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Early Phase Cancer Immunotherapy

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Preface

Immunotherapy: Transforming Cancer Care

1986: "Cancer is a wound that never heals"; 2017: " The patient is both the host and the treatment for their cancer."

While connoting both the social as well as biological consequences of an entity that has plagued mankind for millennia, this sentiment recognizes the central role of the immune system in wound healing, or, in this context, tumor elimination. The critical role that the immune system plays in tumor regression, and therapeutic strategies harnessing the host immune response against tumor, have been recognized since the advent of Coley's toxin over a century ago—based on observations that patients with severe postoperative skin infections after their sarcoma surgery would spontaneously achieve cancer remission. Bacillus Calmette–Guérin (BCG) vaccine has shown durable efficacy in localized bladder cancer with reported responses in metastatic cancers as well. Decades of innovation in medical science would be required to further refine cancer immunotherapy for clinical use.

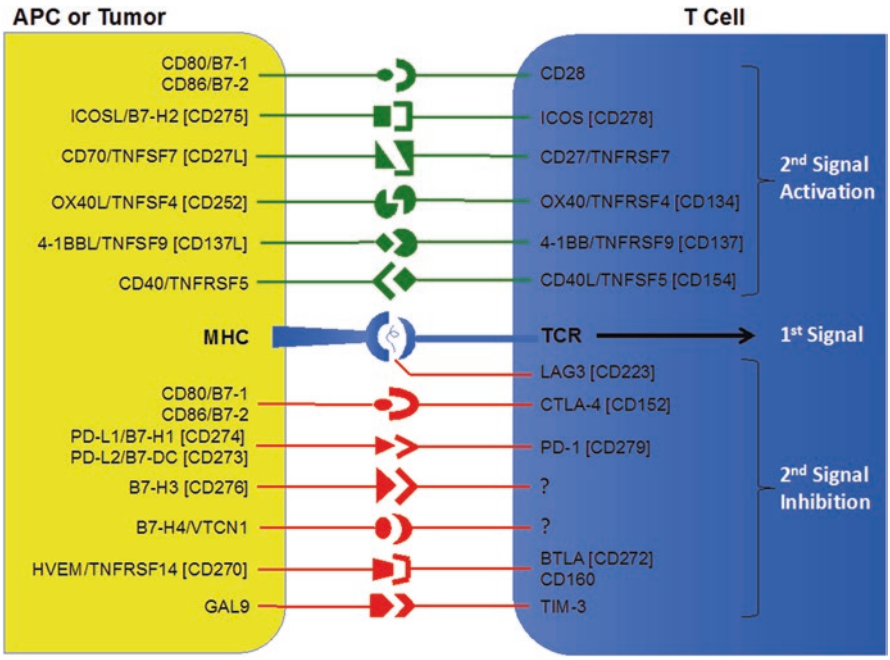
More recently, an improved understanding of the various immune cells within the tumor microenvironment has revealed the importance of immunomodulatory pathways in tumor control and rejection. Both the innate and adaptive arms of the immune system are crucial to tumor control and rejection. The importance of T cell-mediated rejection of tumor was first harnessed in the form of cytokine therapy, in particular interleukin-2, as a therapeutic agent in metastatic melanoma and renal cell carcinoma. Subsequently, advances in cell processing led to the advent of autologous tumor-infiltrating lymphocyte therapy with initial responses in melanoma and subsequently other tumor types. Similarly, immune checkpoint blockade targeting inhibitory T cell axes such as CTLA-4 and PD-1/PD-L1 have revolutionized oncology and can result in durable responses in tumor types ranging from melanoma to lung cancer to Hodgkin lymphoma, among others.

The promise of immunotherapy in achieving long-lasting remissions in advanced disease has unleashed a torrent of drug development, focusing in particular on novel

combinatorial immunotherapeutic strategies. Distinct from chemotherapy and targeted therapy in drug development, response kinetics, toxicity, and biomarker science, early phase clinical trials of cancer immunotherapy have numerous unique characteristics in trial design that are as paradigm shifting as the agents themselves. For example, radiographic pseudoprogression can be seen due to initial immune infiltration of tumor, which, if not appreciated, can result in premature discontinuation of therapy and an incorrect assessment of therapeutic efficacy. Also of importance, residual radiographic lesions may represent inactive cancer or immunologic scars of phagocytosed tumor. Durable stable disease can also be observed resulting in substantial clinical benefit to the patient. This data may not be sufficiently appreciated in early phase clinical trials powered by response rates based on early assessments of tumor shrinkage, relative to often major improvements in symptoms and longer-term survival.

Classes of Cancer Immunotherapy

- Vaccines
  - Peptide/Protein/Tumor cell lysates
  - Viral
  - Dendritic Cell
  - Oncolytics
- Small molecule agonists and inhibitors
  - IDO
  - TGF-beta
- Cytokines
  - IL-2
- Immune checkpoint modulation
  - CTLA-4
  - PD-1, PD-L1
  - TNFSRF agonists
- Cellular therapy
  - CARs, TCRs
  - NK cell



Furthermore, conventional dose-limiting toxicities for early phase clinical trials of cytotoxic chemotherapy based on relatively common cytopenias or end-organ toxicity that are dose-dependent are exceeding rare with immunotherapy. Instead, rarer immune-related adverse events with delayed toxicity kinetics or severe cytokine release syndrome may be observed with the clinical utilization of immune checkpoint blockade and cellular therapy, respectively. These rare and delayed phenomena place heightened importance on pharmacokinetic and pharmacodynamics biomarkers to determine safe dosing, and novel clinical trial designs to best ascertain safe dosing schema for these novel agents. Nuances may exist even within similar pathways—for example dose-dependent immune related adverse events based on increased weight-based dosing of anti-CTLA-4 targeted agents, but similar efficacy and toxicity across dose ranges of anti-PD-1/PD-L1 targeting therapies that result in the latter being more amenable to fixed dosing schema. Dosing of cellular therapeutics such as CAR T-cells may be dependent not only on antecedent conditioning chemotherapy, but on nuances of co-stimulatory factors, individual variances in cell harvest, and ratios of immune cell populations. Whether acting on extant immune cells within the tumor microenvironment via immune checkpoint blockade, or via exogenously engineered cellular therapeutics, novel clinical trial designs to allow for the early investigation of these pharmacodynamically atypical agents are needed.

The advent of next-generation sequencing has revolutionized genomically-based precision medicine, currently utilized and integrated into clinical practice. To date, the use of PD-L1 immunohistochemistry represents the state-of-the-art in clinically

approved immunotherapeutic biomarker science. However, with an improved understanding of novel immunotherapeutic targets affecting alternative immunologic axes and cell types, as well as an improved understanding of the interplay between cancer neoantigens and the adaptive immune system, next-generation immune multiplex assays in development will foster drug discovery and development. Diagnostics assaying tumor mutational burden and transcriptome as predictive biomarkers of response to immune checkpoint blockade are in advanced development and will add substantially to the clinical diagnostic armamentarium to ensure patients are matched to their optimal immunotherapy. Novel blood-based biomarkers for immunotherapeutic response based on cell-free DNA and multiparametric flow cytometry represent active areas of research and an unmet clinical need to date.

Major biomarkers for immunotherapeutic response include:

- PD-L1 immunohistochemistry (IHC)
- Tumor mutational burden (including microsatellite instability, MSI-H)
- Immune infiltrate signature by RNA expression
- PD-L1/PD-L1/JAK2 genomic amplification
- Immune cell infiltrate (CD8+, Th1, memory)

Personalized medicine based on the targeting of important disease pathways has reinvented the field. Cellular immunotherapeutics, based on tumor infiltrating lymphocytes (TILs) expanded from the tumor or chimeric antigen receptor T cells (CAR-T) targeting extracellular cancer targets represent personalized immunotherapy—a form of therapy in which a patient's own cells are mobilized in a manner to fight their particular cancer, and can result in durable remissions. Novel therapeutic targets and cellular engineering methods that maximize efficacy while ameliorating serious toxicities are undergoing rapid clinical development, with the need for equally novel clinical trial designs given the promise of the agents. Given the personalized nature of these cellular therapeutics, paired with currently onerous costs, novel trial designs and regulatory pathways will be needed to ensure continued innovation in this space.

Combinations of immunotherapy with existing cancer approaches has led to novel observations on classical cancer therapeutics. Radiation, typically considered a form of tumor ablative therapy, can be harnessed to modify a tumor microenvironment and unleash cancer neoantigens in combination with immune checkpoint blockade—effectively converting radiation into a cancer vaccination modality. Similarly, cytotoxic chemotherapy can result in immunogenic cell death and heightened efficacy in combinations with immune checkpoint blockade. Efforts to combine these therapies while minimizing autoimmune toxicity and antagonistic chemotherapeutic effects on immune cells are under active clinical investigation. Finally, combinations of targeted therapy and immune checkpoint blockade can result in tumor killing and neoantigen release, as well as cell signaling modulation that can foster enhanced efficacy of immunotherapeutics. Such combinations can harness the relatively rapid response kinetics of targeted therapeutics with the potential for long-term durable benefit from the engendered immune response and sustained with immune checkpoint blockade.

Combinations of immunotherapeutics, in particular immune checkpoint blockade, have resulted in durable responses in melanoma as well as in non-small cell lung cancer, among other tumor types. An improved understanding of the tumor microenvironment and mechanisms of immune tolerance have led to the mechanistic-based use of immunotherapeutics in Hodgkin lymphoma and Merkel cell carcinoma. Further insights and therapies targeting novel immune cell types and pathways will be required in order to expand the promise of immunotherapy beyond the currently known histologies and molecularly-defied cohorts.

With the advent of an ever-expanding cadre of immunotherapeutics, early phase clinical trials investigating these agents will have to be as novel as the immunotherapeutics themselves. Many of the unique challenges related to the investigation of cancer immunotherapy are intertwined with their mechanism of action and inexorably linked to their efficacy. Ultimately, immunotherapeutics are based on the premise that the host immune system can successfully reject tumors—in other words, *the patient is both the host and the treatment for their cancer*. With a growing arsenal of promising immunotherapeutic agents, the inexorable goal of healing the wound that is cancer seems ever closer and reinforces a message well-known to family, friends, and caregivers of those fighting cancer—the most important aspect of any cancer therapy is already within the patient.

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Contents

Chapter 1
Primer on Cancer Immunotherapy and the Targeting of Native Proteins

Valentin Barsan and Paul C. Tumeh

Abstract Immunotherapy has notable potential for achieving durable clinical responses in many cancer types. The ability to readily measure the genomic landscape and infiltrating immune spectra of individual patient tumors offers mechanistic insights for combination therapy selection. Immunotherapeutic approaches through immune checkpoint blockade or stimulation, immune cell therapies, as well as tumor vaccination are being studied as mono and combination therapy in multiple cancer types. Uniquely, many immunotherapies target "native" self-proteins and thus herald a paradigm shift in cancer management in which the drug target is no longer an oncogenic protein but rather a normal signal that impacts the interactions of myriad immune cell types with both cancerous and normal cells. Native proteins in immunology are found in multiple isoforms with distinct interaction partners and at heterotypic transient cellular interfaces. Methods for evaluating the presence and function of native proteins for therapeutic targeting necessitates resolving for tumor–immune cellular interactions to understand which cell type is expressing which native protein isoform in the contextual (variably inflamed) tumor microenvironment. Just as tumor genomics has facilitated the selection of targeted therapies, precision immuno-oncology necessitates a comprehensive understanding of the immune system and the native proteins that govern its coordinated behavior. This primer on the relevant immunobiology, its clinical assessment, and therapeutic implications establishes a framework for conceptualizing the clinical advances in cancer immunotherapy that are the focus of this volume.

Keywords Immuno-oncology • Immunogenomics • Cancer immunotherapy • Checkpoint blockade • Immunobiology • Tumor biology • Adaptive immunity • Native proteins

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1.1 An Intersection of Oncology and Immunology

The tissues that form human organs are composed primarily of two symbiotic cellular components: the parenchyma and the stroma. The parenchyma establishes unique tissue function whereas the stroma comprises an admixture of resident tissue cells (fibroblasts, dendritic and mast cells), vascular and lymphatic endothelial cells, inflammatory cells (lymphocytes, macrophages, myeloid cells), regenerative mesenchymal stem cells, as well as structural matrix proteins and proteoglycans [1]. Healthy tissues maintain a dynamic balance of these composite cellular and structural components across time and despite environmental stressors to achieve resilient "youthful" organ function. However, genomic instability (germline and somatic variants) in cells results in the development of cancer hallmarks [2] and the accompanying loss and compromise of normal tissue function at which time patients present for clinical consultation. Beyond "driver" mutations [3] that establish key mechanisms for neoplastic progression, nonsynonymous somatic mutations (that alter the amino acid sequences of the proteins encoded by the altered genes) can encode the aberrant translation of a diverse set of peptide "neoantigens" that, when recognized as foreign, triggers tumor immunogenicity [4]. Rudolph Virchow first proposed a link between inflammation and cancer in the 1860s when he observed leukocytes infiltrating neoplastic tissues [5]. A century later, it was postulated that lymphocytes can recognize and eliminate aberrant cells [6, 7]. More recently, "immunoediting" has been proposed as an active process in which immune cells both eliminate cancerous cells through immuno-recognition yet simultaneously promote neoplastic progression secondary to collateral inflammation [8]. Each patient's cancer is therefore wholly unique – an evolutionary outcome of successive neoplastic cellular divisions within distinct tumor microenvironments shaped through time as much by the patient's immune system as by successive therapeutic interventions.

The presence, subtype, location, and density of infiltrating immune cells in the tumor microenvironment characterize the degree of tumor inflammation. Diverse immune cell subtypes of varying immune "fitness" within each tissue stroma [9] and in the lymphatic system facilitate the intricate intercellular processes of discriminating self from nonself. Feedback control through suppression of inflammation is equally important in tuning the nature of the immune response to counter neoplastic cellular behaviors with sufficient, yet limited, on-target responses. Cancer immunotherapy and autoimmunity are thus finely related and likely coexist along a clinical spectrum in which the discriminate recognition of self from nonself determines the efficacy and toxicity profile of immunotherapeutics. Cancer immunotherapy therefore entails harnessing the power of the immune system to eliminate cancerous cells while preserving the integrity and function of otherwise healthy tissue. Historically speaking, the cancer drug development paradigm has entailed designing one drug to target one protein which is usually mutated and specific to a tumor type. The paradigm shift in cancer immunotherapy extends beyond targeting immune cells instead of cancerous cells. Rather, coordinating tumor immunity

entails targeting native nonmutated proteins instead of oncogenes. These native proteins are expressed by different immune cell types of varying fitness, in multiple isoforms (with distinct interaction partners), across discrete tissue compartments, and at heterotypic and transient cellular interfaces. Therapies that target the immune system are thus fundamentally different in biologic mechanism, pharmacokinetics, and clinical application than therapies that target key cancer pathways. Conversely, therapies that target driver mutations in oncogene pathways of cancer cells can inadvertently dampen critical intracellular pathways in immune cell activation.

Targeting native proteins introduces a level of biologic and clinical complexity with which we have limited experience in oncology. Methods for evaluating the presence and function of native proteins necessitate resolving for tumor–immune cellular interactions to understand which cell type is expressing which native protein isoform in what contextual (inflamed or noninflamed) tumor microenvironment. Just as each cancer has a distinct mutational landscape so too each patient presents with a unique immune system whose fitness is shaped by genetics, age, vaccination and pathogen exposure history, as well as the environment. For example, epidemiologic studies associate the development of mumps in childhood with protection against ovarian cancer ostensibly due to primed immune surveillance [10]. Important environmental influences on the immune system and cancer progression are intuitive yet complexly interrelated. These include diet and exercise that can modulate gut/airway/skin microbiomes, UV/airborne/ingested carcinogens, and infectious exposures. In health, the immune cells can recognize both pathogens (i.e., viruses, bacteria, fungi, and parasites) as well as mutated cells to effectuate a targeted cytotoxic response with limited collateral inflammatory damage to surrounding tissues. When the immune system cannot effectively discriminate between self and nonself, autoimmune diseases (such as rheumatoid arthritis, diabetes, lupus) develop. The balance between self-tolerance and autoimmunity thus underpins the mechanisms by which immunotherapies have been applied to treat cancer. Our deepening understanding of the immune system at a molecular level has led to broad therapeutic advances in immunomodulatory monoclonal antibodies, cellular therapies, and vaccination strategies that are now being studied in all cancer types alongside conventional approaches of surgery, chemotherapy, and radiation. Understanding when, where, and how the diverse cells of the immune system interact to mount a coordinated cytotoxic immune response against cancer establishes the foundation for implementing these insights in clinical settings.

1.2 Innate and Adaptive Immunity

An effective and specific cytotoxic immune response against a tumor is coordinated by multiple cross-priming agonist and antagonist signals coordinated between varied cells of the innate and adaptive immune systems [11, 12]. These systems are comprised of more than 200 immune cells types and more than 300 immune cell state

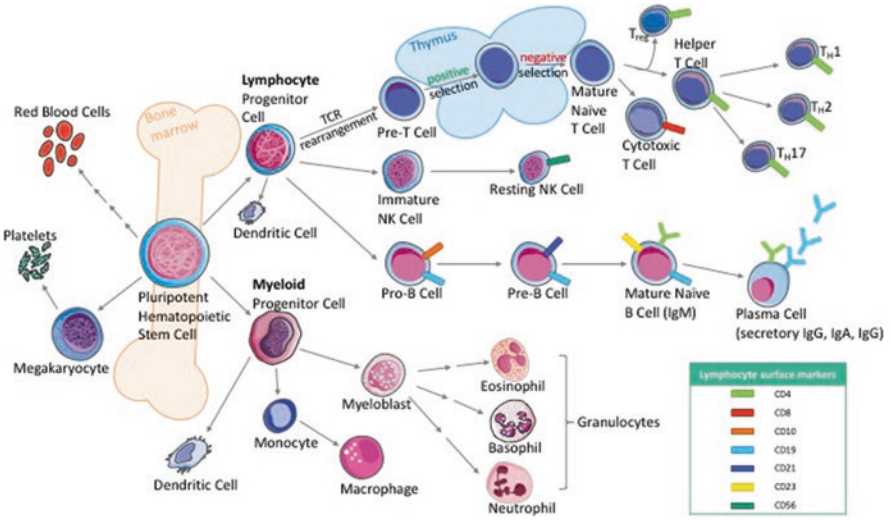

Fig. 1.1 Immune cell growth and differentiation. Cells of the immune system differentiate across myeloid or lymphoid lineages from hematopoietic stem cell precursors in the bone marrow. Hundreds of additional cell types and intermediate states exist. Partial maturation of T cells in the thymus and B cells in the bone marrow is followed by further differentiation in peripheral lymphoid tissues throughout development. Lymphocytes are commonly characterized by the surface expression of cluster of differentiation (CD) markers as well as the types of cytokines or antibodies produced

transitions [13]. All cells of the immune system differentiate (that is, increasingly functionalize) across myeloid or lymphoid lineages from hematopoietic stem cell precursors in the bone marrow (Fig. 1.1). Cells of the myeloid lineage include red blood cells, platelets, granulocytes (eosinophils, neutrophils, basophils), mast cells, and macrophages. Cells of the lymphoid lineage include natural killer (NK) cells, T cells (γδ, NK, $CD4^+$ and $CD8^+$ subsets), and B cells. Antigen-presenting dendritic cells may derive from either myeloid or lymphoid lineages. The myeloid and lymphoid lineages are functionally characterized by innate or adaptive cellular behaviors. The *innate* component includes most immune cells of the myeloid compartment as well as NK cells whereas the *adaptive* component consists solely of lymphoid (B and T) cells and their myriad subtypes. Partial maturation of T cells in the thymus and B cells in the bone marrow in utero is followed by further differentiation in peripheral lymphoid tissues after birth and attainment of immunocompetency under antigenic stimulus. Immune cells and their degree of differentiation are commonly characterized by expression of surface clusters of differentiation (CD) or the types of cytokines they secrete. Adaptive immunity is defined by the ability to discern and remember immunologic threats based on foreign, mutated, or atypically expressed antigens. At baseline, both components of the immune system are “on alert” until a threat has been identified at which time, rapid innate immune activation occurs and

primes an adaptive response. Because each major immune cell type has an active and a regulatory form, the balance between these states characterizes the quality of an immune response.

Cells of the *innate* immune system use generic methods to recognize foreign pathogens based on nonspecific and nonhuman molecular patterns such as single-stranded RNA or lipopolysaccharide. Germline-encoded non-self-reactive receptors on neutrophils, macrophages, natural killers, and mast cells respond to generalized pathogen-associated molecular patterns (PAMPs) such as mannose receptors or toll-like receptors shared by many classes of microbes [14]. Innate cells such as macrophages and neutrophils migrate into tissues though expression of high-affinity integrin, kill microbes through phagocytosis and reactive oxygen species (triggered by interferon-γ), induce inflammation (through tumor necrosis factor, IL-1 and IL-6), activate T cells and NK cells (through IL-12), and initiate tissue repair (through secretion of immunosuppressive interleukins, TGF-ß, and fibroblast growth factors). Innate immunity defense mechanisms further include the complement cascade and inflammation. The complement system is comprised of nine major factors (C1 to C9), most of which are pro-enzymes present in normal serum and not increased by antigenic stimulation. The complement cascade facilitates inflammation, leukocyte recruitment, anaphylatoxin production, mast cell degranulation, opsonization for phagocytosis, secondary signals for B-cell activation, and the formation of membrane attack complexes against pathogenic cells. Tissue inflammation stimulates the adaptive immune response, enables the elimination of invasive foreign pathogens through controlled passage of immune cells, and initiates tissue repair.

Tissue inflammation also influences the resident cells within a tumor microenvironment. In an environment of chronic inflammation, myeloid cell differentiation can be skewed [15] toward the expansion of myeloid-derived suppressor cells (MDSCs). MDSCs are a heterogeneous subpopulation of immune cells (including macrophages, neutrophils, and dendritic cells) with potent immunosuppressive functions. Whereas M1 macrophages release interferon-γ and are responsible for phagocytosis, M2 macrophages release cytokines (IL-4, IL-10, TGF-ß) that curtail inflammatory responses and foster immune tolerance [16]. Macrophages also serve as important regulators of tumor angiogenesis by producing various pro-angiogenic molecules such as erythrocyte growth factor (EGF) and vascular endothelial growth factor (VEGF). Tumors can foster immuno-tolerance in the microenvironment through the manipulation of cytokines (increased secretion of IL-6, IL-10, and TGF-ß; consumption of IL-2) that encourage infiltration of inhibitory immune cells such as MDSCs and regulatory T cells (Tregs). Several therapeutic approaches (PDE5 inhibitors, COX-2 inhibitors, ARG1 inhibitors, bisphosphonates, gemcitabine, and paclitaxel, among others) play a complementary role in promoting antitumor immune responses by inhibiting the function or proliferation of MDSCs [17]. Immune cells also acquire distinct metabolic characteristics [18] that influence the plasticity of their immunological phenotypes and functions.

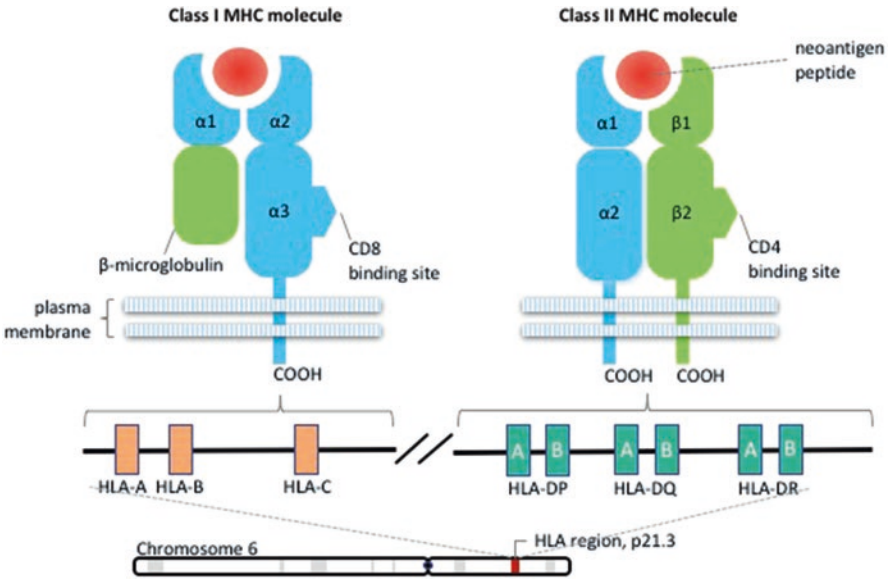

Fig. 1.2 Class I and Class II MHC Molecules. The maternal and paternal HLA haplotypes are located at chromosome 6, on the short arm at position p21.3, and encode the genes for MHC. HLA haplotypes are codominantly expressed. Both MHC Class I and MHC Class 2 consist of an alpha (heavy) and a beta (light) chain. The class I HLA molecule contains an alpha chain anchored to the cell membrane. The peptide antigen of 8 to 11-mer amino acids (red) is presented in a groove formed from a pair of alpha-helicies on a floor of antiparallel beta strands. The class I alpha chains are coded for by genes within the MHC (e.g., HLA-A, HLA-B), whereas the beta chain, beta-2 microglobulin, is encoded on chromosome 15, not in the MHC. The class II HLA molecule is MHC-encoded by both alpha and beta chains each anchored to the cell membrane without beta2-microglobulin. The peptide antigen of ~15-mer amino acids is presented in a groove formed from a pair of alpha helices on a floor of antiparallel beta strands. Class II antigens are constitutively expressed on B cells, dendritic cells, and monocytes and can be induced during inflammation on many other cell types that normally have little or no expression. Genes within the MHC (e.g., HLA-DP/Q/R) code for both chains

1.3 Orchestrating Adaptive Immunity

All human cells express a cell-surface major histocompatibility complex (MHC) that is genetically encoded by the human leukocyte antigen (HLA) locus. HLAs are inherited as haplotypes from both parents and expressed co-dominantly as MHC on all cells (Fig. 1.2). The MHC thus functions as an authenticating cell surface complex that physically presents peptides to adaptive immune cells [19] and enables the immune system to distinguish between self and nonself. HLA typing has thus enabled the matching of transplanted organs [20] and cells to minimize rejection. The HLA locus contains more than half of the four to five million single nucleotide polymorphisms (SNPs) in each individual genome [21]. This genomic variability implies enormous diversity in any given patient's relative immune fitness and

susceptibility to immunologic disorders or infectious agents. MHC diversity explains why tissue transplantation remains so challenging and perhaps as well why autoimmune and infectious susceptibilities cluster by subtype. Proteins encoded by the three key MHC class I genes (HLA-A, HLA-B, and HLA-C) are present on the surface of most cells to present peptides that are internally processed and exported from inside the cell. MHC class I thus facilitates immune surveillance of intracellular pathogens or aberrant proteins. Cells that do not express MHC are indiscriminately attacked by NK cells of the innate immune system. Downregulation of MHC by cancer cells suggests a therapeutic utility of NK cell therapy [22]. The six main MHC class II genes (HLA-DPA, HLA-DPB, HLA-DQA, HLA-DQB, HLA-DRA, and HLA-DRB) encode cell-surface proteins that display peptides derived from circulating, extracellular proteins to the immune system. MHC class II molecules are expressed only on antigen-presenting cells (APCs), such as dendritic cells.

APCs are activated by recognition of antigens that bind surface MHC which induces downregulation of cell-adhesion molecules to facilitate migration from the tissue of residence to a lymph node for antigen presentation to residing adaptive immune T and B lymphocytes. APCs serve as the critical link for priming the adaptive immune cells. Dendritic cells and macrophages are "professional" APCs and critically link the innate and adaptive immune systems. Since their discovery in 1973 [23], dendritic cells have been shown to develop from either myeloid or lymphoid hematopoietic lineages which thereby creates distinctive subsets of dendritic cells that have discreet functions tuned by their tissue of residence and microenvironment (these nuances are especially relevant in vaccine development). The main dendritic subtypes include plasmacytoid DCs (pDCs) and conventional DCs (cDCs). Both pDCs and cDCs are comprised of additional subtypes that have discrete morphology, tissue distribution, surface marker expression, and cytokine production which consequently lead to distinct pathways to T-cell activation. Also, tumor-associated macrophages are ontogenetically diverse [24] and specially tuned to the function of their host tissue. APCs such as dendritic cells or macrophages phagocytose (engulf) and process antigens released from tumor cells to present them to T and B cells. Engagement of the T- or B-cell receptor with MHC peptide is a necessary first step in lymphocyte cell activation. The complementarity determining region (CDR) determines the specificity of a lymphocyte receptor to its cognate antigen. T lymphocytes express clonal T-cell receptors (TCRs) on their surface that recognize antigenic peptides presented by host cells whereas B-cell receptors (BCRs) are secreted as soluble antibodies (immunoglobulins) upon antigen recognition. Lymphocyte receptors also exhibit tremendous genetic diversity to enable the recognition of so many potential antigens presented by MHC. The generation of diverse TCRs and BCRs begins with immature T and B lymphocytes through VDJ recombination, a process in which *germline* DNA is spliced to recombine noncontiguous variable (V), diversity (D), and joining (J) region gene segments and collectively encode the complementarity determining region 3 (CDR3) [25] of a given naïve (antigen inexperienced) lymphocyte. Diversity of the CDR3 region is increased by the deletion and template-independent insertion of nucleotides at the V-D and D-J junctions and further through somatic hypermutation in the BCR.

These receptors recognize residues of peptide antigens in MHC as well as polymorphic residues of the MHC molecule itself. An estimated 100,000–750,000 peptide-MHC class I complexes are expressed for each allelic product (HLA-A and HLA-B loci) [26], and each individual carries ~10^7 different TCRs [27] each detecting up to 10^6 variations of a given peptide sequence [28].

Immature T and B cells must subsequently demonstrate the ability to discern between harmful and innocuous antigens through a tolerance process prior to their release into circulation. B or T cells optimally recognize only one antigen. Developing T cells undergo tolerance and maturation in the thymus whereas B cells do so in the bone marrow. To establish immunologic tolerance in these organs, immature T and B cells undergo positive selection (weak receptor interaction with self-antigen allows for cell survival) and negative selection (lymphocytes that bind too strongly to self-antigens are signaled to die). Randomly generated TCRs and BCRs recognizing endogenously expressed self-epitopes (peptide/MHC "ligandomes") are variably pruned in the thymus [29] and marrow [30], respectively, during their development to limit immunological self-destruction. Mature lymphocytes continuously recirculate between blood and peripheral lymphoid tissues, localizing and extravasating into tissues when guided by chemokine gradients from tissue-resident sentinel innate immune cells. The patient's adaptive immune cells are thus finely tuned within a discrete range of binding affinities – a process that when disrupted can result in autoimmunity or when engineered ex vivo enables potent cellular therapies. Paradoxically, self-reactive adaptive immune cells theoretically comprise an autogenous source of potential anticancer activity. Autoimmunity eliminates cancerous cells.

Through interaction with APCs, the lymphoid cells of the *adaptive* immune system evolve with exquisite specificity to surface and soluble antigens through selective clonal expansion of T and B lymphocytes. The tumor draining lymph node is a more immuno-active microenvironment in which high throughput antigen exposure by APCs to standby lymphocytes occurs. In lymph nodes, naïve T and B cells recognize tumor antigens and can become activated. The mode of cancer cell death (apoptosis versus necrosis) influences the degree and quality of antigen spreading [31], in which previously intracellular immunogenic antigens are released because of cell lysis [32] thereby broadening antitumor responses to additional antigens. T cells exert immune effects through cellular interactions whereas B cells become activated upon antigen recognition to differentiate into antibody-producing plasma cells. Secreted antibody subtypes (immunoglobulins) are frequently measured in infectious diseases as titers and clinically indicate primary versus repeat/historical antigen exposures. B-cell homing areas enable rapid antibody secretion and are found primarily in the splenic follicles, marrow pulp, lymph nodes, and mucosal-associated tissues. Mature B cells are educated (antigen-specific) APCs that present to effector CD4 T cells via MHC-II, who will in turn activate B cells to undergo "class switching" and "affinity maturation" to produce clonal circulating antibodies of varying kinetics and increasing potency. A rapid adaptive immune response is initiated by T and B cells if the presented antigen has been recognized previously.

Both tumor and transplant rejection are mediated mainly by cytotoxic T lymphocytes. T-cell activity is controlled by a combination of antigen-specific signals from the TCR as well as antigen-independent signals from myriad co-receptors [33]. The TCR binds specific short stretches of amino acids (i.e., peptides) presented by MHC molecules located on all host cells, and notably APCs (Fig. 1.3). VDJ recombination produces a TCR that is composed of two different proteins chains (α and ß whose ratios change throughout cellular maturation as well as in diseased states) and CD3 which encodes an invariant transmembrane protein complex that relays surface signals for secretion of pro-inflammatory cytokines such as IL-12 and interferon gamma. The MHC molecules expressed in the thymus restrict a mature T cell to a predetermined spectrum of antigens. Each T cell expresses monoclonal membrane-bound TCRs that all recognize the same specific peptide/MHC complex during physical contact between the T cell and an APC (MHC class II) or any host cell (MHC class I). T-cell subtypes are characterized broadly by their co-receptors: CD4 on helper and regulatory T cells is specific for MHC class II whereas CD8 on cytotoxic T cells is specific for MHC class I. The subtypes of effector $CD4^+$ and $CD8^+$ T cells are often characterized by the specific cytokines (interleukins) produced upon their activation. Activated $CD8^+$ (killer) T cells engage in direct cytotoxic activity whereas activated $CD4^+$ (helper) T cells support other lymphocytes, for example, by promoting the maturation of B cells into plasma cells and memory B cells and activating cytotoxic T cells and macrophages. To mount an effective immune response beyond activation through MHC-peptide and TCR binding, T cells require additional costimulatory signals. A critical priming costimulatory signal in naïve T cells is CD28, which binds to B7-1 and B7-2 (CD80/86) on the APC [34]. Without CD28:B7 interaction, the naïve T cell remains anergic (refractory to activation or unresponsive). The most differentiated effector and memory (antigen-experienced) T cells [35] are least dependent on costimulatory signals due to avidity maturation that reduces the activation threshold of these subtypes.

Once activated, T cells reduce expression of CD28 and upregulate surface immune checkpoint molecules which are native proteins that facilitate feedback inhibition and limit cytotoxic activity. Unrestrained T-cell activation would otherwise lead to malignant proliferation or autoimmune disease. CTLA-4 is one such *inducible* surface checkpoint molecule that is upregulated on T cells after activation, has higher affinity for the ligands CD80 and CD86, and is also constitutively expressed on a variety of Tregs [36]. CTLA acts as an "off switch" when bound to CD80 or CD86 on the surface of APCs. CTLA-4 blockade hence produces both a direct enhancement of T-cell effector function and a concomitant inhibition of regulatory T-cell activity [37]. Programmed cell death-1 (PD-1) is expressed later and functions as an inhibitory homologue of CD28 following T-cell activation. A key mechanism by which cancer cells diffuse the host immune response is the upregulation of PD-1 that bind to PD-1 on tumor-specific CD8+ T cells [38] as well as NK T cells and B cells. PD-1 is a member of the extended CD28/CTLA-4 family of T-cell regulators that is highly expressed on *activated* T cells whose two ligands PD-L1 and PD-L2 have been found to be expressed as immuno-escape behaviors of several cancers. PD-L1 and PD-L2 are also expressed on cells of the immune system

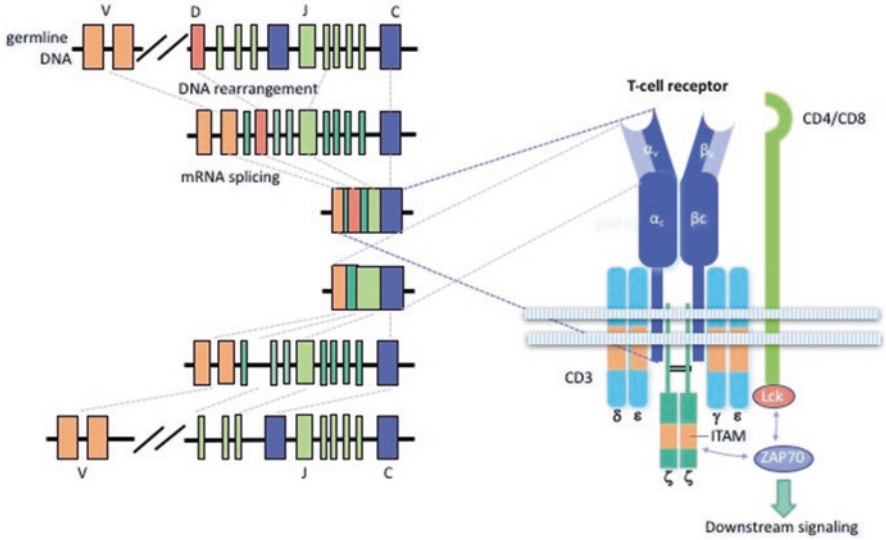

Fig. 1.3 The T cell receptor. The mature T cell heterodimer consists of alpha- and beta-subunit chains that are formed by rearranged germline DNA of variable (V), diversity (D), joining (J), and constant (C) regions. The TCR alpha chain is generated by VJ recombination, whereas the beta chain is generated by VDJ recombination. Signalling is initiated by aggregation of TCR by MHC-peptide complexes on APC. Costimulation is required from CD4 on helper-T cells or CD8 on cytotoxic T cells. The intracytoplasmic region of the TCR is too short to transduce a signal from the cell surface so CD3 facilitates signalling through the TCR. Once MHC-peptide binds the TCR, lymphocyte cell-specific protein tyrosine kinase (Lck) is activated and phosphorylates tryosine residues within the immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3 and zeta chains, enabling zeta chain-associated protein kinase 70 (ZAP-70) recruitment to the TCR which triggers downstream signaling events required for T cell activation

(upregulated on macrophages and DCs in response to bacterial lipopolysaccharide as well as activated T, B, and NK cells). PD-L1 can also interact (like CTLA-4) with the CD80 receptor on T cells, sending a further immunosuppressive signal. In addition, PD-L1 is also expressed constitutively on nonlymphoid tissues such as the heart, lung, placenta, and skeletal muscle where it may serve to downregulate TCR signaling in PD-1+ cytotoxic T-lymphocytes and therefore protect against autoimmune-mediated tissue damage. Multiple additional co-receptors that modulate T-cell activation and inhibition have become the central focus of checkpoint blockade or stimulation (Fig. 1.4). The activation of T-cell subtypes is dependent on the balance of antagonist (e.g., CTLA-4, PD-1, LAG3, TIM3) and agonist native proteins (e.g., GITR, OX40, ICOS) on *both* the APC and T cells [33]. The therapeutic targeting these native proteins implies modulating complex cellular interactions both within the tumor bed and in lymphoid organs where APCs and T cells interact to amplify immune responses. Antibodies that mimic or block the effect of these checkpoint or agonist receptors or ligands aim to enhance the immune response against tumor cells. Chronic recognition of an antigen (such as that present in a malignant clone or