chapter is not possible. While an attempt has been made to cover a large amount of conceptual territory, space does not allow for all of the relevant work to be mentioned here.

2. THE BASIS FOR T CELL RECOGNITION: FRAGMENTS OF ANTIGEN DISPLAYED AT THE CELL SURFACE BY SPECIALIZED “PRESENTING” MOLECULES

2.1. Peptide Binding

While B cells and their antibody products recognize antigens in their native forms, T cells respond to pieces of antigens held at the cell surface by various “presenting molecules” and generated by a variety of intracellular, and even extracellular processes known collectively as antigen processing. Class I molecules are made up of a heavy chain encoded within the major histocompatibility complex (MHC) and a noncovalently associated light chain, $\beta_2$-microglobulin. Class I heterodimers bind peptides that are generally 8–11 amino acids in length and present them to $\text{T}_{\text{CD8}^+}$ whose most appreciated response is killing of the peptide-presenting cell. Class II molecules, comprised of $\alpha$ and $\beta$ chains, both encoded within the MHC, generally bind peptides 11–17 amino acids in length, and present them to $\text{T}_{\text{CD4}^+}$ which respond by elaborating factors that guide and potentiate both B cell and $\text{T}_{\text{CD8}^+}$ responses.\(^1\) The variation in lengths of peptide bound by class I and class II molecules is due to distinct structural differences in the peptide-binding grooves (1). The binding grooves of class I molecules are closed at both ends, with the consequence that a peptide must be a specific length in order to be bound. In contrast, class II binding grooves are open at both ends so that quite large peptides have the capability of binding. Despite this, relatively short peptides are usually isolated from class II molecules, presumably due to the exposure of any extended portions to intracellular and extracellular proteases. As might be surmised from several different crystal structures (2), peptides that directly interact with the binding groove of both class I and class II molecules are resistant to proteolysis, as are the presenting molecules themselves (3–7). Many readers may know that a key feature of class I and II molecules is their tremendous polymorphism, with hundreds of versions of each encoded by many different loci within the MHC existent in the human population. Greatest variation is in the residues that line the peptide-binding grooves, leading to distinct peptide-binding specificities and, thus, differences among individuals in the parts of any antigen that are responded to. This variation is a powerful strategy for a population to counteract the rapid replication and mutation rates that many

\(^1\)CD4 molecules bind to conserved regions of class II molecules and CD8 molecules bind to conserved regions of class I molecules, in both cases participating in activation.
microbes are capable of, but constitutes a major impediment for tissue transplantation and immune-based cancer therapy since both applications may require individually-tailored therapies. The basis for binding specificity is a series of pockets in the floor of any peptide-binding groove into which side chains of the peptide extend. Some of these pockets provide anchoring points that are quite stringent in terms of the side chains that are acceptable, while others are much more permissive. Thus, only specific segments within a protein, with appropriate amino acids properly spaced apart, are able to bind any particular MHC molecule. Those side chains that do not participate in binding to the groove are available for interaction with the T cell receptor. As mentioned at the outset, recognition of peptides by T cell receptors can be highly specific and sensitive. Single amino acid changes in a peptide, including residues that do not directly contact the T cell receptor and even simple phosphorylation of a peptide, can profoundly influence T cell recognition (8–10). In terms of sensitivity, relatively few copies of a particular peptide are required for full T cell activation—on the order of tens to hundreds (11–13). This can be derived from an amount of antigen that cannot be detected using standard biochemical methods (14). Both specificity and sensitivity are highly variable among different T cell clones (15), being determined by both intrinsic factors, such as receptor sequence and density, and extrinsic factors such as the balance of stimulatory and suppressive cytokines. These factors will obviously vary dependent upon the tissue(s) where the antigen is expressed.

From the standpoint of peptide presentation, targets of T cell-mediated tumor immunotherapy can be divided into three broad categories: foreign, mutated self, and nonmutated self epitopes. Examples of the first category (foreign) are epitopes from the growing number of viruses that establish persistent infections and induce transformation, such as the papillomaviruses and herpesviruses. Within the second group are the proteins altered by point mutations, deletions or chromosomal translocation, which are incidentally or coincidentally connected with transformation. All of these can result in new peptide sequences that have the ability to bind to an MHC class I or class II molecule and potentially elicit a response. An emphasis must be placed on the words can and potentially. Such mutations do not guarantee the generation of a neo-epitope that can bind to an MHC molecule and binding does not guarantee T cell stimulation. At least with respect to peptide binding, some level of prediction is possible. Algorithms, based upon known epitopes, have been developed for many mouse and human MHC molecules, such that one can query an open reading frame for the presence of segments with a high likelihood of binding (16, 17). Nonmutated peptides could be of potential interest if they are: 1) derived from antigens, such as carcinoembryonic antigen, that are expressed at low levels or not at all in the adult, but highly expressed in the cancerous cell, 2) expressed by a differentiated (specialized) cell type, such as the melanocyte, that is expendable, 3) expressed by a fraction of a particular cell type, expendable or not, such immunoglobulins, the product of B cell lymphomas, that can provide unique T cell epitopes from the hypervariable regions (18, 19), or 4) altered by cellular processes that have gone awry as a result of transformation. An example of this would be phosphorylation due to aberrant kinase