Molecular Approaches to Tumor Immunotherapy

Editor

Yang Liu

World Scientific

Molecular Approaches to Tumor Immunotherapy

Editor

Yang Liu

Department of Pathology
New York University Medical Center

World Scientific
Singapore • New Jersey • London • Hong Kong

Published by

World Scientific Publishing Co. Pte. Ltd.

P O Box 128, Farrer Road, Singapore 912805

USA office: Suite 1B, 1060 Main Street, River Edge, NJ 07661

UK office: 57 Shelton Street, Covent Garden, London WC2H 9HE

Library of Congress Cataloging-in-Publication Data

Molecular approaches to tumor immunotherapy / editor, Yang Liu.

p. cm.

Includes bibliographical references and index.

ISBN 9810227930

1. Cander -- Immunotherapy. 2. Cancer -- Molecuar aspects. I. Liu, Yang.

[DNLM: 1. Neoplasms -- therapy. 2. Neoplasms -- immunology.

3. Immunotherapy. 4. Gene Therapy. QZ 266 M718 1998]

RC271.I45M67 1998

616.99'406--dc21

DNLM/DLC

for Library of Congress 97-41654

CIP

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Printed in Singapore by Eurasia Press Pte Ltd

PREFACE

This is the best of times. Immunologists can tell us with confidence the basic features of elements involved in specific immune recognition: those of the immune receptors, their ligands, and perhaps a framework of the interactions required to switch on lymphocytes; gene therapists are armed with vectors which allow them to efficiently transfer genes of interests.

This is also the worst of times. In no case has immunotherapy become part of clinical therapy for tumors. By and large, it is still unclear whether gene therapists are intervening immunity, or are intervened by immunity.

Nevertheless, history will likely show that this is the time when ideas matter the most. For this reason alone, interdisplinary conversations may facilitate the birth of new solutions to the most challenging problem of tumor immunotherapy. It is in this spirit that I have invited scientists interested in fundamental immunology, tumor immunology and gene therapy to discuss the state-of-the-art knowledge in their disciplines, and to share with us their vision for possible solutions in the future.

I am most grateful to the authors of the chapters whose expert contributions and enthusiasm have made this book what it is. My loving gratitute goes to Jon, Molly, Pan and my parents, whose love and support have made this book possible and worthwhile.

Yang Liu, PhD
New York University Medical Center

LIST OF CONTRIBUTORS

Appleman, L. J.
New York University School of Medicine
Department of Cell Biology and Kaplan Comprehensive Cancer Center
550 First Avenue, New York
NY 10016, USA

Chamberlain, R. S.
Surgery Branch, National Cancer Institute
National Institute of Health
Bethesda, Maryland 20892, USA

Chen, L. P.
Department of Immunology
Mayo Clinic
200 First Street SW
Roschester, MN 55905, USA

Colombo, M. P.
Instituto Nationale Tumori,
I-2033 Milan, Italy

Forni, G.
CNR-CIOS, University of Turin
I-10126 Turin, Italy

Frey, A. B.
New York University School of Medicine
Department of Cell Biology and Kaplan Comprehensive Cancer Center
550 First Avenue, New York
NY 10016, USA

Guo, Y.
Michael Heidelberger Division of Immunology
Department of Pathology and Kaplan Comprehensive Cancer Center
New York University Medical Center
New York, NY 10016, USA

Hosken, N. A.
Department of Immunology
DNAX Research Institute
901 California Avenue
Palo Alto, Ca 94304–1104, USA

Irvine, K. R.
Surgery Branch, National Cancer Institute
National Institute of Health
Bethesda, Maryland 20892, USA

Janeway, C. A. Jr.
Section of Immunobiology
Yale University School of Medicine
and
Howard Hughes Medical Institute
New Haven, CT 06510, USA

Liu, Y.
Michael Heidelberger Division of Immunology
Department of Pathology and
Kaplan Comprehensive Cancer Center
New York University Medical Center
New York, NY 10016, USA

Maric, M.
Michael Heidelberger Division of Immunology
Department of Pathology
New York University Medical Center
550 First Avenue, New York
NY 10016, USA

Ménoret, A.
Center for Immunotherapy for Cancer and Infectious Disease
University of Connecticut School of Medicine
Farmington, CT 06030, USA

O'Gara, A.
Department of Immunology
DNAX Research Institute
901 California Avenue
Palo Alto, CA 94304–1104, USA

Restifo, N. P.
Surgery Branch, National Cancer Institute
National Institute of Health
Bethesda, Maryland 20892, USA

Rudensky, A. Y.
Howard Hughes Medical Institute
New Haven, CT 06510
and
Department of Immunology
University of Washington
Seattle, WA 98195, USA

Sarma, S.
Michael Heidelberger Division of Immunology
Department of Pathology
New York University Medical Center
550 First Avenue, New York
NY 10016, USA

Srivastava, K.
Center for Immunotherapy for Cancer and Infectious Disease
University of Connecticut School of Medicine
Farmington, CT 06030, USA

Wu, Y
Michael Heidelberger Division of Immunology
Department of Pathology and
Kaplan Comprehensive Cancer Center
New York University Medical Center
New York, NY 10016, USA

Yang, G. C.
Bristol-Myers Squibb
Pharmaceutical Research Institute
P.O. Box 4000
Princeton, NJ 08543-4000, USA

Zhang, X. L.
Cantab Pharmaceuticals Research Ltd
184 Cambridge Science Park
Milton Road
Cambridge CB4 4GN, UK

CONTENTS

Chapter 1

THE MOLECULAR BASIS OF ANTIGEN RECOGNITION BY B AND T LYMPHOCYTES[1]

Janeway, C. A., Jr.

Introduction

The adaptive immune response results from activation of perhaps the most specialized system in the vertebrate body. It is unique in using gene rearrangement to generate a nearly infinite variety of antigen receptors, and it uses genes that are expressed only in lymphoid cells to achieve this feat. Even the olfactory and pheromone receptors operate on a more primitive basis in generating their diversity. In this brief essay, I will try to introduce the reader to this system of receptors in a comprehensible manner. For a more in-depth coverage of these topics, see ref. 1.

Basic Features of Adaptive Immunity

When a person is vaccinated, molecules called antibodies are synthesized in response to vaccination. These proteins bind specifically to the vaccine used to raise them, and not to other vaccines based on distinct pathogens.

[1]Supported in part by NIH grant AI-14579 and by the Howard Hughes Medical Institute.

The same response accounts for the resistance to re-infection, i.e. the natural consequence of an immune response to an initial infection. With each subsequent exposure to a particular pathogen, immunity becomes stronger. These features of the antibody response: inducibility, specificity, and immune memory, illustrate the key features of the adaptive immune response.

Adaptive immunity is based on relatively simple principles. The most important principle is the clonal selection of lymphocytes, originally proposed by Sir Macfarlane Burnet in the mid-1950s. This theory has four main tenets: (1) Antibody responses reflect the activation of cells by specific antigen (from antibody generators) binding to cell surface receptor molecules, (2) such receptors are distributed clonally such that a very low frequency of cells specific for any given antigen will respond, (3) all of the progeny of an antigen-specific cell inherit the same receptor genes, and can secrete antibodies of identical specificity to their receptor, (4) the random generation of specificity requires that cells early in their development be tested for their specificity to proteins prevalent in the site of their development, and those that bind to such proteins are triggered to die before achieving functional activity. The studies of Gowans in the early 1960s showed that the cells mediating antibody responses were small lymphocytes. Thus, the small lymphocyte is taken as the unit of selection. To these four tenets of clonal selection Mel Cohn and others have proposed adding one more: To activate a naive lymphocyte, a second signal is required from another cell, and this is normally triggered by features of pathogens that are recognized by invariant receptors selected over evolutionary time. Cells that only bind antigen but do not receive such a signal are rendered inactive or are programmed to die. This is essential to guarantee the absence of response to those self proteins found only on peripheral tissues.

B Lymphocytes and T Lymphocytes

As Gowans showed, the specific cells of the immune system are lymphocytes, and they are divided into two distinct populations. B lymphocytes, or B cells, are derived from the bone marrow and they

are the only cells that produce antibody molecules. T lymphocytes, or T cells, arise in the bone marrow but mature in the thymus, hence their name. The T cells do not produce antibody. Rather, they recognize antigen when it is displayed on the cell surface in the form of short peptides of degraded proteins bound to a molecule derived from the major histocompatibility complex (MHC) of genes. These genes produce two major types of protein called MHC class I and MHC class II molecules, and the complex of antigenic peptide and MHC molecule is recognized by two major classes of T cells: CD8 T cells mediate the killing of infected target cells and recognize short peptides of 8–10 amino acids derived from the pathogen that are bound to MHC class I molecules; CD4 T cells activate target cells expressing MHC class II molecules that present a specific foreign antigenic peptide. In the third section of this chapter, I will discuss the cellular machinery involved in these two processes that lead to such different consequences. However, we will first need to discuss the generation of antibody molecules by B cells and their specific binding to antigen.

Antibody Molecules and Genes and Antigen Recognition by B Cells

As stated at the outset of this chapter, antibody molecules are produced in response to specific immunization, vaccination, or natural infection, and their most impressive feature is their immense diversity. This diversity allows antibody molecules to distinguish between molecules that would be difficult for the best chemist to discriminate. This specificity can be elicited with a virtually limitless range of compounds, suggesting that antibody molecules must be incredibly diverse in structure. Despite this, all antibodies share certain features. One of the great triumphs of modern immunology has been the discovery of the origin of antibody diversity.

Antibody molecules are plasma proteins with a unique structure, having two heavy chains of 50,000 d and two light chains of 25,000 d. These chains are joined together by disulfide bridges from the two

heavy to the two light chains, and by one or more disulfide bridges between the two heavy chains. They form a **Y**-shaped molecule with two antigen combining sites at the tips of the two arms of the Y that contain the two light chains and a functional moiety in the stem of the Y which contains the C-terminal halves of the two heavy chains. The stem of the Y is identical in all antibodies of the same class or isotype, there being about 10 isotypes in most mammals, including man.

The heavy and light chains were originally studied by amino acid sequencing, and this revealed that the amino terminal 110 residues of both the heavy and the light chains were variable from molecule to molecule, while the remaining regions had identical sequence in antibodies of the same isotype. The variable regions could, upon compilation of sufficient sequences, be further subdivided into regions of great diversity, of which there are three in each variable region, and regions of relatively little diversity, of which there are four in each variable region. It was assumed that the regions of greatest diversity must be involved in the specific binding of antigen, and when the crystal structure of an antibody molecule binding to a protein antigen was solved, it was seen that this is precisely what happens. The rest of the variable region is folded into a pattern characteristic of antibody molecules, now generically called immunoglobulins; such folded regions are termed immunoglobulin domains. The light and heavy immunoglobulin chains pair and bring the six hypervariable loops, three from the heavy and three from the light chain, into a cluster at the outer end of the antibody molecule, where they can contact the antigen. Thus, the loops of greatest variability in the antibody structures are brought to the end of the molecule and can easily bind to complementary antigens. These bonds are non-covalent and consist of electrostatic, hydrogen, van der Waals and hydrophobic interactions, each of which typically contributes to some of the energy of the antigen:antibody bond.

This structure, however, raised a seemingly inexplicable problem: how could each antibody sequenced have part of its sequence variable and part constant? And how could a genome now estimated to contain around 10^5 genes encode such a diversity of proteins? The answer to both questions required a molecular analysis of the antibody genes.

Antibody genes are arranged in gene segments, which undergo recombination in specific somatic cells, those of the B lineage of lymphocytes. Light chain variable regions are encoded in two types of gene segment, called V (for variable) and J (for joining), while heavy chains actually assemble three gene segments, called V, D (for diversity) and J, to form a complete variable region gene. The junctions between the gene segments are the sites of greatest diversity, with the heavy chain actually containing a variable number of non-template encoded or N-nucleotides. In addition, there are substantial numbers of V, D, and J gene segments involved in forming the light and heavy antibody chains, so the total diversity is tremendous. Finally, the pairing of two diverse peptide chains, one heavy and one light, to form a complete antigen binding site increases diversity exponentially. This diversity derived from heavy and light chain pairing is called combinatorial diversity.

The repertoire of distinct antibody molecules in man has been estimated to be at least 10^8, but individual specificities are represented to different degrees, so that some are quite frequent ($\approx 1/10^{4-5}$), while others are quite rare ($\approx 1/10^{10-12}$). This diversity is further amplified in an immune response by a process of somatic hypermutation, which is unique to B cells proliferating in response to antigen. Somatic hypermutation is initiated a few days after B cell responses begin, and occurs with a remarkably high frequency of about one mutation per 10^3 base pairs per generation. As a typical B cell response involves 10–12 divisions, and a typical B cell has around 660 base pairs encoding the two variable regions, this is close to one mutation per cell division. Many of these mutations probably destroy antigen recognition as they are nearly random, but some improve antigen binding significantly, and such mutations are mainly in the complimentarity determining regions. The B cells carrying such mutations are readily selected for antigen binding, with those B cells having receptors with the highest binding affinity continuing the response while B cells with neutral or deleterious mutations in their antigen binding domains are lost.

Thus, B cell antigen receptors and their secreted products, the antibody molecules, are tremendously diverse in just those regions required for

optimal antigen binding. Diversity in other parts of the V region not involved in antigen binding are selected against, while mutation of those genes that encode the constant regions is not observed at all. Therefore, antibody diversity can be encoded in a relatively small number of gene segments: there are approximately 40 heavy chain V gene segments, 25 diversity gene segments, and 10 heavy chain J gene segments in humans, generating a total of 10,000 VDJ joins. Diversity is increased at the VDJ junctions by the insertion of N-nucleotides and further by somatic hypermutation during the antibody response. The actual number of distinct antibody molecules that can be formed is much larger than the number that can be used. The gene rearrangement program also assures that each cell expresses a unique receptor, and that its progeny inherit that same basic receptor, or mutants that are better than the original B cell at binding the antigen.

The B cell antigen receptor consists of a cell surface antibody molecule derived from the same rearranged V(D)J genes as the antibody that the cell will secrete upon activation. The antibody on the cell surface is associated with chains called Igα and Igβ that allow the B cell that binds to antigen to respond by making antibody. These chains lack enzymatic activity of their own, but they do contain so-called immune receptor tyrosine-based activation motifs, or ITAMs, which allow them to interact with cytosolic tyrosine kinases. However, most antigens do not stimulate B cell growth and differentiation directly upon antigen binding. Rather, they are bound by surface antibody molecules and taken up by antigen binding to the B cells receptor, which degrades them into peptides to be presented to T cells. Molecules taken up in this way focus T cells specific for the same antigen on the antigen-binding B cell itself. B cells are one of the few cells in the body that express MHC class II molecules, and therefore can be activated by CD4 T cells which can stimulate the B cell to divide and make antibodies. Thus, all adaptive immunity is T cell-dependent, and we must therefore turn to how T-cells recognize antigen in order to understand the immune response.

Before doing so, however, it is useful to ask how one can use antibodies to trace and target tumors. Antibodies are produced in a variety of

forms even when one uses a very precisely defined antigen. This heterogeneity of antibodies is the result of the great diversity produced by gene rearrangement and somatic hypermutation. It also poses problems for the individual who would use antibodies as a therapeutic tool, because each batch of antibodies prepared will have different properties. However, if one could start from a single B cell and expand it indefinitely, then each cell should make antibodies that are the same. The trick to having homogeneous antibodies is to be able to grow from one cell an immortal clone all of whose antibodies are identical. This feat was accomplished in the mid-1970s by Kohler and Milstein, who invented monoclonal antibodies. They fused spleen cells from a mouse that was immunized with a specific antigen to a mouse myeloma that had all of its own antibody genes removed, and then screened for a cell that secreted the specific antibody. The spleen cell provided the genes encoding the antibody, while the myeloma provided the immortalizing genes and the necessary machinery for synthesizing large amounts of antibody. More recently, even more sophisticated techniques for generating antibodies have been introduced through genetic engineering.

Antibodies have been used against a number of tumors, and on occasion have had the desired effect of attacking the specific tumor. Their potency can be enhanced by coupling them to cytotoxic drugs to form immunotoxins, or to radionuclides to allow the cells in the tumor to be exposed to irradiation. Similar techniques can identify sites where tumor cells are accumulating in the living patient. However, most tumors are not readily recognized by antibodies, and one is left with using T cells in most cases. T cells can find and attack tumors because of their specific receptors, and they can recognize small changes in the tumor cell surface that are not detectable by antibodies. Thus, we must study T cell antigen recognition and differentiation in order to understand why T cells are the focus of so much interest today.

Recognition of Antigen by T Cell Receptors

As stated in the introduction, T cell receptors are distinct from antibody molecules. The major distinction is in how they recognize antigen.

Antibody molecules are responsible for clearing the extracellular fluid of bacteria, viruses and other pathogens. They do so by binding to these extracellular forms of pathogens with high affinity. T cells, by contrast, are responsible for monitoring the integrity of the intracellular milieu of cells. They do this by inspecting the surface of cells for the presence of bound non-self peptides. In this way, T cells can detect infection inside of cells and remove the infected cells in the case of killer T cells, which are mainly CD8 cells, or activate them to destroy pathogens that are growing within them in the case of CD4 T cells. To understand how this happens, it is necessary to divide the cell into two domains: the cytosol, including the nucleus; and the vesicular compartments of a cell: the endoplasmic reticulum, Golgi apparatus, endosomes and lysosomes, which are topograpically separated from the cytosol and exchanged with the extracellular fluid by means of phagocytosis, pinocytosis, and receptor-mediated endocytosis. These two compartments generate peptides in two distinct ways, and present them at the cell surface on two distinct classes of MHC molecule. This process will be described in detail by Rudensky in the next chapter of this book, but a brief description is useful now in order to understand why it is both necessary and important to know how T cells recognize infected or transformed cells.

MHC class I molecules present peptides of 8–10 amino acids to CD8 T cells. Proteins in the cytosol are degraded, probably by proteosomes, which are large multicatalytic proteases present in all cells from the oldest bacteria to present day mammals. These short peptides are transported across the membrane of the endoplasmic reticulum (ER) by an ATP-dependent transporter made up of two subunits called TAP-1 and TAP-2. In the presence of ATP, these transporters pass the peptides into the lumen of the ER where they are met by MHC class I molecules comprising of two chains, an α chain that is a transmembrane protein encoded in the MHC and shows extensive genetic polymorphism, and a light chain called $\beta 2$ microglobulin that is not polymorphic, is encoded on a different chromosome and associates non-covalently with the MHC class I heavy chain. When a peptide of appropriate sequence is presented to the MHC class I molecule, this molecule completes its