CANCER AND THE IMMUNE SYSTEM: The Vital Connection

a publication from

CANCER RESEARCH INSTITUTE
TABLE OF CONTENTS

1..........Introduction
2..........An Enigma Called Cancer
9..........The Human Defense System
9..........The Concept of an Innate Defense Network
11..........The Adaptive Defense System
12..........Brian Pricked His Thumb and His Immune System Jumped Into Action
13..........The Humoral Immune Response
14..........The Cellular Immune Response
17..........The Origins of Immunotherapy
19..........Advances in Human Tumor Immunotherapy
19..........Nonspecific Immunotherapy 1: Bacille Calmette-Guerin (BCG) Therapy
20..........Nonspecific Immunotherapy 2: Cytokines
21..........Nonspecific Immunotherapy 3: Cell therapy
22..........Specific Immunotherapy I: Adoptive Transfer
25..........Specific Immunotherapy II: Vaccination
31..........The Advantages of Cancer Immunotherapy
34..........Techniques Behind the Advances in Tumor Immunotherapy
37..........Epilogue and Acknowledgements
38..........Bibliography
41..........Glossary
INTRODUCTION

The Cancer Research Institute was established in 1953 to foster the field of cancer immunology, which is rooted in the notion that the body’s immune system can be mobilized against cancer. From our inception, we have championed the development of new and effective strategies based on the immune system to complement traditional methods of cancer treatment, such as surgery, radiation, and chemotherapy. We are a non-profit intermediary organization that provides funding for individual and collaborative research projects across the country and throughout the world. Our funding strategy is aimed at providing support to investigators throughout various career development stages encompassing a broad spectrum of research such as basic, preclinical, and clinical sciences.

Today, we are more committed than ever to our long-term goal of fostering cancer immunology. We recognize that further advancement in the field depends on increased public understanding of the enormous power of the immune system and its connection to cancer. To help build that critical understanding, we have prepared this guide, which answers a number of commonly asked questions about cancer, the immune system, and the latest trends in immunotherapy.

In the first chapter, the reader is introduced to the concept of cancer as the defining term for a panoply of diseases underpinned by two common features. This chapter ends with an introduction to the human immune system and how its normal function and cancer prevention are inextricably linked. We move on to chapter 2 for a discussion of the immune system thus setting the stage for the introduction of immunotherapy in chapter 3. The next three chapters (4—6) constitute a tour de force into almost every form of currently available immunotherapeutic regimens and how they are faring in worldwide clinical trials. The seventh chapter is full of hope: reminding us of how far we have come and how much further we must travel on this exhilarating but arduous journey of battling cancer. The remainder of the book provides a brief look at the techniques behind the progress we have made in this field of biomedical science.

We hope that you will find it enlightening.
1.0 AN ENIGMA CALLED CANCER

The word “cancer” is an umbrella term that refers to about 200 diseases that share two common characteristics: first, an uncontrolled growth of cells and second, the ability to invade and damage normal tissues either locally or at distant sites in the body. Since a cell is our body’s basic unit of life, this disease could not have chosen a more effective route to wreak havoc on the entire body. Some human cancers arise in the epithelium (the layers of cells covering the surface of the body and the lining of internal organs and various glands); these cancers are called carcinomas. Sarcomas are cancers of the supporting tissues of the body, such as bone, muscle and blood vessels. Cancers of the blood and the lymph glands are called leukemias and lymphomas, respectively. Gliomas are cancers of the nerve tissue. Melanomas arise from darkly pigmented cells, usually in the skin. There is an imperative to understand and control cancer because to date, apart from heart disease, more people die from cancer than any other disease.

1.1 The genesis of cancer

The number of cells in an average human being is about a hundred trillion (10^14) or beyond. Some of them, for example, brain and nerve cells, are not actively dividing while others like the cells of the skin, gut, bone marrow, and sex organs continually undergo rapid cell divisions to replace aging and dead cells. It has been estimated that about one million cells commit suicide every second in the adult human being.

On an average day, the human body produces and concomitantly eradicates about 60 x 10^9 cells; on an annual basis, this enormous amount of cells is equivalent to an entire body weight. In order to replace a dead cell, an existing cell must divide and for each successful cell division, the entire genetic material of the mother cell in the form of DNA must be faithfully copied by enzymes and handed over to the new daughter cells. If we compare this to the task of photocopying the entire content of the encyclopedia Britannica again and again for over a trillion times a day, one can appreciate the number of errors (due to exhaustion) that would begin to appear in each copied volume after only a few hours.

Fortunately, our cells are equipped with enzymes that not only copy but also proof-
read, edit, and correct errors in the newly manufactured DNA that is destined for the daughter cells. As with most things in life, this system is not perfect and errors do get passed these proofreading, editing, and correcting enzymes. Cancer occurs when the DNA sequence within a gene is altered in such a way that the gene can no longer instruct the cell in which it resides to produce the normal version of the protein it encodes. Scientists call such an occurrence a mutation within the gene. Such alterations can occur more frequently when a gene is exposed to ionizing radiation or certain drugs or chemicals or when some, as yet, unexplained internal switch is flipped on or off. These factors can cause the DNA sequence within the gene to break and recombine incorrectly or to mutate.

The Oncogene: A key factor in development of cancer
Once one of these changes has taken place, certain genes (proto-oncogenes) may be transformed into oncogenes (cancer-causing genes), while other genes (cancer-suppressing ones called anti-oncogenes or tumor-suppressors) may be rendered useless by inactivation. A cell containing mutated genes that result in the loss of its growth control is referred to as a transformed cell. Those who have peered into such a cell report that it contains a veritable gallery of cellular horrors like inactivated genes, extra or missing chromosomes, and a host of other genetic abnormalities that cause cancer.

Say, for example the alteration of the DNA is like the ignition of an unoccupied car being turned on. The transformation of a normal gene into an oncogene is like moving the gear from P (Park) to D (Drive) while the handbrake is still on. Inactivation of anti-oncogenes is akin to the release of the handbrake and the car is now free to wander onto a highway and cause mayhem. That is the ultimate fate of a transformed cell. It is important to note that it normally takes multiple mutations before cancer occurs.

Mutations of our DNA are occurring constantly due to environmental insult. However, a single uncorrected mutation event will not guarantee cancer, rather multiple mutations are required. This is why, for instance, cancer occurs only after years of exposure to a carcinogen (smoking, sun, asbestos, etc). If a gene has become an oncogene, the cell in which it is located may begin to produce unusually large amounts of one of its normal proteins or to manufacture an altered form of that protein. If an anti-oncogene has been rendered inactive, the cell containing it can no longer produce a normal protein whose function is to suppress cancer. On some rare occasions, a normal cell becomes cancerous when a particular virus enters the cell and introduces an oncogene into the genome of the host cell. Once any of these deviations in normal protein production and/or function has occurred, the size, shape, surface characteristics, or morphology and behavior of the cell becomes altered. Thus, it becomes a cancer cell that is distinguishable from a normal cell.

### 1.2 The progress of cancer within the body

Every cancer starts with a single cell that has been unleashed from the growth restraints placed on all normal cells. Because the changes that took place within the cancer cell were directed by the cell’s DNA (the molecular basis of heredity), they are passed on to each of the daughter cells arising from the original cancer cell. As these cells continue to divide, collections of abnormal cells accumulate. Except in the case
of leukemia, these cells form a mass or tumor.

The cells of the tumor then push outward from their boundaries, infiltrating surrounding normal tissues. Small clumps of cells may then dislodge from the tumor (primary site) and migrate to distant (secondary) sites, often by piggybacking on the circulatory system of the blood or lymph. After traveling to a new organ, the cancer cells burrow out of the blood or lymph vessels and invade the surrounding tissues, where they continue to multiply and form secondary tumors. This process of spreading to a distant site is called metastasis. Eventually, either local invasion or metastasis disrupts the body’s normal function and often leads to death.

In the 1980s, the American government declared war on cancer. That war is still raging and the latest report from the frontline is a mixed bag of good and bad news. According to the latest figures from the American Cancer Society, more than half a million Americans will die at the hands of this scourge in this year alone. This makes cancer second only to heart disease as the leading cause of death among Americans. The cost of cancer to the American economy was estimated at almost $200 billion for the year 2000 alone.

According to the latest projections, one out of every four Americans alive today will eventually die of cancer in the absence of major breakthroughs in prevention and control. In terms of mortality rate, lung cancer is by far the most frequent killer among all Americans, followed distantly by cancer of the colon and rectum, the breast, and the prostate. Even when men and women are considered separately, lung cancer is still the biggest killer, although the mortality rate is much higher for men than for women. After lung cancer, prostate and colorectal cancers kill men the most often. Among women, breast cancer is by far the most prevalent form, followed by colorectal and uterine cancers. The next most common killer for men is cancer of the pancreas and for women is ovarian cancer.

A recent and disturbing development is the increasing evidence that suggests that obesity increases the likelihood of colon and prostate cancers in men and breast, ovarian, and gall-bladder cancers in women. With an estimated 97 million American adults classified as obese, this new link does not bode well for the battle against cancer.

1.3 Cancer incidence and mortality rates in the United States

The good news is that the rate of new cancer cases and deaths for all cancers com-
bined, as well as for most of the top 10 cancers in the United States has been declining. The report shows that the incidence rate—the number of new cancer cases per 100,000 persons per year—for all cancers combined declined by an average of 0.8 percent per year between 1990 and 1997. The greatest decline in cancer incidence rates has been among men, who overall have higher rates of cancer than women.
The reduction in deaths from cancer has been attributed to better screening and advances in treatment. These uplifting findings reflect the considerable progress that Americans have made against cancer. This decline in cancer deaths is all the more remarkable if we consider the fact that the size of the American population has been increasing while deaths from cancer have been declining.

1.4 The connection between cancer and the immune system

In 1909, a scientist by the name of Paul Ehrlich proposed that the incidence of cancer would be much greater were it not for the vigilance of our immune defense system in identifying and eliminating nascent tumor cells. This suggestion gave birth to the generally accepted concept that the immune system plays a vital role in the identification and elimination of transformed cells. About 50 years later, two scientists, Lewis Thomas and Frank MacFarlane Burnet, took Paul Ehrlich’s original idea a step further and proposed that a special type of immune cell called a T cell was the pivotal sentinel in the immune system’s response against cancer. This elaboration led to the coinage of the term “immune surveillance or immunosurveillance” to describe the concept whereby the immune system is on perpetual alert against transformed cells.

As dictated by the scientific method, theories must in the course of time either withstand rigorous experimental testing, crumble and be discarded or be improved upon. This basic requirement brought the theory of immunosurveillance under severe attack and great controversy when scientists like Osías Stutman showed in the 1970s that mice supposedly lacking an intact immune system (so-called nude mice) did not become more susceptible to tumor growth as predicted by the theory.

Thus, the theory of immunosurveillance remained controversial until an important scientific article entitled “IFN-gamma and lymphocytes prevent primary tumor development and shape tumor immunogenicity” was published in the journal Nature on April 26, 2001. This breakthrough article was authored by Robert D. Schreiber, Ph.D., and his colleagues at Washington University School of Medicine, St. Louis, MO, in collaboration with Lloyd J. Old, M.D., of the Ludwig Institute for Cancer Research and Memorial Sloan-Kettering Cancer Center, New York, NY. The experimental evidence presented in their paper unambiguously showed that the immune system can and often does prevent tumors from developing, and thus plays a strong protective role against cancer. These researchers also uncovered important new insights regarding the immune system and tumor development that they dubbed “immunoediting.”

Utilizing genetically engineered mice that lacked a functional immune system, the
authors showed that lymphocytes and the immune stimulator, IFN-gamma, cooperate to inhibit the development of both spontaneous and carcinogen-induced tumors. Unfortunately, this natural body defense is imperfect, and some tumor cells escape identification and go on to cause cancer. These renegade tumors are less immunogenic, having undergone a process of immunoselection triggered by the actions of the immune system. Conceptually, in much the same way as bacteria can become resistant to antibiotic treatment and lead to more potent and harmful strains, so too can the body’s own tumor defense system lead to tumors that escape elimination.

Importantly, these researchers went on to demonstrate that there are ways to overcome the “camouflage” of such renegade tumors by increasing their antigen expression and making them visible to the immune system. This suggests that even tumors that have escaped recognition can be turned into targets for an immune response. Further experimentation is underway to test how these results can be used to develop cancer immunotherapies. The Cancer Research Institute (CRI) sponsored this research with a grant to Dr. Schreiber’s lab and provided further funding through its pre- and post-doctoral training programs to two graduate students, one of whom was the first author of the paper, and a postdoctoral fellow.

Despite the tremendous scientific progress (such as that described above) that has been made over the years, a complete and precise understanding of the immune system’s response to cancer remains elusive. The continued exploration of these questions is the province of cancer immunology; the scientific discipline to which CRI is dedicated to supporting and nourishing.
2.0 THE HUMAN IMMUNE DEFENSE SYSTEM

The environment in which we live contains a wide range of organisms called pathogens that view the human body as a rather juicy target to invade and live off till death do us apart. The job of the mammalian immune system is to defend the body against these pathogens, which include bacteria, viruses, fungi and parasitic worms. This task is so complex that mammals have evolved a very sophisticated network of defense units that recognize and attack such a diverse array of potential enemies.

The immune system is characterized by three universal features, which are namely specificity, diversity, and memory. We say the immune system is specific because it only reacts against certain specific molecular targets called antigens. It is very important that the immune system is able to select what it reacts to because this prevents it from attacking components of our own body: a phenomenon called autoimmunity. The immune system is described as diverse because it has the remarkable ability to react specifically to any molecule in the universe. At the end of a successful immune reaction, the immune system assigns a unique group of immune cells, called memory cells, the task of remembering the particular enemy encountered (virus, bacterium, tumor cell, etc). In this way, there will be immune combat units ready to attack and kill off the fresh invaders very swiftly if they are encountered in the future.

The defense system of the human body consists of surface barriers such as skin, internal barriers such as mucus, and special groups of cells, chemicals, and hormones that act in concert to keep the body free of pathogenic invaders. In broad terms, the immune system is divided into two branches called the innate and adaptive defense systems. In evolutionary terms, the innate branch predates the adaptive branch by about 500 million years.

In the next three sections, the reader will be introduced to both the innate and adaptive immune systems. These sections will also examine how the two systems communicate effectively to bring about a coordinated defense of their host organisms.

2.1 The concept of an innate defense network

The innate immune system evolved to protect host organisms about 900 million years ago. It consists of mechanical, chemical, microbiological, and cellular defense
networks. The function of the innate immune defense system is akin to “turning back the barbarians at the gates.” We can view the skin as a huge missile defense shield that prevents the entry of pathogens and foreign substances into the body. In addition, the skin produces acidic substances that make it difficult for bacteria to grow on it. Nevertheless, there is a class of harmless bacteria and fungi that thrive on our skin. These also constitute a defensive mechanism, as they tend to compete with and crowd out pathogenic organisms. Some membranes produce mucus that block the entry of pathogens into the body. Another function of mucus is to trap pathogens that choose the digestive and respiratory routes to invade the body. The hairs in our nose for example, filter out bacteria entering the nasal passage.

Further down the nasal cavity, en route to the respiratory tract are structures called cilia whose job it is to move trapped pathogens away from the respiratory tract. If the cilia fail to do their job properly, coughing and sneezing is induced to expel pathogens from the upper portion of the respiratory tract. Any pathogen that makes it passed all these road blocks into the stomach is assailed by a deadly potion called gastric juice: this is a mixture of concentrated acid and enzymes that chew up the invading pathogens into harmless bits of protein.

Apart from their normal function of lubrication and cleansing, our tears and saliva contain an enzyme called lysozyme that cuts and destroys the bacterial cell wall. Finally, the body produces mild acids into the vagina in order to prevent the growth of pathogens in the female reproductive tract. All these security barriers are part of the innate defense system because they are general protection mechanisms that are designed to keep pathogens out of the body. But as it is often the case in life, security breaches do occur and some pathogens succeed in lodging themselves within the cells and tissues of the body. What happens then?

In the case of the innate defense network, cellular combat units called myeloid cells are deployed to fend off the invading pathogens. The cells that mediate the innate immune response include macrophages, dendritic cells, neutrophils, eosinophils, mast cells, natural killer cells, some B lymphocytes (like B1 B cells and marginal zone B cells) and some T lymphocytes (like TCR-gamma/delta T cells and natural killer T cells). How does recognition occur by the innate immune system? In other words, how do all these cells of the innate defense system tell apart a normal cell such as a red blood cell from an invading bacterial cell such as streptococcus?

The answer to this important question lies in two key evolutionary developments called Pathogen Associated Molecular Patterns (PAMPs) found in all microorganisms
and Pattern Recognition Receptors (PRRs) found in all the cells of the innate defense system. A PAMP is a molecular pattern that is unique to microorganisms. The PRR is like a molecular velcro patch that is capable of recognizing and latching onto each unique PAMP. So, for each PAMP in a pathogen, there exists a corresponding PRR in one or more of the cells within the innate defense network. Examples of PAMPs are LPS (endotoxin), peptidoglycan (cell walls), lipoproteins (bacterial capsules), hypomethylated DNA (such as CpG found in bacteria and other parasites), double-stranded DNA as found in viruses, and a molecule called flagellin that is found in bacterial flagella.

It is estimated that several hundred PRRs exist in the mammalian innate defense system and that they are so vital to the immune defense system that their genes are encoded in germline cells to ensure limited variability in their molecular structures. PRRs are classified as membrane proteins because they are associated with the cell membrane. Examples include mannose binding lectin, pulmonary surfactant protein, C-reactive protein, toll-like receptors (TLRs), C-Type lectin, NOD and MX proteins.

In summary, the immune system recognizes and rapidly responds to microbial pathogens via pattern recognition. A complex example of pattern recognition can be found in our extraordinary ability as human beings to recognize patterns in the environment using cognitive processes to distinguish visual images such as models of cars or species of birds. In the innate immune system, cell surface receptors (like PRRs) that recognize distinct biochemical patterns (like PAMPs) displayed by microbial invaders constitute a receptor-ligand interaction that forms the bedrock of the innate immune system.

2.2 The adaptive defense system

In her infinite wisdom, Mother Nature evolved far more sophisticated and specific responses to pathogenic invasions eons before humans developed complex military systems. The specific immune response involves a repertoire of specialized cells, chemicals, and hormones that work in a highly coordinated fashion to rid the body of these invaders before they get a chance to multiply and cause harm to the body.

The mammalian immune system possesses the ability to recognize every molecule—known and unknown—in the universe! This awe-inspiring feat is even more remarkable when we see that in a healthy human being, the extraordinary recognition capabilities of the immune system does not normally lead to an attack on the body itself. That is why in normal circumstances, my immune cells will tend to guard my
kidney against pathogenic invasions but they will ferociously attack a kidney from you (a donor) that was being transplanted into me unless, of course, you were my identical twin. The only way around this transplantation hurdle is the administration of strong drugs to suppress my immune system. Immunologists refer to the second part of our defense system as the adaptive immune system because it has the potential to modify itself and adapt to any weapon that the enemy (pathogens) throws at it.

The vast majority of immune cells are created in the bone marrow as stem cells. As they mature into specialized cells, they exit from the bone marrow and circulate in the blood. Some of the immune cells are deployed to most of the tissues in the body. In order to maximize the efficiency of this defense unit, the immune cells produce chemicals and hormones that enable them to communicate with each other, for example, to alert another group of immune cells that there is an ongoing invasion in some distant part of the body. The innate and adaptive arms of the immune system work in synergy to defend the body against pathogenic onslaughts. How do they do that?

2.3 Brian pricked his thumb and his immune system jumped into action

Perhaps, we can answer the above question by describing what happens when five-year old Brian pricks his thumb during an apple-picking trip to an orchard. The pricking of Brian’s thumb constitutes a breach of the physical barrier that is part of the innate or natural immune system. In addition, the thorn that pricked Brain’s thumb would have transferred several hundred or so bacterial cells into the wound on Brian’s thumb. The initial response of Brian’s immune system will most certainly involve a type of white blood cell called a macrophage. These cells usually roam the body like sentinels looking for foreign invaders.

The macrophage is able to recognize the invaders as foreign and harmful because the invaders come bristling with PAMPs on their surfaces. Each PAMP can be recognized by the appropriate PRR on the macrophage. In simple terms, it is a bit like recognizing enemy troops by the uniform they wear. In addition, the invading bacterial cells actually produce chemical messengers that can be detected by macrophages: just like invading foreign troops communicating in a coded language on a particular radio frequency that can be picked up and decoded by United States soldiers as foreign. The net result is an aggressive onslaught on the colony of bacterial cells by an army of macrophages that actually eat up the invading cells. A few minutes after sustaining that wound, a casual observer would have noticed that the wound on Brian’s thumb has become red and swollen: a sure indication that an immune response is
in progress! In addition to killing off some of the invading bacteria, the macrophages alert other cells of the immune system that there is an invasion in progress. After the macrophage has swallowed the bacteria, it chews it into tiny bits of protein and then deposits the pieces into the groove of a protein called major histocompatibility complex (MHC). Imagine the MHC molecule as the bun of a hotdog; the antigen to be displayed is placed in the groove where the sausage sits. There are two types of MHC proteins subdivided into class I and class II. Macrophages call on MHC class II molecules to shuttle the fragments of antigens to the surface of the macrophage so that they can be presented to the immune system. In this role, immunologists refer to the macrophage as a professional antigen-presenting cell (APC).

2.4 The humoral immune response

B-cells (so-called because they mature in the bone marrow) are white blood cells that work chiefly by producing soluble substances known as antibodies. Each B cell is programmed to make one specific antibody. When a B cell encounters its specific or eliciting antigen (along with various accessory cells), it differentiates into a plasma cell. The latter is essentially a factory for producing that one specific antibody. Antibodies play a crucial role in a cascade of events called the humoral immune response that ultimately leads to the destruction of some of the invading bacteria. Like all immune responses, the humoral immune response can be subdivided into activation and effector phases.

The activation phase begins when invading bacteria are phagocitized by an antigen-presenting cell (APC), such as a dendritic cell or macrophage. The bacterium is digested and its antigens processed and presented in combination with the MHC Class II complex on the surface of the APC. The antigen-MHC complex is recognized by a type of immune cell called a CD4+ or helper T cell (Th). The helper T cell begins the attack by docking its antigen receptor to the displayed antigen. The docking process requires the presence of co-stimulatory molecules like B7 and CD28.

After successful docking, the helper T cell releases a class of chemical messenger called cytokines. This achieves the following: it causes the helper T cells to multiply, and stimulates both the APC and the helper T cell to exchange chemical messages between themselves and with other cells of the immune system. The helper T cell also releases a cytokine called interleukin-2 (IL-2): this cytokine has a panoply of immune functions, one of which is the proliferation of lymphocytes following activation by a specific antigen. The APC releases cytokines called interleukin-1 (IL-1) and tumor necrosis factor (TNF). The latter steps up the production of IL-1 and performs
many of the same functions as IL-1, including the induction of fever in Brian so that his body can assist in fighting off the bacterial infection more effectively. The proliferating helper T cells release substances that signal another type of lymphocyte, the B cell (that also specifically recognizes the antigen), to begin multiplying and differentiating into antibody-producing cells. This initiates the effector phase of the humoral immune response.

The antibodies released by the B cells bind in a lock-and-key fashion to antigens on the surfaces of bacterial invaders that survived the initial attack by macrophages and bacterial products. The binding of the antibody to the bacterial antigens achieves two things: first, it makes it easier for “killer” cells to attack and destroy the invading bacteria by both phagocytosis and the release of other factors that can directly lyse the bacteria. Second, it activates another immune military unit called complement—a group of proteins that act like the special forces of the immune defense system because their duty is to begin the lethal process of punching holes in the cell walls of the residual invading bacterial army.

*The humoral immune response*

---

2.5 The cellular immune response

As the fruit picking was going on, Brian heard someone shout, “Bless you!” after a fellow fruit-picker let out a loud sneeze. Unbeknownst to poor little Brian, some of
the air he was breathing in the orchard was now laden with particles of the influenza virus. While the humoral immune response is under way, some of the influenza viral particles will have been consumed by phagocytes and neutrophils while others would have began infecting other cells such as Brian’s epithelial cells. So, how does the immune system deal with these infected cells? By a battle plan called the cellular immune response.

*The cellular immune response*

Like the humoral immune response, this is also divided into activation and effector phases. The activation phase begins when an antigen-presenting cell (APC) of the host organism encounters and attacks an invading virus. Meanwhile, other viruses look for nearby epithelial cells to infect. A lysosome containing digestive enzymes combines with the phagosome to process the antigens. The processed antigens combine with MHC class II proteins and are presented on the surface of the APC. The virus also infects Brian’s epithelial cells. Within the infected epithelial cells the virus is processed, attached to an MHC class I protein and is presented on the cell surface. A helper T cell (CD4+) recognizes the displayed antigen on the APC and binds to the MHC class II protein-antigen complex. The activated helper T cell releases chemical messengers such as the cytokine IL-2 and gamma interferon (IFN-g).

The effector phase begins when activated cytotoxic T cells (CD8+) which were stimulated to proliferate by the cytokine IL-2, recognize the MHC class I protein-antigen
complex on the infected epithelial cells. Cytokines also attract other killer T cells to the site of infection. The activated cytotoxic T cell binds to the MHC class I protein-antigen complex on the surface of the infected epithelial cell. The binding causes the cytotoxic T cell to release a potent chemical called perforin. Perforin perforates the cell membrane of the infected cells causing the cells to lyse (burst) and die. As the viral infection is brought under control, regulatory T cells turn off the activated cytotoxic T cells. Memory T cells remain behind to respond quickly if the same virus attacks again.

Finally, as the infection in Brian’s thumb is brought under control, yet another type of T cell, the regulatory T cell, instructs the activated combat units consisting of B cells, helper T cells, and killer T cells to switch from battle mode to stand-by mode. Most of these immune cells will die, but a few will live to fight another day. These cells, called memory cells, will be able to respond more quickly the next time Brian is unfortunate enough to be invaded by the same strain of bacteria. The above account highlights the overwhelming reliance that our body places on the T cell to fight off microbial infections. But accidents do happen (as it is in life in general) within the immune system and sometimes these cells mistake part of our body (self) for a microbe (non-self) and the resulting “friendly fire” can lead to autoimmune diseases such as multiple sclerosis and juvenile diabetes. To avoid this kind of “collateral damage” nascent T cells are subjected to a strict training program in the thymus. As part of their education, the developing T cells are exposed to as many self-proteins as possible and any T cell that displays any reactivity is eliminated. This rigorous training regime ensures that the remaining T cells will react only to non-self molecules.

Although the foregoing description of the immune response applies mainly to viruses and bacteria, it is important to note that the immune system reacts in a similar manner when it encounters cancer cells, which it also recognizes as foreign or “non-self” and therefore, must destroy. Scientists have observed in the laboratory that the cells and other components of the immune system are capable of destroying malignant tumor cells. They have found that certain antibodies that recognize tumor cells help the macrophages and the natural killer cells to accomplish their mission. Over the years, further study of the immune system has demonstrated that the body defends itself against cancer in much the same way that it seeks to eliminate other intruders such as bacteria and viruses. Further study of the immune system is expected to reveal ways to bolster it, allowing the body to become a more active partner in the fight against cancer.
3.0 THE ORIGINS OF IMMUNOTHERAPY

As it applies to cancer, immunotherapy might be considered a revolutionary form of medicine but its roots actually go back as far as 1778, when Edward Jenner, an English physician, administered the first vaccine, which was targeted against smallpox. Jenner observed that milkmaids who contracted cowpox, a relatively mild disease, seemed protected from the deadly smallpox infection. To test this hypothesis, he injected some material from a pustule on the body of a milkmaid infected with cowpox into the arm of a small boy. After the boy had recovered from the cowpox infection, Jenner inoculated him with smallpox. As Jenner had expected, the boy never developed the disease. Jenner named his technique “vaccination,” a term derived from the Latin word “vacca” for cow. Even without a scientific understanding of why his method worked, Edward Jenner had discovered an effective way to prevent people from developing a serious disease.

It was not until the late 19th century that medical science disclosed the reason: Jenner had created a condition of acquired immunity in the boy. When the child contracted the less serious disease of cowpox, his immune system had mounted an attack against the invading virus. Later, when the boy was inoculated with the smallpox virus he did not contract the disease because the memory lymphocyte (T and B cells) of his immune system “remembered” the cowpox infection and were able to stimulate the immediate production of the specific antibodies needed to kill the related but more deadly smallpox virus. This pioneering immunological work eventually gave rise to a number of other vaccines against such diseases as rabies, diphtheria, yellow fever, polio, mumps, hepatitis B, measles, rubella, influenza, whooping cough, and tetanus. These days, this type of immunotherapy is in widespread use to protect against microbial infection.

The connection between cancer and the immune system was first uncovered nearly 100 years ago; long before an in-depth knowledge of the intricate workings of the immune system existed. In the early 1890s, Dr. William B. Coley, a New York physician, became intrigued by the dramatic disappearance of malignant tumors that he observed in cancer patients who had contracted acute streptococcal infections. Suspecting that the onset of bacterial infection was in some way responsible for the regression of the tumor, he decided to try an experiment in which he injected live streptococci into a patient with inoperable cancer to see whether the patient’s tumor
would regress. After he had tried administering three different bacterial cultures to the patient, he finally injected a fourth that resulted in the complete disappearance of the tumor.

Dr. Coley continued to pursue his approach and ultimately developed a mixture of killed bacteria that became known as Coley’s mixed bacterial toxin. He and other physicians treated over 1,000 cancer patients with this substance, with varied success. His results were unpredictable however, and neither he nor the medical community at large could explain precisely why his mixture worked in some patients. This was due to the fact that the science of immunology was in its rudimentary stages at that time. Thus, his results were disregarded and virtually forgotten for years.

Scientific interest in Coley’s work has been accumulating since his daughter, Helen Coley Nauts, started compiling and disseminating information on his remarkable observations. Gradually, scientists began to understand why Dr. Coley’s preparation worked—the bacterial products of which it was composed had acted as immune potentiators. In other words, they had stimulated certain immune cells to kill the cancer cells directly or through cancer-killing factors. With the founding of the Cancer Research Institute by Mrs. Nauts in 1953, resources were provided to pursue research into the link between cancer and the immune system. Today, cancer immunology is a rapidly advancing field and Dr. Coley has come to be regarded as the “father of cancer immunotherapy.”
4.0 ADVANCES IN HUMAN TUMOR IMMUNOTHERAPY

Tumor immunotherapy is an anticancer approach in which the patient’s immune system is either prodded or cajoled to fight tumors. Over time, our understanding of the immune system and tumor immunology has increased and this has enabled scientists to develop the ability to apply specific immunotherapies designed to enhance the immune response of a particular patient against unique targets. The prevailing techniques of tumor immunotherapy can be divided into two broad groups called non-specific and antigen-specific therapies. The latter can be attained by either adoptive transfer or vaccination.

Adoptive transfer means the physician directly transfers into the patient, the actual components of the immune system that are already capable of producing a specific immune response. Vaccination on the other hand involves the administration of a particular antigen to induce a specific immune response. Nonspecific immunotherapy refers to therapies that can stimulate the immune system by using a substance that activates or enhances immune cell function regardless of their antigen specificity. In the early days of immunotherapy, many non-specific immunostimulants were tested as antitumor reagents in their own right, but today their use in this way has declined. The majority of these substances are now recognized for the supporting roles they play such as enhancing cellular communication between immune cells and therefore are being tested for use in combination with antigen-specific immune stimulation. In the next section, we will consider three examples of nonspecific immunotherapeutic reagents.

4.1 Nonspecific immunotherapy 1: Bacille Calmette-Guerin (BCG) therapy

In 1975, the Cancer Research Institute presented its first Award for Distinguished Research in Immunology to a group of 16 scientists it called the “Founders of Cancer Immunology.” The award, which was renamed the William B. Coley Award for Distinguished Research in Basic and Tumor Immunology in 1993, is now presented annually to scientists who have made outstanding achievements in the fields of basic and cancer immunology. Their work has deepened our understanding of the response of the immune system to disease, including cancer, and holds forth the promise of further progress in the development of novel and effective immunotherapies. In 1993, one of the recipients of this award was Dr. Alvaro Morales.
In the early 1970s, it was found that the administration of weakened forms of a mycobacterial strain called Bacille Calmette-Guerin (BCG) had anticancer effects. During this period, the CRI awarded one of its grants to Dr. Alvaro Morales for his BCG research. His work led to the publication of a seminal paper in the Journal of Urology on the use of intravesical BCG for the prophylaxis of transitional cell carcinoma of the bladder.

The work of Dr. Morales paved the way for the use of this agent as standard therapy worldwide. Prior to its use as an anticancer agent, the live and potentially infectious form of BCG was used as an effective vaccine against tuberculosis. After extensive clinical testing, the efficacy of BCG against a few cancers such as metastatic melanoma and certain types of early bladder cancer were established: so much so, that today BCG is the treatment of choice for early forms of bladder cancer.

Like other nonspecific immunotherapeutic agents, scientists do not know the exact mechanism by which BCG generates anticancer immune responses in certain patients but they speculate that BCG probably activates both macrophages and lymphocytes. With modern techniques, scientists have been able to separate BCG into separate components, which on their own have been shown to have anticancer effects. Despite this, the most useful application of BCG (either used whole or fractionated) is as an adjuvant or supplement to other forms of therapy. Now, let us turn our attention to the cytokines.

4.2 Nonspecific immunotherapy 2: Cytokines

Laboratory studies have shown that the use of cytokines in immunotherapy can lead to the destruction of tumors by one of two general mechanisms: first, a direct antitumor effect or second, an indirect enhancement of the antitumor immune response. In the first, cytokines, such as tumor necrosis factor (TNF) alpha, interferon (IFN) alpha, IFN-beta, interleukin-4 (IL-4), and IL-6, interact directly with tumor cells, inducing the latter to either commit suicide or stop further growth. Although these cytokines are effective when given as single agents, the administration of a cytokine cocktail can be even more potent as an anticancer agent due to the synergistic effect accruing from all the different cytokines. A note of caution: some cytokines can have dangerous side effects. For instance, TNF-alpha and IL-6 are able to suppress the growth of some tumors while actually promoting the growth of others so the immunotherapeutic use of cytokines demands great care.
The cytokines that fight tumors via the indirect mechanism do so by stimulating immune cells to fight tumors through a variety of different pathways. For example, a cytokine such as IL-2 promotes T-cell and natural killer (NK) cell growth. Other cytokines such as the interferons and granulocyte–macrophage colony-stimulating factor (GM-CSF) can act on professional antigen-presenting cells (APCs) and increase the production of important immune molecules such as MHC molecules and immune co-stimulators such as B7 that have important roles in facilitating the activation of lymphocytes. Although more clinical trials are necessary to determine the right dosage and to predict immunological responses in the more complex environment within humans, currently, a number of cytokines have proven effective in cancer therapy.

For example, in patients with metastatic melanoma or renal cell carcinoma, the administration of intravenous IL-2 can induce objective tumor regression in 17 percent and 20 percent of cases, respectively. Also, clinical studies have shown that combining IL-2 with other cytokines, such as IFN-alpha, can lead to a synergistic response. IL-2 has been approved by the US Food and Drug Administration (FDA) for the treatment of both of these cancers. IFN-alpha is also FDA approved for the treatment of malignant melanoma, chronic myelogenous leukemia (CML), hairy cell leukemia, and Kaposi’s sarcoma. The FDA has approved both GM-CSF and G-CSF because they have been shown to promote the revival of the immune system (following chemo/radiation therapies) and improve patient survival. Recently, advances in biotechnology have allowed scientists to clone the genes of cytokines leading to the large-scale production and administration of cytokines to cancer patients. Due to this advance in biotechnology, a large number of cytokines, including IL-1, IL-4, IL-6, IL-7, IL-11, IL-12, macrophage inflammatory protein (MIP)-1-alpha, IFN-beta, and IFN-gamma, are currently being tested in humans for anticancer therapy. For the next topic on nonspecific tumor immunotherapy, let us turn our attention to cell therapy.

4.3 Nonspecific immunotherapy 3: Cell therapy

The transfer of live, whole cells into patients can also be used to achieve non-specific immunotherapy against cancer. For example, in patients with metastatic melanoma, human peripheral blood mononuclear cells (PBMCs) can be isolated and fed with IL-2 to generate a class of cells called lymphokine-activated killer (LAK) cells. When a combination of LAK cells and IL-2 are given to patients with either advanced metastatic melanoma or renal cell carcinoma, complete tumor regression can be achieved in about 10 percent of cases. Many of the above therapies now provide the basis for specific immunotherapeutical approaches that are currently undergoing clinical trials.
5.0 SPECIFIC IMMUNOTHERAPY I: ADOPTIVE TRANSFER

As mentioned earlier, the term adoptive transfer applies to all the therapies that consist of the transfer of components of the immune system that are already capable of mounting a specific immune response. This takes us straight into the effector phase of the immune response. Examples of adoptive transfer include both the transfer of antibodies and also, specific types of cells that are capable of mediating antigen-specific tumor regression such as LAK and T cells. Now, let us take a closer look at antibodies therapy and how they can act as anticancer agents.

5.1 Antibody therapy

Monoclonal antibodies (MAbs) constitute highly pure populations of immune system proteins that attack specific molecular targets. Their role in anticancer therapy can be likened to that of heat seeking or guided missiles. In this sense, monoclonal antibodies tipped with poisons or radioactive isotopes can home in on tumor cells and deliver their deadly payloads; thus, selectively wiping out cancer cells. By the late 1970s, scientists had shown that monoclonal antibodies could be targeted to tumor cells. Since then, numerous animal and human studies have shown that antibody administration can mediate tumor regression in some patients.

Although scientists are not quite sure about the exact manner by which antibody therapy works, they think the antitumor effect may be achieved via two different mechanisms: first, the activation of the complement system and second, by the use of antibodies to mark these tumor cells for destruction. These two mechanisms need not be mutually exclusive. It has been speculated that the binding of antibodies to the target tumor cells may compel the latter to either stop growing (antiproliferative effect) or commit suicide (apoptosis). The interest in antibody therapy has been such that by the 1970s, a revolutionary technique called hybridoma technology had been developed to mass produce antibodies (further discussed in chapter 8, section 8.2). Since this breakthrough, there has been considerable progress in our ability to exploit antibodies therapeutically against cancer.

To date, two antibodies have been approved by the FDA for use in cancer treatment. Rituxan is specific for an antigen called CD20, which can be found on the surface of both normal and malignant B-lymphocytes. In a phase III clinical study, 50 percent of patients with non-Hodgkin’s lymphoma (NHL) responded to Rituxan. Herceptin is
the other FDA-approved antibody and it is specific for the human epidermal growth factor receptor 2 (HER2) protein. HER2 is over-expressed in 25-30 percent of primary breast cancers and it has been shown to be effective against this disease in phase III clinical studies.

Despite these promising advances, antibody therapy has certain disadvantages that have limited its use as a tool against cancer. The main limitation is that since bulky tumors tend to be inaccessible to antibodies, the use of this technique has been limited to tumors that are relatively small in size. On the plus side, antibodies are almost never toxic so this bodes well for their continuous use in the fight against cancer.

5.2 Adoptive transfer of T cells

After decades of experiments conducted on mice and other animals, scientists have shown that they can isolate antigen-specific T cells from a cancer patient, expand them to large numbers in a test-tube, and re-infuse them back into the patient to kill off the remaining tumor cells. When this technique was used in a clinical study to treat patients with metastic melanoma, it was observed that the tumor regressed in about 34 percent of the patients. The drawback of this therapy is that the killer instinct of the transferred killer or cytotoxic T cells (CTLs) is rather short-lived after infusion into patients. This is because the patient cannot provide all the accessory immune molecules that the killer T cells need to be maintained and to finish the job. If the original immune response to the tumor cells had been effective, immune cells responsible for producing these supplementary molecules would have been called into action and this problem would not exist.

In the immune system, these accessory molecules are sometimes called adjuvants or co-stimulatory molecules. Examples include cytokines like IL-2 and co-stimulators like CD28. These days scientists are using genetic-engineering techniques to boost the production of co-stimulators for infused T cells within cancer patients. As we saw in the earlier chapters of this booklet, IL-2 is a vital molecule that ensures the growth and multiplication of antigen-specific killer T cells. The CD4-helper T cell is responsible for the production of IL-2. Some researchers have demonstrated that they could ensure the continuous supply of IL-2 to infused CD8 killer T cells by transferring the gene for CD28 into CD4 helper T cells. This enabled the CD8 killer T cells to grow, multiply and seek out tumor cells for eradication. The main advantage of this modern approach is that it is not limited to a particular patient or type of cancer. In the future, we can hope that the broad applicability of this technique will produce T cells at a lower cost in a shorter time for widespread use in adoptive T-cell therapy.
A great deal of basic and clinical research is being conducted on how to use vaccination to combat cancer. Instead of transferring T cells that have been stimulated in vitro (outside the patient), as in the case with adoptive T-cell transfer therapy, vaccination relies on the injection of known cancer antigens into the patient with the hope of provoking an immune response against that particular cancer. In this case, the T cells are stimulated in vivo (within the patient).
6.0 SPECIFIC IMMUNOTHERAPY II: VACCINATION

The aim of cancer vaccination is to place an antigen within the body of a cancer patient so that the immune system can be provoked to unleash the wrath of the killer T cells on the patient’s tumor cells. Armed with the knowledge of how T cells interact with antigens at the atomic level, scientists are able to design antigens that can selectively activate specific T cells to eradicate cancer cells. In general, the success of vaccine strategies depends on the mode of antigen delivery, the choice of adjuvant, and the particular antigen being used. For the rest of this section, let us examine a few examples of anticancer vaccination therapies including tumor-based, virus-based, peptide-based, and professional APC-based vaccinations.

6.1 Tumor-based vaccines

A relatively ancient but still useful form of anticancer vaccination strategy is to extract whole tumor cells, mash them up, and inject the crude extract back into the patient. These days, this method has been refined somewhat: whole tumor cells are extracted from the patient, blasted with radiation to weaken them substantially before they are transferred into the patient in the presence of an adjuvant such as BCG. Although the exact mechanism of action remains unknown, scientists think that the BCG supplement creates a suitable environment within the patient so that the antigens associated with the tumor cells can be properly presented to the immune system for the subsequent generation of T cells that can seek and destroy tumor cells.

In a study conducted in 1999, patients with stage II cancer of the colon who had undergone surgery to remove part of the tumor were given a tumor-BCG mixture to fight against the reappearance of the tumor and it was observed that this approach reduced the risk of recurrence by about 61 percent. Other scientists have shown via studies in mice that the use of cytokines, such as GM-CSF, or co-stimulatory molecules, such as B7, can dramatically improve the efficacy of tumor-based vaccinations. Adjuvants, cytokines and co-stimulators are all believed to improve this anticancer therapy by creating an optimal in vivo environment for the presentation of tumor-associated antigens to the immune system.

The main advantage of this method is that scientists do not have to isolate a specific antigen: the use of a crude cancer cell extract is good enough. A disadvantage is that it is difficult to measure specific immune responses without knowing the stimulating
antigen within the crude mixture. This limitation, therefore, makes it more difficult for researchers to learn from these tests if the vaccine fails to generate a tumor regression. Also, isolating and persuading cancer cells to grow in a test-tube is a tedious and costly job whose application is limited to the single patient from which the tumor cells were originally isolated. As we shall see in section 6.3, modern techniques have rendered the use of crude cell extracts almost obsolete in the fight against cancer.

6.2 Virus-based vaccines

In 1910, physicians observed that the tumor cells of a woman suffering from a cancer of the cervix went into remission while she was receiving a rabies vaccine. This has become one of those serendipitous discoveries in medical science that provided the impetus for a form of anticancer strategy called in vivo viral oncosylate vaccination that involves the direct injection of viruses into tumor sites. Scientists rationalized the success of this technique as follows: the viral proteins are foreign to the body and as such, they are highly immunogenic whereas the tumor proteins that arose from the body’s own cells are weakly immunogenic. The association of the two types of protein makes the tumor proteins immunogenic enough for them to elicit a tumor-specific immune response.

Although earlier clinical trials of this technique were encouraging, the results were deemed too inconsistent. This led to a change in the strategy whereby the tumor cells were infected with the virus in vitro. After the virus had successfully infected and broken up the tumor cells, scientists isolate parts of the mixture that lack nuclei and use that to vaccinate cancer patients. This approach has led to results that are more consistent and its efficacy has been demonstrated in patients suffering from melanoma, colon and ovarian cancers.

These days, scientists are attempting to further improve this technique with the help of genetic engineering techniques. This involves the isolation of the human genes that code for tumor antigens and then genetically engineering them into viral vectors. The latter is a gene courier that delivers the gene to a particular address within the body. Infecting a patient with such an engineered virus will hopefully, lead to an immune response against both the virus and the tumor antigen. In one example of this approach, scientists equipped the vaccinia virus with the gene of a human cancer antigen called carcinoembryonic antigen (CEA). After injecting this into patients with CEA-expressing tumors, the scientists observed that an effective immune response was generated and this led to the production of CEA-specific T cells that fought off
the CEA-expressing tumors.

6.3 Protein and peptide-based vaccines

If we cast our minds back to how the immune system works (chapter 2), we will recall that during the activator phase of both the humoral and cellular responses, antigens need to be processed into peptides before they are presented to the immune system as MHC-peptide complexes. Recently, one of the most remarkable advances in immunology has been the understanding at the atomic level of how T cells actually recognize and dock onto processed peptides sitting in the groove of MHC molecules. Armed with this knowledge, scientists think that for a particular tumor antigen, they can home in on just the specific region called the epitope that is presented via the MHC molecule. By supplementing this epitope with the appropriate co-stimulatory molecule(s), they can activate tumor-specific T cells into action. This technique is essentially peptide-based vaccination.

A cancer antigen can be defined as an antigen that is selectively or abundantly expressed in cancer cells. Human cancer antigens that are recognized by our T cells include cancer testis (CT) antigens such as MAGE-3, BAGE, GAGE, and NY-ESO-1; melanocyte differentiation antigens such as Melan-A/MART-1, tyrosinase, and gp100; protein products of point mutant genes like beta-catenin, MUM-1, CDK4, p53, and ras; overexpressed “self” antigens such as Her-2/neu, p53, and MUC-1; and viral antigens such as the Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), and the human papilloma virus (HPV). Researchers have amassed a wealth of evidence that shows that some human tumors tend to overproduce certain proteins in either their normal or mutated forms.

Of all the examples mentioned above, NY-ESO-1 represents one of the most potent naturally occurring cancer antigens. With the exception of the testis, this protein is completely absent from normal tissues hence its categorization as a CT antigen. NY-ESO-1 is found in about 30 percent of breast, prostate and ovarian cancers as well as melanoma. These desirable features—that is, rarity in normal tissues, high immunogenecity, and significant presence in a relatively broad range of cancers—have made NY-ESO-1 a highly attractive target for specific immunotherapy in certain cancer patients. The identification of antigens such as NY-ESO-1 that are selectively or abundantly expressed in cancer cells has set the basis for the design of a large number of cancer vaccine trials around the world. The CRI and the Ludwig Institute for Cancer Research have created a new partnership with the objective of developing a cancer vaccine collaborative (CVC) program involving a number of internationally
recognized medical centers. Specific information on this program and participating institutions can be obtained from the CRI website.

The main advantage of a peptide-based vaccine is that it provides a method for monitoring a specific immune response for a particular antigen. This affords researchers the opportunity to obtain important information for evaluating the efficacy of other tumor antigens. Other advantages are as follows: first, it bypasses the need for antigen-presenting cells to process a whole cell before presenting the antigen to the immune system. Second, administration of a peptide antigen does not carry the risk of introducing dangerous substances into the patient—unlike other vaccines that rely on weakened pathogens or crushed tumor cells. The potential of peptide vaccination as an anticancer therapy will be brighter when current loose ends (such as peptide dose, adjuvant, cytokine combination, method of delivery, optimized peptide sequences and maybe the synergistic use of MHC class I and Class II peptide combinations) are tied up by scientists.

6.4 Antigen-enhanced, APC-based vaccines

As mentioned in chapter 2, section 2.5 of this book, T cells only recognize antigens that have been properly processed and presented on the surface of the APC bound to a protein called MHC. Recently, scientists have demonstrated that they can exploit the unique skills of APCs for vaccination against cancer. This can be achieved by isolating a tumor antigen from a cancer patient and then loading or pulsing APCs (also isolated from the patient) with the tumor antigen ex vivo (outside the patient). The transfer of these pulsed APCs into the cancer patient elicits a significant tumor-specific immune response that attacks the tumor cells. In essence, the tumor antigen is hitching a ride into the patient’s immune system from the APCs. Currently, there are three different methods for letting tumor antigens piggyback on APCs into the human immune system. First, growing APCs in the presence of a tumor-associated protein; second, using genetic engineering techniques to introduce the gene that codes for a tumor-associated protein into APCs, and third, pulsing APCs with fragments (peptides) isolated from a tumor antigen.

The main advantage of APC-based vaccination is that DCs produce all the molecules required for eliciting an immune response, unlike other forms of cancer immunotherapy where adjuvants and co-stimulatory molecules are required to boost the ensuing immune response. The potency of DCs as vehicles for delivering antigen and achieving a tumor-specific immune response has been demonstrated in a number of clinical trials around the world. For example, in a recent clinical study, involving 16 patients,
DCs pulsed with tumor-associated peptide or lysate were shown to be effective in treating metastatic melanoma among five of the patients. However, a recent study has reminded us of the need for caution in utilizing DCs for clinical trials.

A group of Rockefeller University scientists, including CRI-funded researchers Drs. Madhav Dhodapkar, Ralph Steinman, and Nina Bhardwaj has undertaken a clinical study testing the capacity of mature versus immature dendritic cells (DCs) to stimulate immune responses. Immature and mature dendritic cells simply refers to a state of development just like a teenager (with certain unique traits) on the way to becoming an adult with a different set of unique characteristics. As described in a report published in the January 14, 2001, issue of the *Journal of Experimental Medicine*, this group found that in contrast to prior findings using mature DCs, injection of immature DCs into healthy subjects led to the specific inhibition of antigen-specific T-cell function. They found that immature DCs were not simply weaker adjuvants, but led instead to silencing of preexisting immune effectors. When the T cells elicited by immature DCs were boosted in culture, they were dysfunctional as they lacked the ability to kill target cells and exhibited a reduced level of interferon-gamma production. Additionally, the use of immature DCs stimulated a population of antigen-specific regulatory T cells that produced a cytokine called IL-10, which suppresses the immune system.

While DCs are currently under active investigation, mostly for their immunostimulatory properties in cancer and viral infection, this study suggests that caution must be utilized in the use of immature DCs when trying to enhance tumor and microbial immunity. The suppressive properties of immature DCs observed in this report suggest that these DCs may also be valuable for antigen-specific inhibition of T-cell function in the setting of autoimmune diseases and organ transplantation in humans.

6.5 The future of cancer vaccination

As we saw in chapter 2 of this booklet, the immune response can be broadly divided into a cellular and a humoral response. The latter relies on T cells whereas the former involves antibodies. Currently, the vast majority of cancer vaccines are directed at achieving a cellular immune response. Scientists are now working on anticancer strategies that will result in a humoral immune response because recent studies have demonstrated that the presence of tumor-specific antibodies can lead to tumor regression in some patients.

Some of the potential antigens that researchers have their eyes on include molecules
like p53 and gangliosides. The gangliosides are carbohydrate molecules that are normally present on cell membranes. In one clinical study, patients with metastatic melanoma who were vaccinated with a ganglioside called GM2 followed by BCG treatment showed a 14 percent increase in overall survival rate compared to those who received BCG alone. None of the patients had antibodies to GM2 before the trial began. Scientists are conducting trials in which they are experimenting with other adjuvants such as keyhole limpet haemocyanin. Also, the search is on for alternative ganglioside targets such as GD2 and GD3.

Although cancer vaccines that activate the humoral immune response remain a vital strategy in the toolkit of cancer immunotherapists, the current method of choice for practitioners involves the activation of the cellular immune response. Some of the strategies that researchers are using to achieve this effect involve vaccines based on genetically engineered bacteria and DNA. Scientists have known for a while that bacteria such as salmonella, BCG, and listeria are very good at infecting professional APCs. We can turn this to our advantage by genetically altering these bacteria so that they can carry tumor antigens directly to the sites within a patient where an optimal immune response can be guaranteed.

A new and exciting anticancer vaccination strategy involves DNA vaccines. The efficacy of this approach was first demonstrated in animal studies where the administration of DNA effectively protected the mice against the influenza virus. This approach involves using genetic engineering techniques to put the DNA for a tumor antigen into a plasmid (see chapter 8). By disabling the ability of the plasmid to replicate, it can be safely injected into animals and it has been demonstrated that this results in the expression of the plasmid-encoded tumor antigens. This means that physicians will have the ability to direct the DNA vaccines to the exact location that they think will lead to the best immune response within the patient. DNA vaccination against cancer is a very promising field in immunotherapy because it is relatively safe, practical, and affordable.
7.0 THE ADVANTAGES OF CANCER IMMUNOTHERAPY

We hope the reader would have noticed from the preceding discussions in chapters 4—6 that the most salient point of immunotherapy as an anticancer agent is its exquisite specificity. The reliance of this technique on naturally occurring biological molecules to augment the immune system means that of all the scientifically validated methods for treating cancer, immunotherapy seems to be the most natural and friendly to the patient: therein lies the uniqueness and promise of cancer immunotherapy. It is well established that early detection of cancer is extremely important in the management and successful treatment of the disease. By taking advantage of the tremendous recognition capacity of the immune system, immunotherapists hope to develop much more sensitive and effective cancer diagnostic tools.

Due to the vital role that the immune system plays in the body’s defense, the kind of basic and applied research that CRI supports has many spin-offs that benefit other diseases. For instance, upon initial infection by HIV, it is common for the immune system to mount an attack on the virus resulting in the generation of HIV-specific CD8 killer T cells to destroy some of the viral invaders. As the disease progresses, it has been observed that CD4 helper T-cell populations begin to decrease and there is a direct correlation between the symptoms of an infected individual getting worse and the decline in CD4 helper T cells.

It has been shown that the individuals who get infected with HIV but remain symptom-free for a long time have large and healthy populations of HIV-specific CD4 helper T cells that can produce enough IL-2 to keep the HIV-specific CD8 killer T cells growing and multiplying to fight off the deadly onward march of HIV. AIDS patients and others with compromised immune systems tend to develop a form of cancer called Kaposi’s sarcoma. It is believed but not proven that Kaposi’s sarcoma is caused by a Kaposi Sarcoma Herpesvirus (KSHV) infection. For sometime now, attempts by researchers to study how KSHV induces cancers in humans have been hampered because KSHV is unable to grow and divide outside the human body. Scientists are turning to immunotherapeutic approaches to circumvent these research obstacles.

In one study, scientists isolated CD8 killer T cells from people experiencing chronic infections with cytomegalovirus (CMV) and Epstein Barr virus (EBV). Comparative analyses of these cells with those from healthy individuals showed that CD8 killer T cells from the virus-infected individuals lacked a key co-stimulatory molecule called...
CD28. The scientists discovered that the loss of CD28 correlated with the lack of IL-2 in these individuals. However, when the researchers introduced the gene for CD28 via a gene courier (called a vector) into the CD8 killer T cells of these individuals, they were able to produce IL-2 independent of CD4 helper T cells, multiply and fight off the viral infections. One of the reasons why incurable viral infections like HIV, CMV and EBV become chronic is because our immune system cannot produce enough IL-2 to keep CD8 killer and memory T cells in a state of growth and proliferation. By the use of genetic engineering techniques to enhance the efficacy of adoptive T-cell immunotherapy, scientists have developed a vital tool that can be deployed against cancers and other cancer-causing viral infections like human papillomavirus, which causes genital warts and cervical cancer. These exciting results are very encouraging and they provide a compelling example of how active research in cancer immunotherapy has the potential to generate cures for other immune-related diseases.

7.1 The future of cancer immunotherapy

The future of cancer immunotherapy continues to be a promising one with an increasing number of new discoveries and techniques. Although our understanding of the human immune system is at a very advanced stage compared to the state of immunology during Coley’s lifetime, we are yet to obtain a complete understanding of this complex system. The reason why cancer immunotherapy is not yet in widespread use is that we do not know all there is to know about the human immune system.

Despite this limitation, a lot of progress has been made in the field since the advent of Dr. Coley’s vaccine. For instance, adoptive T-cell transfer and vaccination is proving effective in the treatment of metastatic melanoma: a disease that tends to cause death within six months of initial diagnosis. Intensive research in the last decade has provided vital new therapies for diseases such as bladder cancer, renal cell carcinoma, colon cancer, and some leukemias. Not surprisingly, these great strides in cancer immunotherapy have coincided with improvements in techniques such as genetic engineering and monoclonal antibody generation (discussed in chapter 8) and further discoveries in immunology.

In the future, cancer immunotherapies are expected to become a treatment option for cancer alongside the traditional methods such as surgery, radiation and chemotherapy. Used in combination with these three traditional methods, immunotherapies may increase the likelihood of long-term remissions for cancer patients. For example, the administration of immunotherapies to patients who are at a high risk of recurrence after surgery and other treatments may stimulate the immune system to destroy cancer
cells left behind (micro metastases) and responsible for future recurrences. Studies are already underway that examine the use of immunotherapy in conjunction with radiation and chemotherapy to increase the effectiveness of patient responses. Now, let us take a brief look at two techniques that have ushered cancer immunotherapy into a new dawn that is full of promise.
Progress in the field of biotechnology in the last three decades has ushered in a new era in science. Researchers are now able to reproduce natural body products that can be used as drugs in the treatment of many diseases, including cancer. Some of the techniques used to accomplish this feat are genetic engineering and hybridoma technology.

8.1 Genetic engineering

Genetic engineering or recombinant DNA technology is the ability to tinker with and manipulate genes and organisms for a specific goal like the production of therapeutic proteins like interleukin-2. The first step of this methodology is to isolate the gene that produces the human protein of interest. This gene is then spliced into a circular piece of bacterial genetic material called a plasmid: the latter must have been cut with a specific cutting or restriction enzyme (see Figure 5). This is simply a cut and paste experiment and the resulting product is called a recombinant plasmid to distinguish from a natural plasmid. The recombinant plasmid carrying the human gene is then placed in a bacterium such as \textit{Escherichia coli}. Any bacterium that successfully takes up the recombinant plasmid is said to be transformed. After careful selection, the transformed bacterium is grown in a bioreactor where it is induced to produce large amounts of the desired human protein. Finally, the product (called a recombinant protein to distinguish it from a natural protein) is isolated and in most cases, the protein turns out to be identical or similar enough to function equivalently to that produced in the body. These days, scientists can produce human proteins in several different cells including human cells, in the milk of cattle and goats, and in plants like corn. The advantage of using a human cell is that our vigilant immune defense system will recognize the therapeutic protein as “self” and thus tolerate its presence within the body.

8.2 Hybridoma technology

As we saw in chapter 5 under section 5.1, monoclonal antibodies (MAbs) are one of the prized fighters in the battle against cancer and other diseases. The use of MAbs has proven to be so vital that in 1984, the Nobel prize in Physiology or Medicine was awarded to Georges J.F. Köhler and César Milstein of the Medical Research Council’s Laboratory of Molecular Biology in Cambridge, United Kingdom, for their invention in 1975 of hybridoma technology: the technique for manufacturing MAbs. The
first step in preparing a particular monoclonal antibody is to inject the mouse with the foreign substance to which the desired antibody will react. This inoculation stimulates an immune response in the mouse, and its B lymphocytes begin producing antibodies that will recognize and inactivate the foreign substance. Next, the spleen of the mouse, which contains a concentrated source of B cells, is removed, and the B cells are fused with fast-dividing cancer cells (called myelomas) to produce hybridomas. The B-cell component of each hybridoma tells it what kind of antibody to make, and the myeloma cell provides the machinery for producing the antibody and for continuous growth of the hybridoma. Hybridomas are able to generate antibodies that react to only one antigen—the antigen associated with the foreign substance originally injected into the mouse. Since hybridomas can be grown indefinitely in culture, one hybridoma can produce large amounts of one type of antibody. A variation of this method is being developed to produce human monoclonal antibodies.

8.3 Humanization of monoclonal antibodies

In the early 1980s, the unbridled optimism with which monoclonal antibodies were greeted suffered a setback in clinical trials when patients who were infused with therapeutic MAbs suffered a series of symptoms dubbed the HAMA response. These included the development of skin rashes, swollen joints, and life-threatening kidney failure. It turns out that because the MAbs were generated in a mouse cell, the infused patients developed human antimouse antibodies (HAMA) against the infused MAbs. Ultimately, this led to the destruction of the infused MAbs. To overcome this problem, scientists have developed ways to “humanize” MAbs generated from hybridoma technology. This entails the use of genetic engineering to replace parts of mouse MAbs with human components so that our powerful immune system can be fooled into thinking that the infused MAbs were “self” rather than “nonself.”

8.4 Overcoming the HAMA response via modern technology

Once again, researchers have had to call upon our old friend “genetic engineering” to assist them in overcoming yet another research obstacle. Another way around the HAMA response is via a technique called phage display. A phage is a long, stringy virus that infects bacteria. With a specific antigen like the receptor on a cancer cell in mind, scientists first isolate a gene from human B cells and then create a recombinant plasmid that can be transformed into a bacterium like E. coli. While the transformed bacterial cells are growing in culture, the researchers introduce so-called filamentous phages to infect the transformed bacteria. The phages take advantage of the nutritious soup they find themselves in and commence proliferating. As they make copies
of themselves, the phages also make the proteins of the human B cells they have infected and decorate the surface of the new or baby phages with these antibody proteins. The researchers then use the antigen they had in mind as bait to fish out the new phages containing the gene for the most specific antibody to that gene product. Once the target antibody has been isolated, the scientists can mass produce this particular antibody for therapeutic use as we saw in chapter 5.

So, we have seen that invaluable techniques such as genetic engineering, hybridoma technology, and modern forms of monoclonal antibody manufacture allow for the efficient production of naturally occurring immune proteins in a pure enough form for therapeutic applications. These technologies and their products are expected to continue to play pivotal roles in cancer immunotherapy.
9.0 EPILOGUE

We hope we managed to fulfill our mission spelled out at the beginning of this booklet as that of enlightening the reader about the immune system, its connection to cancer, and how a detailed understanding of these two subjects is an absolute pre-requisite for the application of immunotherapy as an effective anticancer agent.

It is particularly pleasing that the founder of the Cancer Research Institute lived to see parts of her vision begin to come true before she passed away. Now, we must soldier on because although great strides have been made, the task ahead remains daunting.

This is where the CRI needs your help in the form of contributions. You may visit our website or call 1-800-99CANCER for further information on how make donations to the institute.

10. ACKNOWLEDGEMENTS

We would like to express our gratitude to the following people for reading this manuscript and offering helpful criticisms: Ms. Lynne Harmer, (Cancer Research Institute), Dr. Ellen Puré (The Wistar Institute and Ludwig Institute for Cancer Research center, USA), Dr. Carl Nathan (Weill Medical College of Cornell University, USA), Dr. Leonora Leigh and Dr. Nebojsa Milanovich (Colgate-Palmolive, USA), Dr. Augustine Alifo (Palisades General Hospital, U.S.A), Dr. Lloyd J. Old (Ludwig Institute for Cancer Research, USA). With regards to errors, omissions, and other shortcomings of this booklet, the buck stops with the authors.
BIBLIOGRAPHY


Glossary

Antigen: A substance that is foreign to the body and is capable of eliciting an immune response. Antigens are to immune cells what red cloths are to mad bulls.

Antibody: A blood protein that is produced by white blood cells when the body recognizes that foreign invaders and their associated antigens have trespassed into the body. The job of the antibody is to fight the invading bacteria or viruses by attaching themselves to these invaders. This marks the invaders for destruction.

Lymphocyte [B lymphocyte (cell) and T lymphocyte (cell)]: A type of white blood cell: there are several types of white blood cells including B and T cells. Together, lymphocytes account for up to a quarter of the white blood cell population in the body. The function of a B lymphocyte is to make antibodies in response to a foreign invader or any substance that the body perceives as foreign.

T Cell: T cells can be sub-divided into four categories: killer T cells, helper T cells, memory T cells, and regulatory T cells.

Killer T cell: As the name suggests, the function of a killer T cell is to kill other cells that the body perceives as foreign. The target of killer T cells includes cells infected with bacteria or viruses, cancer cells, and other harmful cells.

Helper T Cell: Type of white blood cell that helps or stimulates B cells to make antibodies as part of the immune response. They also stimulate killer T cells and cells of the innate immune system.

Immune Response: A cascade of mechanisms that the body activates in order to fight off foreign invaders. Examples of foreign invaders may be bacteria, viruses, and cancer cells. The immune response ranges from phagocytosis (where a group of cells called phagocytes swallow up whole cells) through the manufacture of antibodies by B cells to the stimulation of killer T cells by helper T cells.

Immune System: The entire defense system of the body. It involves the lymph glands, spleen, and a plethora of white blood cells. Our immune system fights infection and also causes allergic reactions.
**Immunity:** To be immune to a disease such as flu refers to the ability of one’s body to resist infection upon exposure to that particular pathogen (the influenza virus, in this particular case). Upon sensing an invasion by bacteria or a virus, the body activates the immune system to fight off the infection. One of the remarkable features of the immune system is that once the body has been exposed to a particular disease, it remembers the encounter. If the disease were to strike again, the immune system deploys the memory cells and these react fast enough to keep the enemy at bay.

**Macrophages:** A type of white blood cell within the immune system. They can be found in the lymph nodes, throughout the circulatory system, and in almost all tissues where they act as sentinels. Macrophages can swallow up and kill whole cells such as bacteria, viruses, and cancer cells.

**Memory cell:** A type of white blood cell that remembers an infection so that when the immune system encounters such a pathogen in the future, the response will be much swifter and more effective.

**Regulatory T Cell:** As part of the immune response, regulatory T cells specialize in telling B cells when to stop making antibodies. They also instruct T cells to call off an assault at the end of an immune reaction.