CHAPTER 1
The Immune Response to a Transplanted Organ: An Overview

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Basic definitions

Organs transplanted between two members of the same species are rejected unless the donor and recipient are genetically indistinguishable (identical twins in the case of humans). Rejection is caused by the recipient's immune response to foreign elements present on the transplanted organ. These elements are usually proteins that differ between the donor and recipient and are called "alloantigens." The transplanted organ itself is referred to as the "allograft" and the immune response mounted against it as the "alloimmune response" or "alloimmunity." The prefix "xeno," on the other hand, is used to denote the transplantation of organs between members of different species, as in the terms xeno-antigens, xenografts, and xenotransplantation.

The principal players

The T lymphocyte is the principal mediator of the alloimmune response [1, 2]. Experimental animals devoid of T cells do not reject tissue or organ allografts [3, 4]. Similarly, T cell depletion in humans prevents rejection effectively until T cells return to the circulation [5]. T cells cause direct injury to the allograft through a variety of cytotoxic molecules or cause damage indirectly by activating macrophages and other inflammatory cells (Chapter 3). T cells also provide help to B lymphocytes to produce a host of antibodies that recognize alloantigens ("alloantibodies"). Alloantibodies inflict injury on the transplanted organ by activating the complement cascade or by activating macrophages and natural killer cells (Chapter 4). An exception to the T cell requirement for allograft rejection is the rapid rejection of organs transplanted between ABO blood-group-incompatible individuals. In this case, allograft destruction is mediated by preformed anti-ABO antibodies that are produced by B-1 lymphocytes, a subset of B cells that are activated independent of help from T cells. Another potential mechanism of T-cell-independent rejection is graft dysfunction mediated by monocytes. This has been observed in renal transplant recipients after profound T cell depletion [5], but it is unlikely that monocytes lead to full-blown rejection in the absence of T cells or preformed antibodies.

The principal alloantigens recognized by T cells, B cells, and antibodies are the human leukocyte antigens (HLAs). These are cell-surface proteins that are highly variable (polymorphic) between unrelated individuals. Two main classes of HLA proteins have been identified. Class I molecules (HLA-A, -B, and -C) are expressed on all nucleated cells, whereas class II molecules (HLA-DP, -DQ, and -DR) are present on cells of the immune system that process and present foreign proteins to T cells;

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these are referred to as antigen-presenting cells (APCs) and include B cells, dendritic cells, macrophages, and other phagocytic cells (Chapter 2). In humans, activated T cells and inflamed endothelial cells also express class II molecules. Since HLA inheritance is codominant, any given individual shares one haplotype (one set of alleles) with either biological parent and has a 25 % chance of being HLA-identical (sharing both haplotypes) with a sibling. The chance that two unrelated individuals are HLA-identical is less than 5 %, because of the highly polymorphic nature of the HLA. Although HLA matching between donor and recipient confers long-term survival advantage on grafts [6], it does not in any way obviate the need for immunosuppression. The immune system is, in fact, capable of recognizing any non-HLA protein that differs between the donor and recipient as foreign and of mounting an alloimmune response to it that is sufficient to cause rejection. Non-HLA proteins that trigger an alloimmune response and are targeted during allograft rejection are referred to as "minor histocompatibility antigens" (Chapter 2). It is likely that a large number of minor antigens exist, making it very difficult to match for them.

Types of rejection

Pathologists have traditionally divided allograft rejection into three groups based on the tempo of allograft injury: hyperacute, acute, and chronic. Hyperacute rejection is a very rapid form of rejection that occurs within minutes to hours after transplantation and destroys the allograft in an equally short period of time. It is triggered by preformed anti-ABO or anti-HLA antibodies present in the recipient [7, 8]. Blood typing and clinical cross-matching, whereby preformed anti-HLA antibodies are screened for by mixing recipient serum with donor cells, or more commonly nowadays by sensitive flow-cytometric methods, has virtually eliminated hyperacute rejection. Acute rejection, in contrast, leads to allograft failure over a period of several days rather than minutes or hours. It usually occurs within a few days or weeks after transplantation, but it could happen at much later time points if the immune system is "awakened" by infection or by significant reduction in immunosuppression. Chronic rejection is a slow form of rejection that primarily affects the graft vasculature (or the bronchioles and bile ducts in the case of lung and liver transplants respectively) and causes graft fibrosis. Chronic rejection may become manifest during the first year after transplantation, but more often progresses gradually over several years, eventually leading to the demise of the majority of transplanted organs, with the exception perhaps of liver allografts. Since acute and chronic rejections are caused by T cells, antibodies, or both, it is increasingly common to label rejection by its predominant immunological mechanism, cellular or antibody mediated, in addition to its temporal classification (Chapters 3 and 4). Rejection is also graded according to agreed-upon criteria known collectively as the Banff classification [9]. These are important advances in transplantation pathology, as they often guide the choice of anti-rejection treatment and are used as prognosticators of long-term allograft outcome.

Distinguishing features of the alloimmune response

Although alloimmune responses resemble antimicrobial immune responses in many ways, they are distinguishable by several salient features. These features are highlighted here, as they have direct implications for the development of anti-rejection therapies.

Alloimmune responses are vigorous responses that involve a relatively large proportion of the T cell repertoire

Humans carry a large repertoire of T lymphocytes that recognize and react to virtually any foreign protein with a high degree of specificity. The diversity of T cell reactivity is attributed to the random rearrangement during T cell ontogeny of genes that code for components of the T cell receptor (TCR) for antigen (Chapter 3). The same applies to B cells, leading to an immense variety of

antibodies that detect almost any conceivable foreign antigen (Chapter 4). The high specificity of T cells is explained by the fact that TCRs do not recognize whole antigens; instead, they recognize small peptides derived from foreign proteins and presented in the context of HLA molecules on antigen-presenting or infected cells (Chapter 2). This leads to fine molecular specificity in which only a very small proportion of T cells react to a non-self peptide. It is estimated that only 1 in 10 000 or less of all T cells in a human being recognize peptides derived from any given microbe. The small proportion (or precursor frequency) of microbe-specific T cells is nevertheless sufficient to eliminate the infection because of the ability of T lymphocytes to proliferate exponentially (a phenomenon referred to as clonal expansion) before differentiating into effector cells. In sharp contrast, the immune response to an allograft involves anywhere between 1 and 10 % of the T cell repertoire [10, 11] – essentially 10–100 times more than an antimicrobial response. The large-scale participation of T cells in the alloimmune response can be readily demonstrated in the mixed lymphocyte reaction (MLR), a laboratory test in which coculturing recipient peripheral blood mononuclear cells (PBMCs) with donor PBMCs results in conspicuous proliferation of recipient T lymphocytes. Detecting T cell proliferation against microbial antigens, on the other hand, is a much more difficult feat because of the low precursor frequency of microbe-specific lymphocytes. Alloimmune responses, therefore, are especially vigorous responses because of the participation of a significant proportion of T cells with a wide range of specificities. The reasons for this phenomenon, perhaps the dominant obstacle to improving allograft survival without unduly compromising the recipient's immune system, are explained next.

T cell alloreactivity is cross-reactivity

The immune system has evolved to protect animals against infection. It is not surprising, therefore, that humans and most other vertebrate species are armed with T cells that recognize microbial antigens. Why is it, then, that we also carry a disproportionately large proportion of T cells that react to alloantigens? Based on cellular and molecular studies in humans and experimental animals, it has become evident that TCRs specific for a microbial peptide (presented in the context of self-HLA) are also capable of recognizing allogeneic, non-self HLA [11]. This phenomenon is known as cross-reactivity or heterologous immunity and has been best demonstrated for T cells specific to Epstein–Barr virus (EBV) antigens [12]. The same is likely to be true of T cells specific to other viruses. The inherent ability of developing T cells to bind to HLA molecules also contributes to the high precursor frequency of alloreactive T cells in the mature T cell repertoire [13]. The inherent bias to generate TCRs that "see" HLA is attributed to the fact that T cell education in the thymus and the ultimate development of a mature cellular immune system are dependent on recognition of peptides bound to HLA (Chapter 3). Therefore, alloreactivity is an unintended side effect of an immune system that has evolved to effectively fend off foreign, generally microbial, antigens.

T cell alloreactivity is in large part a memory response, even in naive individuals not previously exposed to alloantigens

The primary immune response to a foreign antigen not previously encountered by the host is mediated by naive T lymphocytes (Chapter 3). Naive T cells specific to the foreign antigen are present at a low precursor frequency, have a relatively high stimulation threshold (e.g., stringent dependence on costimulatory molecules), can only be activated within secondary lymphoid tissues (e.g., the spleen and lymph nodes) [14], and are, therefore, slow to respond. In contrast, the secondary immune response to an antigen previously encountered by an individual (e.g., after vaccination or infection) is mediated by memory T cells and is significantly stronger and faster than a primary response. Antigen-specific memory T cells are long-lived lymphocytes that exist at a greater precursor frequency than their naive counterparts, have a low stimulation threshold and high proliferative capacity, and can be activated within secondary lymphoid tissues or at non-lymphoid sites – for

example, the site of infection or in the allograft itself [15]. Memory B cells and plasma cells share some of the properties of memory T cells thus, endowing vaccinated individuals with the ability to rapidly produce high titers of antigen-specific antibodies upon reinfection (Chapter 4). Immunological memory, therefore, provides humans with optimal protection against microbes.

Humans for the most part are not exposed to alloantigens, with the exception of mothers who may have been sensitized to paternal antigens during pregnancy or individuals who had prior transfusions or organ transplants. Yet all humans, including those presumably never exposed to allogeneic cells or tissues, harbor alloreactive memory T cells. Accurate quantitation of alloreactive T cells has demonstrated that approximately 50 % of the alloreactive T cell repertoire in humans is made up of memory T lymphocytes [11, 16, 17]. This finding can again be explained by the phenomenon of cross-reactivity, whereby memory T cells specific to microbial antigens also recognize alloantigens and contribute to the high precursor frequency of alloreactive T cells. Therefore, the extent of one's alloreactivity is intimately shaped by one's immunological memory to foreign antigens not necessarily related to the graft.

The distinguishing features of alloimmunity summarized above have important implications for both the immunological monitoring of transplant recipients and the development of anti-rejection therapies. It is becoming increasingly clear that measuring anti-donor memory T cells or donor-specific antibodies either before or after transplantation could predict rejection incidence and graft outcomes [18]. Moreover, T-lymphocyte-depleting agents used to prevent rejection invariably skew T cells that repopulate the host towards memory [19, 20]. These memory T cells arise from antigen-independent, homeostatic proliferation of undepleted naive or memory T cells – a phenomenon known as lymphopenia-induced proliferation [21]. Lymphopenia-induced T cell proliferation is responsible for early and late acute rejection episodes in lymphocyte-depleted transplant recipients and creates an obstacle to minimizing immunosuppression [22]. Another clinical implication of alloreactive memory T cells is that anti-rejection agents that inhibit naive lymphocyte activation or migration are not expected to be as effective as those that suppress both naive and memory lymphocytes. Targeting memory T or B cells, therefore, is desirable but leads to the important conundrum of how to inhibit alloreactivity without compromising beneficial antimicrobial memory. Overcoming this challenge could pave the path towards developing the next generation of immunotherapeutic agents in transplantation.

Immune regulation

The alloimmune response is subject to regulatory mechanisms common to all immune responses. Four principal regulatory mechanisms have been described: activation-induced cell death (AICD), regulation by specialized lymphocyte subsets known as T_{REG} and B_{REG}, anergy, and exhaustion. These mechanisms ensure that "collateral damage" to the host is kept to a minimum during or after a productive immune response.

Primary and secondary T cell responses are characterized by exponential proliferation of antigen-specific T cells followed by a "crash" phase in which the majority of activated or effector T cells die by apoptosis (Plate 1.1). This process prevents unnecessary immunopathology while allowing T cells that escape apoptosis to become memory lymphocytes. The same is true for B cells, where the process of expansion followed by death allows for the selection of B lymphocytes with the highest affinity to their target antigens (affinity maturation) (Chapter 4). Most immunusuppressive drugs available for clinical use target lymphocyte proliferation and in some cases (e.g., calcineurin inhibitors) prevent AICD [23], leaving the possibility of developing agents that selectively enhance the apoptosis of activated T cells open. Such a strategy would be more specific than pan-T-cell depletion, as only T cells that have been activated by alloantigens are killed.

The isolation of T and B cell subpopulations that downregulate immune responses in vitro and in vivo has led to a resurgence of studies on regulatory lymphocytes (Chapter 6). T_{REG} and B_{REG} populations

have been identified in rodents and, in the case of the former, in humans as well. Regulatory lymphocytes suppress mixed lymphocyte reactions in vitro and prolong allograft survival in rodent transplantation models. The mechanisms by which T_{REG} suppress immune responses are varied. They include cytokines (e.g., IL-10 and TGFβ), inhibitory membrane molecules (e.g., CTLA-4), and possibly direct cytotoxicity to naive or effector lymphocytes. In addition to interest in isolating and expanding T_{REG} for adoptive cell therapy in transplant recipients, there has been an important focus on developing or exploiting existing immunosuppressive drugs that spare or enhance regulatory lymphocytes. One example is the mTOR inhibitor rapamycin, which in mice generates a favorable T_{REG} to effector T cell ratio that may contribute to long-term allograft survival. It is not certain, however, whether the salutary effects of rapamycin on T_{REG} in rodents will translate to longer allograft survival in humans because of the pleiotropic functions of mTOR signaling in different cells of the immune system.

Anergy and exhaustion refer to the state in which T cells or B cells become unresponsive to restimulation with antigen. Anergy occurs when naive lymphocytes encounter antigen in the absence of critical costimulatory signals necessary for their full activation. A prime example of costimulation is the B7–CD28 pathway (Chapter 3). B7 molecules expressed on antigen-presenting cells engage CD28 on T cells concurrent with T cell stimulation through the TCR. Blocking B7-CD28 interaction renders T cells anergic and/or induces their apoptosis [24, 25]. CTLA4-Ig, a fusion protein that binds B7 molecules and prevents them from engaging CD28, is currently approved for use in renal transplant recipients. Published data suggest that CTLA4-Ig may be an effective substitute for calcineurin inhibitors. Finally, exhaustion occurs when effector or memory T cells repeatedly encounter a persistent antigen, as would occur during chronic viral infection or in the case of an allograft. Repeated antigenic stimulation induces the expression of inhibitory molecules that keep T cells hypo- or un-responsive. One example of such inhibitory molecules is PD-1, shown in rodents to suppress alloreactive effector T cells [26]. These regulatory pathways provide interesting opportunities for developing novel strategies to inhibit T cells that have been activated by alloantigens. By targeting activated but not naive T cells, these strategies may prove more selective than currently available immunosuppressive therapies.

The innate immune system in transplantation

The mammalian immune system consists of two integrated arms: the innate and adaptive.

The adaptive immune system (the subject of discussion of this chapter so far) consists of T and B lymphocytes which express diverse and highly specific antigen receptors brought about by gene rearrangement, expand clonally, and generate immunological memory. Unlike the adaptive system, the innate immune system is made up of inflammatory cells (dendritic cells, monocytes, macrophages, neutrophils, eosinophils, basophils, and other cells) that do not express rearranging receptors, have limited proliferative capacity, and, for the most part, do not generate memory. Cells of the innate immune system instead express nonrearranging, germ-line-encoded receptors that detect conserved molecular patterns present in microbes but not shared by mammalian cells [27]. A representative example of innate receptors is toll-like receptor (TLR)-4, which recognizes lipopolysaccharide on Gram-negative bacteria (Chapter 5). It should be noted that the innate immune system also encompasses noncellular mediators capable of microbial recognition – for example, complement proteins. Activation of the innate immune system by microbial ligands causes inflammation, the first line of defense against infection, but more importantly induces the maturation and migration of antigen-presenting cells to secondary lymphoid tissues where they trigger primary T cell and B cell responses. The latter function of the innate immune system is critical for initiating adaptive immunity to infection and vaccines in the naive host. The innate immune system, therefore, is responsible for the first self–non-self recognition event that ultimately leads to productive T and B cell immunity.

Although the innate recognition pathways required for establishing antimicrobial immunity have been uncovered for many infectious diseases, how the innate immune system triggers the adaptive alloimmune response is not as straightforward. Several endogenous ligands released by dying cells in the graft participate in ischemia–reperfusion injury (Chapter 6), but it is not clear whether any single ligand has a dominant role or whether any are critical for triggering either naive or memory T cell activation. These uncertainties could be due to the release of myriads of redundant activators of the innate immune system by the graft at the time of transplantation or due to the possibility that memory T cell activation, an important component of the alloimmune response, could occur independent of innate immune activation. Nevertheless, it is generally accepted that inflammation influences the migration of effector and memory T cells to the transplanted organ and increases the intensity of rejection [28]. Prolonged cold or warm ischemia not only predisposes allografts to delayed function after transplantation, but also to increased risk of acute and chronic rejection [29]. Recent studies have suggested that the innate immune system may be capable of distinguishing between self and allogeneic non-self [30, 31], akin to its role in detecting microbial non-self. This intriguing possibility could imply that an innate allorecognition system that precedes allorecognition of HLA by the adaptive immune system maintains immunity against allografts long after the early inflammatory phase has subsided. The nature of such innate allorecognition and whether it contributes to either acute or chronic rejection remains to be determined.

Concluding remarks

The immune system is composed of rich layers of cellular and humoral mediators that work in concert to protect humans against potentially fatal infections. One price that humans pay for this highly developed defense system is the rejection of life-saving organ transplants. Better understanding of the regulatory mechanisms embedded in the immune system and of the subtle distinctions between antimicrobial and alloimmunity should pave the path towards selective immunotherapies that prevent rejection but preserve beneficial immunity against infection. Studying the immune system is like peeling an onion: beneath each layer we find another; "chopping the onion will bring tears … only during peeling does it speak the truth" [32].

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