

Logic of the Immune System

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Abstract

Our immune system evolves under continuous selection to protect us against pathogens, a trait that can be used to deploy the immune system or its component parts for diagnostic and therapeutic purposes in cancer. What follows is a thumbnail sketch of the logic that underlies immune recognition, so that those not directly active in the field of immunology may grasp both possibilities and pitfalls when considering applying the tools and concepts of immunology to cancer and cancer therapy. *Cancer Immunol Res*; 1(1): 5–10. ©2013 AACR.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

CME Staff Planners Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

On completion of this activity, the participant should understand the overall schema of immune surveillance, including the basic components of the host immune system and the logic that underlies immune recognition, and be able to begin to identify opportunities for immune intervention.

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Introduction

Pathogens prey on all forms of life: bacteriophages attack bacteria, whereas viruses, parasites, and bacteria target multicellular organisms. Survival requires defensive countermeasures, their evolution sculpted by interactions between pathogens and their hosts. Compared with the hosts under attack, viruses and bacteria have much shorter replication times and can adjust to selective pressures rapidly. It follows that our immune system deployed in defense against these challenges is a work in progress, evolving under continuous selection. An unfortunate corollary is the emergence of pathogens such as HIV, uniquely capable of evading immunity, with grave consequences for more than 20 million individuals infected.

This short piece strives to provide a thumbnail sketch of the logic that underlies immune recognition, so that those not directly active in the field of immunology may grasp both possibilities and pitfalls when considering applying the tools and concepts of immunology to cancer and cancer therapy. It could not possibly aspire to completeness or avoid bias. Vaccines—witness the elimination of small-pox and rinderpest, with polio close behind—exploit the immune defenses against pathogens and will stand as one of immunology's

greatest foundational principles. With notable exceptions such as economic advancement, elimination of armed conflict or environmental pollutants, modification of dietary habits, and matters as seemingly mundane as safe drinking water, when it comes to public health and avoiding premature death, vaccines have delivered bang for the buck. Immune intervention in diseases such as cancer is a more challenging—and far more expensive and resource-intensive—proposition. Our understanding of the complexities of immune recognition, the intricacies of the integrated networks of cells, soluble mediators, and transcriptional circuitry that together regulate host defense, has improved in leaps and bounds. It has yielded concrete results in the form of successful interventions, with monoclonal antibody-based therapies, or cytokine-based treatments of autoimmune disease and inflammatory conditions as shining examples. Notwithstanding its recent successes or its importance as an academically and clinically challenging discipline, an immunologic approach to cancer treatment that is affordable in the long run remains an enormous challenge to science and to the providers of the care based on its discoveries.

When cancers arise, immune recognition by the host has failed as a possible countermeasure. Therapeutic intervention is therefore aimed at enabling the immune system to do things it would not or could not do on its own accord. Is immune recognition impossible because access to the tumor is denied? Is the cancerous cell too similar to normal tissue to allow the clear distinction that maintains self-tolerance while allowing immune attack? Has an immune response been blunted by regulatory mechanisms that might perhaps be unblocked by intervention, thus enabling an attack that does not

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compromise the need to avoid damage to healthy cells and tissues? Could we entice a heterologous immune system, say that of mouse or other rodent, to distinguish between a human cancerous cell and its normal counterpart and adapt this capability to a therapeutic setting in man by "humanizing" this response?

Host defense evolved to deal with the onslaught of pathogens. Our cells and tissues come equipped with defense mechanisms that are hard-wired and innate, capable of an immediate response to the invader. Receptors responsible for making these necessary early decisions recognize rather broad macromolecular patterns that distinguish a pathogen from its host. These receptors may function at the cell surface, or may work inside the cell. Stressed or even cancerous cells may display structures that allow them to serve as targets for recognition by innate immune cells, which may then kill them as part of a return to homeostasis.

Lymphocytes are the best-known immune cells. They take time to mount a response, but when they do, they do so with exquisite precision in what is called adaptive immunity. All lymphocytes use complex genetic machinery for the construction of their antigen-specific receptors. Such receptors comprise 2 subunits, one of which requires assembly of V, D, and J DNA elements, whereas the other subunit uses only V and J elements. Random pairing of the rearranged VDJ- and VJ-bearing products yields an already impressive degree of combinatorial variability, but diversity is massively increased because of imprecision and DNA modifications inherent in the

rearrangement process itself: at the junctions of each of the newly combined elements (D to J, V to DJ, and V to J) nucleotides may be added or deleted, thus altering the amino acid sequence of the receptor in a manner that is not template-encoded. These segments of the receptor polypeptide chain display maximal diversity and correspond to the regions of the folded receptor that most intimately contact antigen. Together, combinatorial diversity and junctional imprecision account for a sheer limitless number of distinct antigen receptors, each with its own unique capacity to recognize antigen. In principle, antigen receptors can bind to almost anything, including self-antigens or structures that are man-made and do not occur naturally. Combined with innate immune recognition, the ability of the adaptive immune system to recognize and interpret this complex antigenic universe affords a natural measure of protection against premalignant and malignant cells, a concept referred to as immune surveillance (Fig. 1).

The assembly of complete antigen receptors occurs in stepwise fashion. Early intermediates in the assembly of antigen receptors execute early lymphocyte fate decisions, intermediates that serve as diagnostic traits to stage lymphoid malignancies. Only fully assembled receptors expressed by mature lymphocytes are used to detect invaders. For full activation, proliferation, and terminal differentiation, lymphocytes not only require a signal delivered via their antigen-specific receptor, they also need input from costimulatory molecules and rely on exposure to cytokines. Activation of the innate immune system triggers the expression of

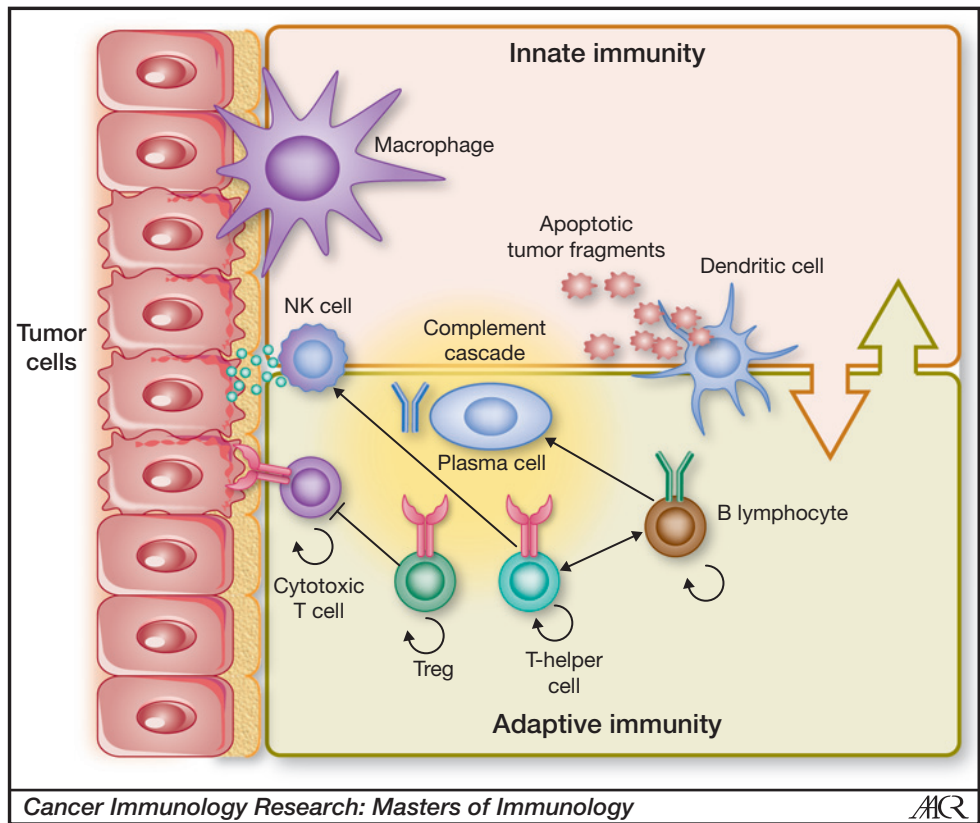


Figure 1. Schema of immune surveillance (Adapted from ref. 1).

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costimulatory molecules and relevant cytokines. Withholding these additional signals may compromise lymphocyte function and incapacitate them. Innate and adaptive immunity are thus highly integrated and inextricably intertwined protective mechanisms. The salutary contribution of our commensal microbiota to immune homeostasis is only now gaining in understanding (and acceptance) and will undoubtedly force us to modify our approaches to immune intervention in general.

Immunoglobulins

Antigen receptors on B lymphocytes are called immunoglobulins, capable of recognizing antigen without the involvement of any other additional molecule. Immunoglobulins comprise 2 chains, a light chain (L) produced upon VJ rearrangement and a heavy chain (H) requiring VDJ rearrangement, each present in 2 copies held together by disulfide bonds: H₂L₂. A B cell produces 2 forms of immunoglobulin: membrane-bound and secreted. The former serves as a unit that captures antigen and transmits signals to the B cell that carries it, allowing the B cell to proliferate (clonal expansion), whereas the latter can act at sites distant from where the immunoglobulin is produced. Although the VJ and VDJ combinations generate the antigen-binding unit, other portions of the immunoglobulin heavy chains are encoded as tandem blocks in our genome, accessible in modular fashion, so that different classes of immunoglobulin—each with unique functional properties—can be produced, while maintaining the original VJ and VDJ combination, and thus retention of antigen specificity. Although the variable segments bind antigen, the constant region, referred to as the Fc region, which distinguishes each class, does not vary in sequence, as its name implies. Cells of the myeloid lineage carry receptors for these Fc regions. Upon engagement of these Fc receptors, cells may kill cancerous cells or pathogen-infected cells to which the immunoglobulin latched on via its variable region. The ability to access the different constant regions is a differentiation event carefully controlled in the life of a B lymphocyte. For a newly generated B cell, the constant region used is the one proximal to the rearranged VDJ combination. The functional distinctions that characterize different classes of immunoglobulin include the ability to bind and activate proteins of the complement cascade. The complement cascade is a collection of serum proteins not unlike the blood clotting cascade, where an initial trigger of a single molecule leads to the successive proteolytic activation of several downstream elements in the cascade, proteases all, with massive amplification as the result. Activation of complement leads to physical damage of membranes onto which the activating antibody adsorbs. It also generates chemoattractants that recruit inflammatory and phagocytic cells to the site of activation. Macrophages have receptors for complement-modified structures to facilitate their internalization by phagocytic uptake, with improved entry into the antigen-processing pathways (*vide infra*) as outcome.

Antibodies are both therapeutic modalities and diagnostic tools. Overexpression of self-antigens can occur on malignantly transformed cells and be gauged through the use of appropriate antibody-based assays. Entire cell lineages, such

as B cells and malignancies derived from them, may be wiped out through the use of monoclonal antibodies that target a cell type-specific antigen. As long as cancerous cells and the metastases derived from them bear a unique set of diagnostic markers, antibodies can detect them. Radiolabeled preparations of antibodies or fragments derived from them provide new opportunities for diagnostic imaging procedures and have been proposed as therapeutics as well. The clinical success of Erbitux (a monoclonal antibody directed against the EGF receptor) in the treatment of colorectal and head and neck cancers, or the treatment of B-cell malignancies with rituximab, are early examples of antibody-based therapies (Fig. 2).

TCR and MHC restriction

Unlike B lymphocytes, T cells display antigen-specific receptors that use a fundamentally different mode of antigen recognition, although they, too, rely on pairs of VDJ and VJ recognition units. T-cell receptors (TCR) bind to the products of the MHC, a large family of glycoproteins that shows extreme polymorphism, with thousands of alleles—each encoding a distinct MHC product—present in the human population. MHC products evolved to bind short snippets of protein by accommodating them in a peptide-binding cleft, the design of which allows a single allelic MHC variant to interact with a diverse set of peptides.

In the absence of any outside intruders, the source of these peptides is always protein made by the host. In the cytosol, proteins are continuously checked for their structural integrity and destroyed if they fail to meet quality control, or when targeted specifically for degradation. The proteasome is the key protease responsible for generation of peptides in the cytosol, which are then delivered via specific peptide transporters to the endoplasmic reticulum, where they are assembled together with the class I MHC subunits to yield a stable class I MHC molecule. All nucleated cells synthesize class I MHC products, or can be induced to do so. One particular class of T cells, the killer cell or cytotoxic T cell, is uniquely centered on recognition of class I MHC products and the peptide cargo they carry. Only when pathogen-derived proteins make their appearance, they will be converted into peptides for display by class I MHC products and be recognized as foreign by the TCRs on cytotoxic T cells. Such cells use the CD8 glycoprotein marker to ensure engagement with class I MHC products.

The second major route of protein turnover involves lysosomes, intracellular compartments rich in proteases that break down proteins that enter through endocytosis or—in specialized cells—phagocytosis. Damaged cytoplasmic organelles may end up in lysosomes through autophagy. A second type of MHC product, the class II MHC molecules, expressed on all professional antigen-presenting cells, samples the lysosomal compartment for the presence of antigen. Similar in overall design to class I MHC products and with a similar function, namely to bind peptides in their peptide-binding pocket, the means of assembly and peptide loading are distinct from those used by class I MHC products. Class II MHC products with their peptide cargo serve a class of T

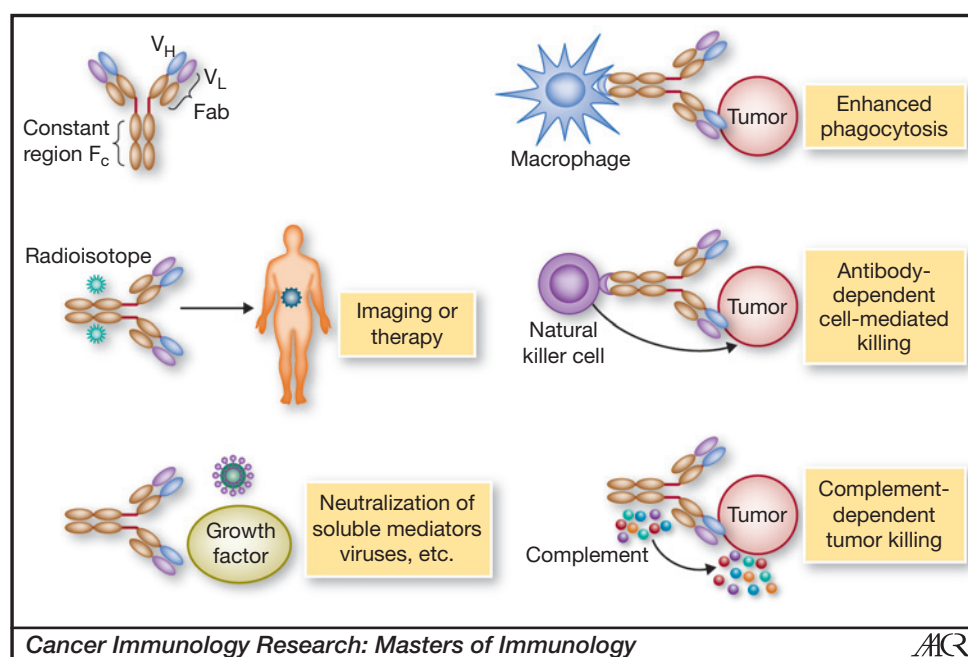


Figure 2. How antibodies function and their potential use in cancer diagnostics and therapies.

cells characterized by expression of the CD4 glycoprotein marker. The CD4 T cells represent a functionally heterogeneous set of lymphocytes, distinguished primarily by their ability to secrete unique combinations of cytokines. Depending on the types of cytokines released, CD4 T cells help B cells execute fate decisions and become immunoglobulin-secreting or memory B cells. Other CD4 T-cell subsets not only provide help to B cells, but also provide cytokines that kick-start a CD8 T-cell response, in collusion with the cytokine output of professional antigen-presenting cells. Yet, other CD4 T cells regulate the responses of other T cells. These regulatory T cells (Tregs) are important in avoiding self-reactive T cells from running amok. Some CD4 T-cell subsets produce cytokines that help combat infectious disease but at the risk of promoting autoimmunity. Similar heterogeneity of function might apply equally to the CD8 T-cell compartment.

The cells that display class I and II MHC products present self-antigens constitutively, and thus report to the immune system the types of structure that should best be left alone—these self-antigens are nonetheless recognized by T cells and used as survival signals. When pathogens enter and the host's antigen-presenting cells acquire pathogen-derived material, peptides derived from them begin to contribute to the sets of displayed MHC-peptide complexes, and thus serve as a guidance system for the incoming immune attack. Not only that, antigen-presenting cells also carry receptors involved in innate immunity: double-stranded RNA, unmethylated DNA, peptidoglycan, lipopolysaccharides, and other pathogen-derived materials serve as ligands. The result of this engagement is cytokine production, including IFNs, which itself is a protective response. In addition, cells thus activated upregulate expression of the component parts of the antigen-processing machin-

ery and begin to display sets of proteins referred to as costimulatory molecules. T cells must recognize not only a peptide MHC complex that fits their clonotypic receptor, they also require engagement of these costimulatory molecules as a prerequisite for full functional activation.

Conversion of proteins into peptide-MHC complexes is referred to as antigen processing and presentation. No adaptive immune response can take place without involvement of properly loaded MHC products. Several types of cancer and their metastatic lesions are characterized by a partial or complete loss of MHC products, caused by attenuation of the transcripts that encode MHC products, their accessories, or of proteins involved in ensuring their assembly and trafficking. This is evidence for selective pressure exerted by CD8 T cells, and suggests that methods to harness a cytotoxic T-cell response (induction, amplification, and recall) to eliminate cancerous cells deserves high priority.

Selective pressures exerted by our immune system allow the emergence of pathogen variants that escape detection, either through mutation of the antigen recognized or through more sophisticated mechanisms that compromise activation of antigen-specific lymphocytes, such as downregulation of essential recognition elements on the target cell or through more sophisticated mechanisms. This logic applies to immune responses against malignantly transformed cells as well; even if the tumor cell were to display a structure that could be recognized by an antigen-specific receptor, the tumor cell might escape elimination through a mutation or through downregulation.

Many viruses use a similar strategy of reducing or eliminating expression of class I MHC products to avoid detection by CD8 T cells. Luckily, the mammalian immune system deploys measures to counteract such evasive maneuvers.

Natural killer (NK) cells are bone marrow–derived cells that carry receptors capable of recognizing class I MHC products, and their functions are calibrated such they can diagnose the absence of self-MHC products and proceed to kill such MHC-deficient targets, or respond by production of cytokines that activate macrophages. In this manner, they can both help eliminate malignant cells that escaped recognition by CD8 T cells for lack of MHC products or contribute to an inflammatory microenvironment that might even be beneficial for cancers. Although NK cells are kept silent in the face of adequate levels of self-MHC products, the expression of stress-induced molecules such as the MHC-encoded MIC-type surface antigens, as found also on certain malignant cells, may activate NK cells and create an environment conducive to tissue repair or elimination of cancerous cells.

The context of lymphocyte activation determines the outcome of the response. In the absence of any microbial or other inflammatory stimuli, engagement of antigen receptors may silence the cells that carry them. Only if the composite of all inputs is interpreted as a response to an invader or an infectious event will lymphocytes become fully active and exercise their effector functions. The quality and the intensity of such signals are likely to span a wide range and may thus account for the rather heterogeneous functional outputs recorded for lymphocytes. Dendritic cells are the most important professional antigen-presenting cells, capable of displaying the full panoply of signaling molecules and soluble mediators required for starting an adaptive immune response. Surface molecules required for full activation are referred to as costimulatory molecules. A key task ahead will therefore be to understand these networks of interactions, their interdependencies and the consequences of specific perturbations. For example, persistent activation of antigen-specific T cells, a situation evoked by chronic infections, autoimmunity, and responses against different types of cancers, can lead to their exhaustion, a state reflected by the expression of surface molecules such as the costimulatory molecule PD1. By antibody-mediated masking of the PD1–PD-L1 interaction, the exhausted state is at least partly reversible. Interference in the CTLA4 and PD1–PD-L1 axes is proving to be an extremely promising strategy to enhance beneficial T-cell responses against melanoma and prostate cancer.

Tolerance

The very nature of the recombination processes that yield the functional VJ and VDJ combinations (the repertoire) not only anticipates encounters with any representative from the diverse world of pathogens, given its randomness it also unavoidably produces antigen receptors that can bind to the host's own building blocks (protein, carbohydrates, lipids, and nucleic acids). Lymphocytes that display overtly self-reactive receptors can cause autoimmunity. The receptor repertoires of lymphocytes are therefore edited by a combination of negative selection, accomplished by physical removal of the offending lymphocytes, and through adjustment of their highly complex signaling apparatus so that requirements for full activation will not be met.

All lymphocytes require engagement of their antigen receptors for survival. In the absence of any infectious agent, this implies recognition of self-antigens in the course of lymphocyte development. At the same time, recognition by the developing lymphocyte of self-antigens that would cause tissue damage is forbidden, and a state of self-tolerance thus characterizes the normal immune system. Maturing lymphocytes are test-driven on self-antigens in processes that include selection for desirable properties, such as the ability of T cells to recognize one's own MHC products, whereas being purged of undesirables such as overtly tissue-damaging lymphocytes, a situation that applies to both T and B cells. Imposition of tolerance is imperfect; witness the incidence and severity of autoimmune disease. To avoid damaging self-reactive responses, additional checks are in place in the form of regulatory cells with innate-like features. Myeloid-derived suppressor cells and Treg and regulatory B cells fit this category.

A state of self-tolerance does pose a hurdle for immune recognition of malignantly transformed cells. The differences between a normal cell and its malignant counterpart are relatively few. A tumor-specific immune response is most easily envisioned for those malignancies with a viral etiology. Because immunity evolved to protect against insults caused by pathogens, virus-induced tumors obviously fit this category. The distinction between prophylaxis and therapy is key: the success of the HPV vaccines as a preventive measure to reduce incidence of cervical carcinoma rests on the ability of the vaccine to elicit neutralizing antibodies that deal with incoming virus before it has had a chance to gain a foothold. Therapeutic interventions are a different matter. Mutations in oncogenes or the production of fusion proteins derived from chromosomal translocations could conceivably be scored as "foreign" by lymphocytes or their products, and therefore serve as targets for immune elimination. Dysregulation of gene expression may result in elevated levels of protein products not seen in normal healthy cells or tissues. Crossing this threshold may provoke an immune response, or could create a window, a therapeutic opportunity that allows the discrimination between normal and transformed cells. Tolerance may be broken by removing the checks and balances exerted by regulatory lymphocytes, for example through elimination of Tregs. Relief of inhibitory constraints on T-cell activity through therapeutic antibody-mediated interference with inhibitory molecules such as CTLA4 or the PD1–PD-L1 axis has enabled successful treatment of malignant melanoma and prostate cancer.

Cells of the innate and adaptive system interact and exchange signals: CD8 T cells can produce the cytokine IFN- γ , a potent activator of macrophages. Activated macrophages can inflict tissue damage and create a proinflammatory environment. An inflammatory environment, in turn, assists in the effective induction of adaptive responses, but persistent inflammation is counterproductive. In some situations, T cells may acquire another function, for example by switching to a different set of cytokines. Such T cells can produce anti-inflammatory molecules such as interleukin (IL)-10. These diverse types of response help shape the tumor

microenvironment and in certain cases may enhance tumor growth. Tumor cells themselves may produce chemoattractants and produce cytokines that limit the efficacy of T cells that arrive on site. What is urgently needed is a better understanding of exactly how the tumor microenvironment affects the properties of both the tumor cells themselves, the products they release and of immune cells recruited to these sites. The presence and activity of Tregs, for example, would be detrimental to an effective immune attack.

Integration

The blood stream transports lymphocytes and NK cells to sites from which they may exit the circulation. These trafficking patterns are controlled by chemotactic signals in the form of chemokines, for which leukocytes must carry the appropriate receptors. Exit from the circulation and migration patterns within lymphoid organs and tissues not only rely on chemokine gradients laid down on extracellular matrix components, but also require the presence on leukocytes of the proper adhesion structures, to ensure the type of productive encounter, for example, with antigen-presenting cells that lead to initiation of an adaptive immune response, primarily in the lymph nodes. These specialized anatomic structures receive antigenic input via lymphatic flow from peripheral sites. Having received activation signals and cues that confer distinct functional properties on newly activated lymphocytes, they may leave the lymph node and reenter the circulation. Now fully armed and ready to carry out their function, T cells survey

peripheral sites for the presence of their cognate antigen in the form of MHC-peptide complexes. Access of lymphocytes to tissue resident malignant cells or clusters of cells is carefully controlled and certain types of cancer such as pancreatic cancer may complicate immune recognition simply by denying effective access.

The ability to interrogate lymphocytes, their subsets, and even individual cells for their functional properties has led to numerous opportunities for immune intervention. Antibodies that recognize differentiation antigens diagnostic for certain cell lineages and expressed on their malignant derivatives serve as targets for antibody-based elimination. Antibodies can also modulate the properties of T cells for therapeutic gain, for example by reversal of exhaustion, or other types of relief from inhibitory signals. Because we know relatively little about the interplay between external factors such as our own microbiota and the complex network of interactions of immune cells, tumor cells, and stroma, a major challenge is to decipher these networks and identify the nodes that can be exploited therapeutically. With many if not most of the component parts in hand or at least known, to understand the logic of this circuitry is the next big challenge.

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