Basic concepts of immunology and neuroimmunology

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The immune system is a complex network of specialized cells and organs that defends the human body against attack from foreign pathogens. The major lymphocytes involved in protecting the body against potential infections are B and T cells, which also play an important role in combating tumor growth. The cells of the immune system patrol the tissues and organs through both blood and lymphatic vessels, but some organs—including cornea, testes, and brain—are usually not patrolled by these cells. The brain has been thought to be an immune-privileged site because of the tight blood–brain barrier (BBB) that protects it. Few cells migrate to the brain under normal circumstances, because the BBB permits only certain molecules to cross into brain tissue. Recently, however, studies have shown that activated T cells exposed to antigen can cross the intact BBB and migrate into brain. This finding opens the path to developing effective means of immunotherapy for lesions of the central nervous system. The authors discuss basic facets of the immune system, review the current knowledge about human neuroimmunology, and survey current strategies for developing immunotherapy-based treatments for human brain tumors.

KEY WORDS • brain • brain tumor • vaccine • dendritic cells • immunology • cancer

BASIC IMMUNOLOGY

The Components of the Immune Systems

The human blood is made up of red blood corpuscles (erythrocytes) that transport oxygen, platelets (thrombocytes) that trigger clotting, and white blood corpuscles (leukocytes), which are an important element of the immune system that defends the human body against attack from foreign pathogens. White blood cells originate in the bone marrow from pluripotent cells that give rise to common lymphoid progenitor and myeloid progenitor (Fig. 1). Common lymphoid progenitor cells, in turn, give rise to B cells and T cells, which are essential components of the immune system.

Innate immunity is conferred essentially by macrophages and neutrophils, which provide the first line of immune defense where phagocytes eliminate infectious organisms. However, those cells cannot always recognize foreign organisms that need to be eliminated. Adaptive immunity is conferred by B and T lymphocytes, as they recognize and eliminate microorganisms that are missed by phagocytes.

B Lymphocytes

The B cell originates from lymphoid precursor cell in the bone marrow. The earliest B-lineage cells are known as “pro-B” cells because they are progenitor cells with only a limited capacity for self-renewal. Pro-B cells are derived from pluripotent hematopoietic stem cells that are identified based on the presence of characteristic cell-surface proteins, or antibodies. Those antibodies are antigen-specific molecules produced by the B cells. The production of antibody in response to infection is the main contribution B cells make to adaptive immunity.

Differentiated B cells have antibodies that are either present on their surface or are secreted. The successive stages of B-cell differentiation in humans, as in mice, are marked by successive steps in the rearrangement and expression of the Ig genes, as well as by changes in the expression of molecules within and on the cell surface of cells. Immunoglobulin genes undergo rearrangement and encode for antibodies. There are five main types of antibodies: IgG, IgM, IgE, IgD, and IgA. Immunoglobulin-G is the most common type of Ig molecule present in human body.

Antibodies for IgG are large molecules composed of two different polypeptide chains: one chain, which weighs approximately 50 kD, is termed the heavy (H) chain; the other chain, which is called the light (L) chain has an approximate weight of 25 kD. The two heavy chains are linked to each other by disulfide bonds, and each heavy chain is linked to a light chain by a disulfide bond (Fig. 2). In any single IgG molecule, the two heavy chains are identical to each another, as are the two light chains.

Antibodies have two main functions: 1) to recognize a specific antigen and bind specifically to molecules from the pathogen that elicited the immune response; and 2) to engage the effector mechanism, recruiting various cells and molecules to destroy pathogens.

Abbreviations used in this paper: BBB = blood–brain barrier; CAM = cell-adhesion molecule; CNS = central nervous system; Ig = immunoglobulin; MHC = major histocompatibility complex.
The antigen-binding region on antibody molecules varies extensively among molecules and is thus called the variable, or V, region. Genetic recombination events that encode for the variable region give rise to an enormous variation among individual antibody molecules, each of which recognizes a particular antigen. Thus, antibody molecules are individually antigen specific, but collectively they recognize a vast number of antigens. The region of the antibody involved in the effector function is called the constant, or C, region.

Antibodies play an important role in eliminating bacteria and other pathogens by complement-mediated cell killing. Cells infected with virus can also present antigens on their cell surface that are recognized by antibodies. Cells bound by such antibodies can then be killed by natural killer cells. This destruction of antibody-coated target cells by natural killer cells is called antibody-dependent cell-mediated cytotoxicity. Antibodies also play an important role in the elimination of pathogens that cause infection that recurs after a prolonged period of time.

**T Lymphocytes**

The T lymphocytes develop from bone marrow stem cells but migrate to the thymus, where they mature. When mature, they are termed thymus-dependent (T) cells. As the lymphocytes proliferate and mature into T cells, they pass through a series of distinct phases marked by changes in the status of T-cell receptor genes. The thymus provides a specialized microenvironment for the maturing of T cells. Immature T cells, termed double-negative cells, lack expression of CD3, CD4, and CD8 receptors. These cells give rise to either CD3+/CD4−/CD8− cells or CD3+/CD4+/CD8+ cells. Cells that are CD4+/CD8+ are initially large and active, and they later give rise to small and resting double-positive CD4+/CD8+ cells. A large percentage of those cells die of apoptosis, and less than 5% of them give rise to CD4+/CD8− and CD4−/CD8+ cells. The cells then migrate to patrol the tissues to eliminate foreign pathogens.

There are two main classes of T lymphocytes. On activation one class differentiates into cytotoxic T cells, which are distinguished by the cell-surface molecule CD8 and which kill cells that are infected with virus. Pathogens
and their products in the vesicular compartments of cells are detected by a different class of T cells, which are distinguished by surface expression of the molecule CD4. There are two kinds of CD4 cells: inflammatory T cells and helper T cells. Helper T cells activate macrophages and activate B cells to produce antibodies. Bacterially infected cells are killed mainly by T cells.

Like B cells, T lymphocytes bear highly diverse receptors on their surface that allow them to recognize antigen, and whereas they are highly diverse in their antigen specificity collectively, an individual lymphocyte is equipped with receptors that recognize only one particular antigen. The T cells recognize only antigen that is associated with MHC.

Structure of the T-Cell Receptor

The function of T cells to protect against cells that are infected with pathogen relates to their ability to recognize peptide fragments of pathogen that are present in a complex with MHC molecules on the surface of the antigen-presenting cell. The generation of peptides from an intact protein involves a modification of native protein and is referred to as antigen processing. “Antigen presentation” is the term used when the peptide is displayed at the cell surface by the MHC molecules.

The T cell receptor consists of two polypeptide chains, termed α and β. The α and β chains are bound together by a disulfide bond (Fig. 3). These heterodimers are very similar in structure to the Fab fragment of an Ig molecule, and they account for antigen recognition by all the functional classes of T cells. The T-cell receptor has a variable region, a constant region, a hinge region, a transmembrane region, and a cytoplasmic tail; as described earlier, it is the variable region of the T-cell receptor that is directly involved in antigen recognition.

Major Histocompatibility Complex Molecules

For a T cell to recognize antigen on the surface of an antigen-presenting cell, the antigen has to be appropriately associated with the MHC molecules. There are two major types of MHC molecules. Major histocompatibility complex class I molecules consist of two polypeptide chains, an α, or H chain encoded in the MHC, and a smaller noncovalent-associated chain, β2-microglobulin, which is not encoded in MHC. There are three α subunits, α1, α2, and α3. Alpha-1 and α3 molecules come in direct contact with the β2 microglobulin (Fig. 4a). Cytotoxic T cells eliminate all viruses and some bacteria replicate in the cytosolic compartment. Their antigens are presented by MHC class I molecules to CD8 T cells. Peptides that bind MHC class I molecules are usually eight to 10 amino acids long.

Major histocompatibility complex class II molecules consist of a noncovalent complex of two chains, α and β, both of which span the membrane (Fig. 4b). Peptides that are bound to the MHC class II molecule are 13 amino acids long. Some bacteria and parasites are engulfed into endosomes, usually by phagocytic cells such as macrophages, and are able to proliferate within the endocytic vesicles. Peptides resulting from bacterial and parasitic degradation are presented by MHC class II molecules to CD4 T cells.

NEUROIMMUNOLOGY

For many years, the brain has been thought to be an immunologically privileged site, where no immunosurveillance of lymphocytes occurs. This assumption gained support from the existence of a BBB, which excludes components of the immune system. Additional support of the brain’s immunoprivileged nature is its lack of lymphatic vessels and lymphatic drainage. Moreover, except for astrocytes, there is no constitutive expression of MHC molecules in the cells of the CNS. The most convincing evidence of all is that tissue transplanted from one individual into the brain of another individual survives for extended periods of time.

Although the CNS does lack a fully developed lymphatic vasculature, because there is evidence that CNS extracellular fluid drains into the deep cervical lymph nodes, the possibility exists that antigen presentation could occur in the CNS. It has also been shown that, in response to inflammatory reactions, neoplasms, or brain
injury, the expression of MHC class I and II molecules can be induced on several cells that reside in the brain; such cells include astrocytes, endothelial cells, microglial cells, pericytes, and choroid plexus epithelia. In recent studies the authors have also shown that an immunological reaction occurs in the brain in response to a number of disease processes that affect the CNS and spinal cord. Moreover, extensive migration of lymphocytes into the CNS and expression of MHC molecules on vascular endothelial cells, astrocytes, microglial cells, and pericytes have been observed in patients with viral encephalitis, multiple sclerosis, and allergic encephalomyelitis.

Wekerle, et al., 17 and Hickey, et al., 6 have shown that C14-labeled CD4+ myelin basic protein–specific T-cell lines, when activated either by presentation of specific antigen or by concanavalin A, do migrate to the brain through intact BBB. The first phase of entry occurs within 24 hours after injection, and the second phase occurs 96 hours postinjection. It has become evident that CAMs are actively involved in mediating the recruitment of specific lymphocyte subsets into different tissues. Several cell adhesion molecules, such as RANTES, selectins, α4, MCP-1, MIP-1α, ICAM-1, ICAM-2, VCAM-1, and LFA-3 are expressed on cerebral endothelial cells. Overexpression of LFA-1, α4, CD44, and CD2 molecules on activated T lymphocytes contributes to adhesion and to their ultimate migration through the BBB (Fig. 5). 4

What happens to T cells after they are injected into the brain? In recent studies the authors have suggested that activated cells, when injected into the cannulated CNS, preferentially follow white matter tracts and sometimes are detected in gray matter along the perivascular spaces. The existence of afferent connections from the CNS to the immune system have been supported by investigators who showed that injecting antigen into CNS tissue elicits a systemic immune response. 7 If cells of the immune system migrate to the brain, do they protect against human tumors? This question requires addressing the use of additional immune components in developing a vaccine against human brain tumors.

DEVELOPMENT OF A VACCINE FOR HUMAN BRAIN TUMORS

Brain tumors affect approximately 17,000 people annually in the United States, and they are a leading cause of death among children and are a devastating disease in adults. Glioblastoma multiforme and astrocytomas, which account for more than 40% of all CNS neoplasms, are the most common brain tumors affecting adults between 45 and 60 years of age. The incidence of these gliomas is increasing at a slow but steady rate. Surgical removal followed by radiation therapy is currently the most common treatment. Although these therapies are beneficial to a certain extent in the initial treatment of gliomas, they rarely eradicate all the tumor cells. The poor prognosis for patients with glioblastomas (a survival time of approximately 2 years) is primarily related to recurrence of resected tumor and to the spread of tumor cells into surrounding regions of the brain. 10,13,14 Among patients in whom low-grade gliomas initially develop, most experience tumor recurrence. These therapies and adjuvant chemotherapy and gene therapy also have detrimental side effects. Thus, there is an essential need to develop long-term treatments that are not only tumor specific but also effective in removing all brain tumor cells.
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MOLECULAR CHANGES DURING THE GENESIS OF HUMAN BRAIN TUMORS

Neoplastic transformation in the normal human brain occurs as a result of a series of genetic alterations, including the loss, gain, or amplification of different chromosomes. These alterations lead to changes in the expression of proteins that play important roles in the regulation of cell proliferation. Several common genetic alterations at the chromosomal level have been observed, including loss of chromosomes 17p, 13q, 9p, 19, 10, 22q, and 18q and amplification of chromosomes 7 and 12q. These alterations lead to changes in the expression of several genes during the genesis and progression of human gliomas; genes affected include \( p53 \), \( RB \), \( INF\alpha/\beta \), \( CDKN2 \), \( MMAC1 \), \( DCC \), \( EGF\), \( PDGF \), \( PDGFr \), \( MDM2 \), \( GLI \), \( CDK4 \), and \( SAS \). Analysis of recent studies suggests that altered expression of several other genes and proteins is associated with the genesis of human gliomas; those genes include \( MET \), \( MYC \), \( TGal \), \( CD44 \), \( VEGF \), \( hNr-CAM \), \( NCAM LI \), \( p21^{\text{ind/Cip1}} \), \( trkA \), \( MMRs \), \( C4-2 \), and \( D2-2 \); and the proteins include cathepsins, tenasin, matrix metalloproteases, tissue inhibitors of metalloproteases, nitric oxide synthetase, integrins, IL13 receptor, connexin 43, uPARs, extracellular matrix proteins, and heat-shock proteins. Taken together, these findings point to the accumulation of multiple genetic mutations coupled with extensive changes in gene expression in the development of human glioma.

CURRENT STRATEGIES FOR CNS TUMOR VACCINE DEVELOPMENT

Activated T cells (CD4+ and CD8+) have been shown to cross the BBB due to differential expression of a set of cell-surface molecules. The T cells that do enter the brain are capable of recognizing their prospective antigens among CNS constituents. Based on this observation, extensive effort has been made to develop an immunotherapy-based strategy for treating human brain tumors. Development of an effective vaccine, however, requires an antigen-presenting cell that can elicit an immune response to activate the T cells that can migrate to the brain (Fig. 6).

Except for germ cells and placental cells, all cells express MHC class I molecule, and they present antigen to CD8+ cells. In contrast, expression of MHC class II molecules is restricted to a few cell types, such as dendritic cells, the function of which is antigen presentation. Within the CNS, astrocytes may express MHC class I and II molecules and present antigens. However, most brain tumors do not express MHC class I molecules, and approximately 40% of brain tumors express MHC class II molecules.

Because of the reduced expression of MHC class I and class II molecules, cancer cells are not good antigen-presenting cells. During the past 5 years, however, dendritic cells have received considerable attention in the immunotherapy studies of several different kinds of tumors. These cells are professional antigen-presenting cells that can induce a T cell–based immune response several times more effective than a normal response. Two main strategies have been proposed by which a vaccine against human brain tumors could be developed. In one strategy, tumor cells are genetically modified to secrete cytokines that will induce a T cell–based response. In the second strategy, antigen-presenting cells are pulsed with tumor antigens to induce an immune response.

Vaccine Development By Using Genetically Modified Tumor Cells

Tumor cells are poorly immunogenic and have poor antigen-presenting capability, primarily due to reduced MHC expression. They also secrete factors that can impair the host’s humoral and cell-mediated immunity against tumor cells. Thus, in developing therapeutic techniques, it is important not only to make gliomas more immunogenic but also to induce a strong immune attack against them. Cells transfected with genes for several cytokines (IL-2, IFN-\(\gamma\), IL-4, IL-1, IL-3, IL-6, IL-7, TNF\(\alpha\), IL-12, and GM-CSF) can induce rejection of tumors in syngeneic animals. These cytokines will enhance MHC expression in tumor cells, making them more immunogenic. The goal of transflecting these genes into tumor cells is to repair one or several defects, which will lead to the restoration of a strong and specific immune response. A major problem with this approach is that persistent expression of these genes for a long period of time needs to be ensured. One cytokine expression may not be sufficient to overcome the suppressive factors secreted by tumor cells. With the availability of new vector systems, it is now possible to express two to five genes in one cell.

Development of Brain Tumor Vaccine Through a Dendritic Cell-Based Approach

Dendritic cells, originally identified by Banchereau and Steinman, are the pacemakers of the immune response. These cells are an excellent source of presenting antigenic peptides and proteins to T and B cells. Generated in large number in the bone marrow every day, dendritic cells course through the blood stream, migrate into tissues, and complete their journey by responding to local cytokines, picking up antigens at sites of inflammation, carrying antigens to the resident lymph nodes, and selecting those few
T and B cells that are capable of responding to the antigen in an optimum fashion. Dendritic cells can be distinguished from other circulating cells by their surface markers (for example, S100, p55, CD83, and OX62). These markers have been used to isolate and purify these cells. In cancer research, dendritic cells have recently been used in developing novel therapeutic strategies. With simple cell-culture techniques, it is now possible to isolate and purify large quantities of dendritic cells from human blood.

Dendritic cells have been used as antigen-presenting cells in developing vaccines against melanomas and prostate cancer. These professional antigen-presenting cells are used to develop vaccines in four major ways: they can be exposed to tumor cell lysate, exposed to purified peptide, infected by virus containing a gene, or fused with the tumor cells (Fig. 7). In all these approaches, dendritic cells present on their surfaces peptides that are cancer-cell specific. In clinical efforts to treat melanoma and prostate cancer, a single peptide or few peptides from cancer-specific antigen were used to pulse the patient’s own dendritic cells. Those stimulated dendritic cells were then returned to the same patient, and an effective immune response against tumor cells was observed. This technique was also successful in eradicating a melanoma tumor implanted in rat brain. Because dendritic cells will come in direct contact with T cells, an immune response is induced against tumor cells carrying the same peptide on their cell surface.

For treating brain tumors, experimental strategies have been developed to pulse dendritic cells with many peptides, thus fusing them with glioma cells. Currently, only a few glioma markers are known that can be used in developing a dendritic cell-based vaccine approach. These markers include EGFR, EGFRvIII, L1, and tenascin. Experimental and clinical studies are being designed to investigate this approach. (unpublished data).

Fig. 7. Strategies for initiating the pulsing dendritic cells (DCs) with antigens.

CONCLUSIONS

The human immune system consists of cells that can mount a strong immune response against invasion by foreign pathogens. The major role in the body’s immune system is played by B and T cells. Because it is now known that activated T cells can cross the BBB to migrate into the brain, an effective immunotherapy-based strategy can be developed to treat disorders affecting the brain, including tumors. With the isolation and characterization of dendritic cells, it is now possible to induce a much stronger T cell–based immune response. The more glioblastoma-specific antigens that become isolated, the more possible it will be to take advantage of the effective antigen presentation properties of dendritic cells to eradicate deadly tumors by using the body’s own defense system.

References


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