3 The Immunobiology of Transplant Rejection and Acceptance

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3.1 Introduction

The immune system normally functions to mitigate infectious and neoplastic risk. In the absence of immunosuppression, the transplantation of allogeneic tissue constitutes a challenge as allorecognition triggers injurious effector mechanisms culminating in graft destruction. This chapter will focus on innate immunity, the basic mechanisms of allorecognition, co-stimulation, T cell amplification, effector mechanisms, and antibody production.

3.1.1 Immune Response to Transplanted Tissue

To adequately understand the response to transplanted tissue, it is helpful and important to review the general immune response. The immune system can be divided into two core components (see Table 3.1). Innate immune system is nonspecific and non-adaptive while the adaptive immune system is antigen specific and exhibits memory, or secondary, immune responses.

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3.2 The Innate Immune System

Innate immunity refers to the nonspecific natural immune system that involves macrophages, dendritic cells, neutrophils, NK (natural killer) cells, cytokines, toll-like receptors, and complement components. Innate immune system provides immediate albeit incomplete protection against intruders and, at best, has only short-term memory.

3.3 Role of Innate Immune System in Allograft Rejection

How the innate immune system recognizes allogeneic non-self is incompletely understood. It has long been established that cells of the innate immune system do not directly participate in allorecognition. Rather, NK cells respond to inflammatory ligands released by dying cells [1, 2]. These inflammatory ligands include uric acid and nuclear protein high-mobility group box 1, among others. Such mediators are allograft nonspecific and relate more to hypoxic injury and signal through innate pattern recognition receptors [3, 4]. Such receptors include Toll-like receptors and various components of the inflammasome, all of which also participate in the recognition of microbes.

NK cells are activated by stimulatory ligands such as the MHC I- related proteins MICA and

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Table 3.1 Comparison of Innate and Adaptive Immunity

Innate immunity	Adaptive immunity
Non-specific	Specific
Involves physical and chemical barriers, macrophages, phagocytic leukocytes, dendritic and NK cells	Involves B and T lymphocytes
No memory cells	Involves generation of "memory cells" against specific antigens for future enhanced response
Respond to any foreign antigen	Responds to a specific antigen
Inherited	Can't be inherited
Faster response	Slower response
Activation of alternative and lectin pathways	Classical pathway

MICB which have been detected in solid organ allografts as well as the absence of inhibitory signals delivered by self-MHC [5]. However such stimulatory signals are generally insufficient for complete activation of NK cells as is seen after a viral infection [6]. Interestingly, studies on T and B cell deficient RAG −/− (recombination-activating gene) mice have shown that a specific alloimmune response to allogenic non-self was mounted independent of NK cells [7]. The findings provide direct evidence that monocytes mediate a response to allogeneic non-self, a function not previously attributed to them, and suggest the presence of mismatching at loci unlinked to MHC. The allo-determinants for these loci unlinked to MHC may be polymorphic genes that are outside the MHC as Polymorphic Ig Domain containing genes that are expressed in cells of the innate immune system in mice and humans [8]. Recently a study [9] on the innate response of $Rag2^{-/-}\gamma c^{-/-}$ mice, which lack T, B, and NK cells, to grafts from allogeneic showed that donor polymorphism in the gene encoding signal regulatory protein alpha (SIRPα) is a key modulator of the recipients innate allorecognition response.

3.4 The Adaptive Immune System

The adaptive immune system recognizes non-self-antigens to initiate immune responses. Unlike the innate immune system, which functions based on the identification of general threats, adaptive immunity is activated by exposure to pathogens or alloantigens, and uses immunological memory to learn about the threat and enhance the immune response accordingly. Adaptive immunity is often lifelong. In general terms, the adaptive immune response is slower to respond to threats and infections than the innate immune response, which is primed and ready to combat threats at all times.

Adaptive immune responses are triggered when APCs activate antigen-specific T cells within secondary lymphoid organs leading to effector cell generation and their migration to the allograft where they mediate rejection. The majority of effector T cells eventually undergoes apoptosis and the few that survive become long-lived memory T cells that endanger the survival of a subsequent organ transplant.

3.4.1 Cells of the Adaptive Immune System

The adaptive immune system mainly relies on *T cells* and *B cells*. Both T cells and B cells are lymphocytes that are derived from bone marrow derived multipotent hematopoietic stem cells.

3.4.1.1 T Cells

Naïve T cells are formed in the bone marrow, and then migrate to the thymus (hence the name "T cell") in order to mature. While in the thymus, the developing T cells start to express T cell receptors (TCRs), and either CD4 or CD8 receptors.

Unlike antibodies, which can bind to antigens directly, T cell receptors can only recognize antigens that are bound to Major Histocompatibility Complex class 1 (MHCI) or class 2 (MHCII). These MHC molecules are membrane-bound surface receptors on professional antigen-presenting cells, such as dendritic cells and macrophages. CD4 and CD8 play a role in T cell recognition and activation. Class 1 MHC molecule present peptide antigens to CD8-positive T cells while MHC class-II Molecules presents antigen to CD4-positive T cells.

T cells undergo two selection processes:

1. Positive selection ensures MHC restriction by testing the ability of MHC-I and MHC-II to distinguish between self and non-self-proteins. In order to pass the positive selection process, cells must be capable of binding only self-MHC molecules. If these cells bind non-self-molecules instead of self-MHC molecules, they fail the positive selection process and are eliminated by apoptosis.
2. Negative selection tests for self-tolerance. Negative selection tests the binding capabilities of CD4 and CD8 specifically. For example, T cells only bind to self-MHC molecules presenting a foreign antigen. If a T cell binds to a self-MHC molecule that isn't presenting an antigen, or alternately, binds to a self-MHC molecule presenting self-antigen, it will fail negative selection and be eliminated by apoptosis. These two selection processes mitigate autoimmunity risk.

The end result of positive and negative selection includes: Helper T cells, Cytotoxic T cells, and T regulatory cells.

3.4.1.2 Helper T Cells

T helper (Th) cells are divided into two main populations: Type 1 (Th1) and Type 2 (Th2) cells. A third T-cell subset (T helper cells Type 17) has also been identified. These cells are involved in early response to pathogens, in autoimmunity and tissue inflammation.

3.4.1.3 Type 1 Helper Cells

Th1 cells produce interleukin (IL)-2, gamma-interferon (IFN-gamma) and tumor necrosis factor-beta and are involved in delayed type hypersensitivity reactions. In addition, they are the main cells involved in acute allograft rejection.

3.4.1.4 Type 2 Helper Cells

Th2 cells express IL-4, IL-5, IL-6, IL-10 and IL-13 and provide help for B-cell production of antibody, and particularly IgE response (parasitic infections). This IgE response is mediated by IL-4 which acts as a growth factor for B cells antibody production while directly inhibiting the T cell maturation into Th1 pathway.

3.4.1.5 Cytotoxic T Cells

Cytotoxic T Cells express CD8 and are principally involved in the killing of tumor and virally infected cells. Activation of Cytotoxic T cells involves interactions between molecules on the surface of cytotoxic T cells and APC's. The first signal is interaction between peptide bound MHC class 1 molecule on APC and the TCR on CD8+ T cells. The second signal is interaction between CD28 molecule on T-cell and either CD80 or CD 86 (also called B7–1 and B7–2) on APC. Activation of cytotoxic T cells leads to killing of the infected cells by either delivering a "lethal hit" or alternatively by inducing apoptosis. After activation, CD8+ T cells release cytoxins, perforins, granzyme B, and granulysin. Through the action of perforin, granzymes enter the cytoplasm of the target cell and their serine protease function triggers the interleukin-1-beta converting enzyme (ICE) mediated protease pathway that eventually lead to cell death. This pathway is critically important for eradication of microbial infection. A second way to induce "activation-induced cell death is by utilizing the FAS pathway [10–13]. When a cytoxic T cell is activated it starts to express the surface protein FAS ligand (FasL) (Apo1L)(CD95L), which can bind to Fas (Apo1) (CD95) molecules expressed on the target cell. Engagement of Fas with FasL allows for recruitment of the death-induced signaling complex (DISC) which comprises activated caspases leading to cleavage of death substrates such as lamin A, lamin B1, lamin B2, PARP (poly ADP ribose polymerase), and DNA-PKcs (DNA-activated protein kinase) [14].The final result is apoptosis of the cell that expressed FAS. The FAS pathway is of importance in limiting T-cell proliferation in response to antigenic stimulation. Cell-mediated cytotoxicity has been shown to play an important role in acute, although not chronic, allograft rejection [15].

The diagnostic utility of measurement on mRNA encoding cytotoxic attack proteins granzyme B and perforin in urine specimens obtained from renal allograft recipients, has been investigated and reported that mRNA levels of perforin

and granzyme B were significantly higher in the urinary cells obtained from renal allograft recipients with a biopsy confirmed episode of acute rejection than in the patients without an episode of acute rejection. Analysis involving the receiver-operating-characteristic curve demonstrated that acute rejection can be predicted with a sensitivity of 83% and a specificity of 83% using perforin mRNA levels, and with a sensitivity of 79% and a specificity of 77% using granzyme B mRNA levels [16]. Similarly, reverse transcriptase-polymerase chain reaction (RT-PCR) has been used to identify intrarenal expression of cytotoxic attack molecules (granzyme B and perforin) and immunoregulatory cytokines (IL-2, IL-4, IL-10, IFN-gamma, and TGF-beta 1) in human renal allograft biopsies. Molecular analyses revealed that intragraft display of mRNA encoding granzyme B, IL-10 or IL-2 is a correlate of acute rejection, and intrarenal expression of TGF beta 1 mRNA, of chronic rejection [17].

3.4.1.6 T Regulatory Cells

Regulatory T cells (Tregs) play a pivotal role in regulating other cells in the immune system. Tregs mediate their regulatory function through multiple soluble and cell surface markers. The most widely used markers for Tregs are (see Fig. 3.1): CD25, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR), lymphocyte activation gene-3 (LAG-3) and forkhead/winged-helix transcription factor box P3 (Foxp3) [18–20]. However accumulating evidence suggests that these markers are not strictly Treg-specific. For example, CTLA-4/TCR interactions with their co stimulatory ligands on APC's and CD25 with IL-2 involvement leads to production of soluble messengers TGF-beta, IL-10 and adenosine (Fig. 3.1) which in turn suppresses activation, proliferation and cytokine production of CD4+ T cells. CD8+ T cells and are thought to suppress B cells and dendritic cells [21, 22]. Tregs impact immune responses to self-antigens, allergens, and commensal microbiota as well as immune responses to infectious agents and tumors. In addition, T regulatory cells by distinguishing between self and non-self-molecules, play an important role in reducing the risk of auto immune diseases. When regulatory T cells (Tregs) emerged as a mechanism in control of autoimmunity [23, 24], considerable interest focused on their role in organ transplantation and their potential for cell-based therapy. Such studies often incorporate forkhead box P3 (FOXP3), a forkhead-winged helix transcription factor expressed on the X chromosome, important in the development and function of Tregs. Such studies [25–27] were facilitated by the discovery of Foxp3 loss-of-function mutations in humans

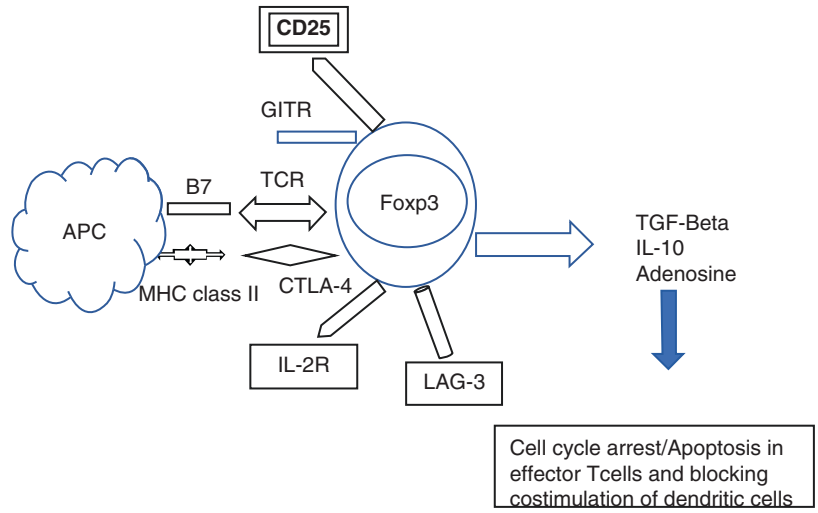

Fig. 3.1 Regulatory T cell receptors and activation

leading to a severe multi-organ autoimmune and inflammatory disorder IPEX (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-Linked) and a similarly devastating widespread lesions in a mouse mutant strain, *scurfy*. Thus demonstrating the importance of FOXP3 in cells which regulate self-tolerance. The majority of Foxp3-expressing regulatory T cells are found within the major histocompatibility complex (MHC) class II restricted CD4-expressing ($CD4^+$) population and express high levels of the interleukin-2 receptor alpha chain (CD25) [28].

3.4.2 Role of Tregs in Experimental Allograft Tolerance

In the past decade numerous reports have revealed the importance of Tregs in the promotion of transplant tolerance in animal models of heart, kidney, and skin transplantation [29–31]. In a mouse model of liver transplantation, recent studies have demonstrated the critical role that Tregs play in the establishment of tolerance [32, 33]. The presence of Tregs was increased in the periphery and in the tolerant graft from day 5 after transplantation to day 100. The increased number of Tregs was associated with the heightened expression of Tregs effector molecules (TGF-b and CTLA4) and IL-4 production. Treatment of tolerant mice with Tregs-depleting anti-CD25 antibodies resulted in acute allograft rejection, which was associated with a reduced Tregs/T effector cell ratio, decreased production of IL-4, and increased production of IL-10 and IL-2. Furthermore, anti-CD25 antibody–treated mice displayed reduced numbers of apoptotic alloreactive T cells, and suggesting that Tregs mediate their activity through the induction of apoptosis of activated T cells. The engagement of CTLA4 was found to be important for the induction of spontaneous tolerance in a mouse model of liver transplantation and treatment with antibodies to CTLA4 prevented the induction of tolerance. The anti-CTLA4 antibody treatment was associated with the increased activity of donor-specific T cells and natural killer cells in both the liver and the spleen. Furthermore, blocking CTLA4 with antibodies led to the protection of alloreactive T cells from apoptosis, and this suggests that the Tregs effector molecule mediates its tolerogenic effect by killing the target cells in the tolerant graft and the periphery [33].

Monitoring the expression of Tregs effector molecules has been used experimentally to predict tolerance and rejection. The expression of a panel of Tregs effector genes with a novel multiplex real-time polymerase chain reaction platform (GeXP analysis system, Beckman Coulter) in murine models of rapamycin-induced cardiac tolerance, spontaneous hepatic tolerance, and cardiac rejection has been analyzed [34]. The increased expression of fibrinogen-like protein 2, killer cell lectin-like receptor G1, and Foxp3 was found to be associated with tolerance in both tolerant cardiac and liver allografts, whereas in rejected cardiac grafts, the increased expression of CD25, granzyme B, and interferon-c was associated with rejection.

3.5 Mechanisms of Allograft Rejection

Allorecognition is the first step of a series of complex events that leads to T-cell activation, antibody production, and allograft rejection. Donor antigen can be recognized directly or indirectly by T cells.

In the direct pathway, recipient T-cells recognize intact allogeneic HLAs expressed by donor APC (Antigen Presenting Cells). In the indirect pathway, T-cells recognize donor MHC peptides presented by recipient APC (Fig. 3.2). The direct and indirect pathways are well understood in organ transplantation. The direct pathway is very important in the immediate post-transplant period. Without appropriate immunosuppression, a strong and effective alloimmune response ensues, due to the high number of recipient T-cells that will recognize the graft antigens and leading to acute rejection. While the indirect pathway of allorecognition may also participate in acute rejection, it is usually predominant in the late onset of rejection, and especially chronic rejection. As long as the allograft is present in the host, the recipient APCs

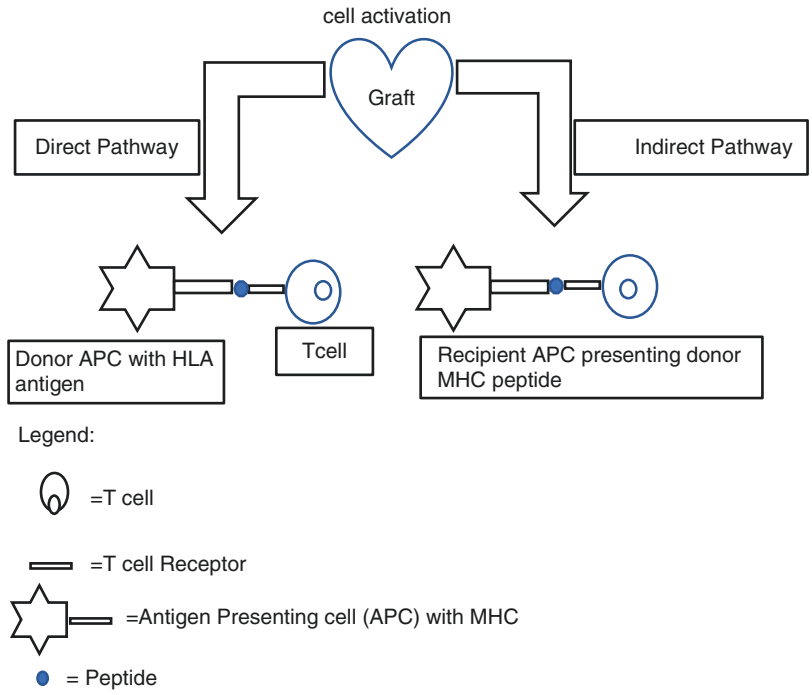

Fig. 3.2 Direct and indirect pathway of T cell activation

can pick up the alloantigen shed from the graft initiating the alloimmune response.

3.5.1 Three-Signal Model of T-Cell Activation

The key process of allograft rejection is the T-cell activation. The most common sited mechanism of T cell activation includes interaction of two cells (Dendritic and T cells) and involves 3 signals (Fig. 3.3).

3.5.1.1 Signal One (Recognition)

During an immune response, extracellular antigen is endocytosed and processed by the endoplasmic reticulum before translocating back to the cell surface in the context of self MHC. In the lymph nodes, the antigen peptides are presented by MHC-class II molecules present on APC's to naïve T-cells for activation. The initiation of intracellular signaling (signal 1) is transduced through the TCR-CD3 complex. CD3, formerly a target of OKT3, a now discontinued murine monoclonal formerly used to treat severe rejection episodes [35].

3.5.1.2 Signal Two (Co-stimulatory Signals)

The second co-stimulatory signal depends on the receptor-ligand interactions between T-cells and APCs (signal 2). Numerous co-stimulatory pathways have been described and blockage of these pathways can lead to antigen-specific inactivation or death of T-cell [36]. The best-studied ones are the CD28-CD 80 and CD154-CD40 pathways. CD28 and CD154 are expressed on T-cells, and their ligands B7 and CD40 are expressed on APCs. CD28 has two ligands, B7–1 (CD80) and B7–2 (CD86). T-cells also express cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) only when they are activated as compared with to CD28 which is present on T-Cells in resting state. CTLA-4 is homologous to CD28 and has a higher affinity than CD28 to bind B7. However, when CTLA-4 binds B7 (both CD80 and CD86), it produces an inhibitory signal resulting in Tc anergy. CTLA-4-Ig (belatacept) is a novel immunosuppressive medication, which is a recombinant fusion protein that contains the extracellular domain of soluble CTLA4 combined with an immunoglobulin G1 (IgG1) heavy chain [36]. CTLA4Ig is a competitive inhibitor of CD28

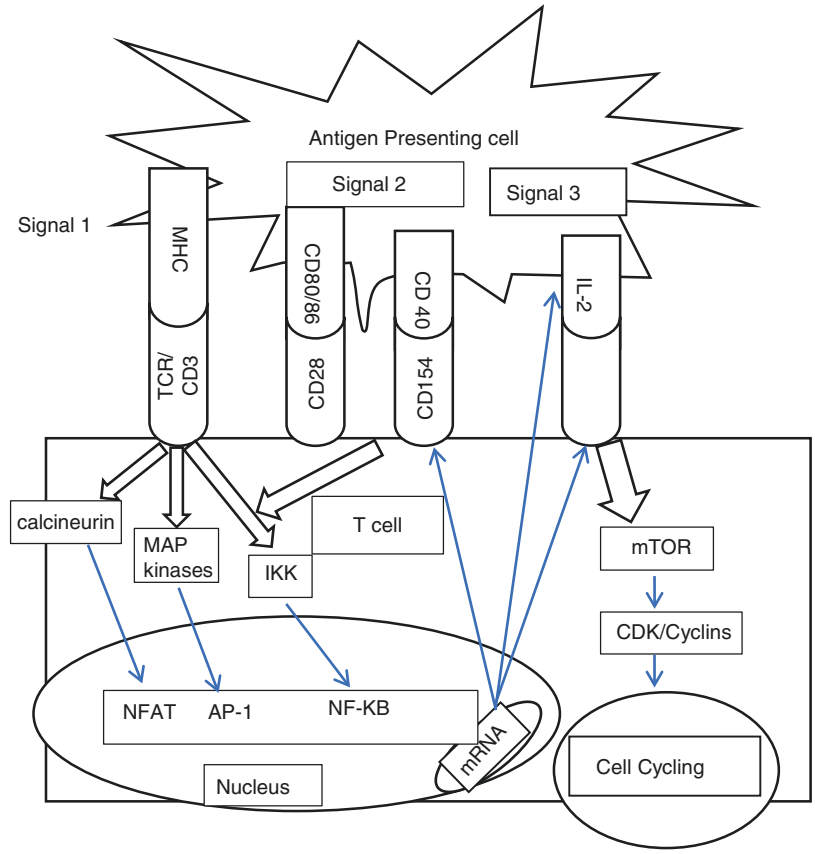

Fig. 3.3 3 Signal model of allo-recognition

binding, resulting in T-cell anergy in vitro. In addition, the importance of CTLA-4 can be emphasized by observation of development of lymphoproliferative disease in genetically engineered mouse in which gene for CTLA-4 has been knocked down. Similarly, exacerbation of autoimmune disease by administration of anti-CTLA-4 monoclonal antibodies is another practical illustration that the CTLA-4 induced signal play an important role in the activation of T cells.

The other best studied co stimulatory pathways is the interaction between CD40 and its ligand CD40L (CD 154). CD40, a member of the tumor necrosis factor (TNF) receptor superfamily, is expressed on B cells and other APCs, including dendritic cells while CD40 ligand, CD40L (CD154), is expressed early on activated T cells. CD154-CD40 inhibition has also been shown to prevent allograft rejection in animal models, including anti-CD154 antibody and molecules that target CD40.

The combination of signal 1 and 2 activates three downstream signal transduction pathways: the calcium-calcineurin pathway, the RAS-mitogen activated protein kinase pathway, and the IKK-nuclear factor κB (NF-κB) pathway. These three pathways further activate transcription factors including the nuclear factor of activated T cells, activated protein-1, and NF-κB, respectively. Several new molecules and cytokines including CD25, CD154, interleukin (IL)-2, and IL-15 are subsequently expressed.

3.5.1.3 Signal Three- Proliferation

IL-2 and IL-15 deliver growth signals through the mammalian target of rapamycin pathway and phosphoinositide-3-kinase pathway, which subsequently trigger the T-cell cycle and proliferation. The fully activated T-cells undergo clonal expansion and produce a large number of cytokines and effector T-cells, which eventually produce $CD8^+$ T-cell mediated cytotoxicity, help

macrophage-induced delayed type hypersensitivity response (by $CD4^+$Th1), and help B cells for antibody production (by $CD4^+$Th2). A subset of activated T-cells becomes the alloantigen-specific memory T-cells. For Th1 and Th2 differentiation, initiation of signal three requires the presence of IFN-gamma and IL-4 respectively. Dendritic cell and naive Th cells are unable to produce IFN-gamma or IL-4 themselves.

3.5.1.4 T Cell Migration

Naïve T cells and central memory cells circulate between blood and secondary lymphoid tissue. Leukocyte trafficking is critical for immunosurveillance purposes. This migration pattern is guided mainly by the cell surface expression of specific homing molecules, such as selectins, integrins, and chemokine receptors. (See Table 3.2). Activation of naïve lymphocytes occurs within secondary lymphoid tissue. Upon activation and differentiation, marked changes in the homing behavior of lymphocytes are observed as a direct result of changes in the cell surface expression of homing molecules. The interactions between these molecules and their ligands or receptors triggers a sequential and coordinated series of events which summarized in Fig. 3.4.

Table 3.2 Adhesion molecules involved in T cells migration

Steps in T cell migration	Adhesions molecules
Rolling	Selectin mediated (L, E and P selectins)
Triggering	Chemokine mediated (CCR1, CCR3 and CCR5)
Firm adhesion	Integrin mediated (ICAM-1, VCAM-1)
Transmigration	PECAM and integrin mediated

3.5.1.5 Rolling

The rolling step is mediated by selectins, a closely related family of Ca (2+)-dependent lectins (L, E and P selectins, respectively). They are found on leukocytes, inflamed vascular endothelial cells and platelets. L-selectin is expressed constitutively on leukocytes. The interaction between selectins and its ligand are loose, reversible, and occur in settings of shear flow. Because rolling precedes (and appears to be essential for) the integrin-mediated firm arrest before extravasation in response to inflammatory or infectious stimuli, inhibition of selectin function has potential for anti-inflammatory therapy, but also presents some significant challenges because of the complexity of the processes involved.

3.5.1.6 Triggering

Leukocyte activation or triggering is mediated by chemokines which are produced by both leukocytes and endothelial cells. Chemokines, are a family of chemotactic cytokines that signal through G-protein-coupled receptors, play critical roles in regulating the leukocyte recruitment cascade. The signals basically convert the loose selectin mediated rolling into integrin mediated

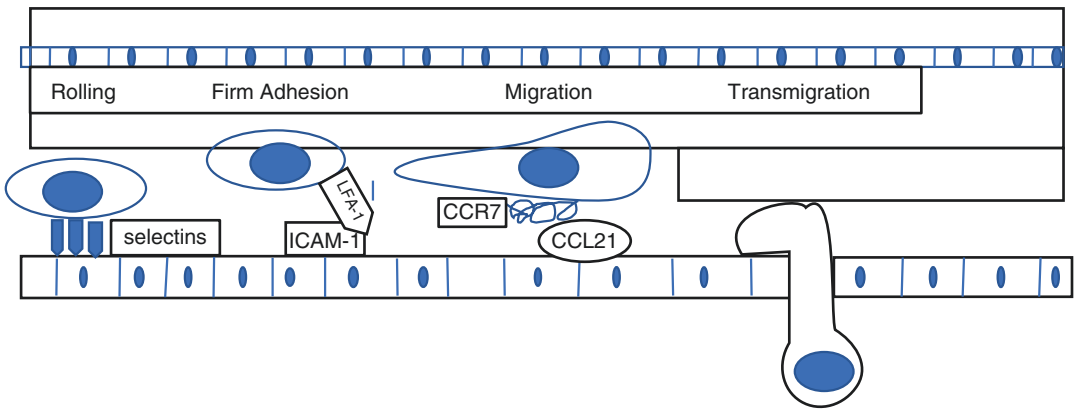

Fig. 3.4 Steps in extravasation of T cells

leukocyte-endothelial adhesions. Chemokines can be transported and immobilized on the surface of vascular endothelial cells, where they activate leukocyte subsets expressing specific receptors. These include chemokine receptor 1 (CCR1), CCR2, CXCR3, and CCR5. These receptors are predominantly expressed during an allograft rejection. CCR5 which is a high affinity receptor for chemokines has been shown to play a significant role in leukocyte trafficking in transplanted allografts in animal models and clinical observations [37]. In addition, much work has been done to characterize the chemokines expressed in the rejection of heart allografts. The specific chemokines found to be important for lymphocyte trafficking in rejecting heart grafts include CXCL9 (MIG), CXCL10 (IP-10), and CXCL11 (I-TAC) [38]. Neutralizing chemokines or blocking their receptors has been shown to prolong graft survival and prevent graft infiltration in animal models [39, 40]. Chemokines can also direct migration of adherent cells across the endothelium, and control segregation of cells into specific microenvironments within tissues. The regulated expression of chemokines and their receptors is a critical determinant for homing of specialized lymphocyte subsets, and controls both tissue and inflammation-specific immune processes.

3.5.1.7 Firm Adhesion

Firm adhesion of leukocytes to the endothelium is induced by chemokine stimulations and high-affinity integrin activation. The resulting integrin conformation change after activation can lead to as much as a 10,000-fold affinity increase of lymphocyte function-associated antigen-1 (LFA-1) to its ligand ICAM-1 [41]. In addition to LFA-1, Very late antigen-4 (VLA-4) ligation of endothelial vascular cell adhesion molecule-1 (VCAM-1) also provides the principal interaction leading to adhesion.

3.5.1.8 Transmigration

The final and less well understood step in the homing cascade is transmigration. Though traditionally thought of as the passage of the lymphocyte between endothelial cells (paracellular), it is becoming more apparent that lymphocytes can also migrate directly through endothelial cells (transcellular) [42]. This process is mediated predominantly by the platelet endothelial cell adhesion molecule (PECAM). Migration along the endothelium is primarily dictated by chemokine signals that direct cell chemotaxis via chemotactic gradients. In addition, both β1 and β2 integrins are also thought to be involved in these processes via interaction with endothelial junction integrin ligands such as JAM-B and JAM-A, respectively [43].

3.5.1.9 B Cells

There are two lineages of B-cells; B1 cells are part of innate immune system and develop during fetal and perinatal life; B2 cells are part of the adaptive immune system and develop during post-natal life.

3.5.1.10 B Cell Development

B1 cells are self-renewing and form part of 'Natural Memory'. B2 cells are formed from pluripotent hematopoietic stem cells that mature in bone marrow. At this time IgM is expressed, forming B cell receptor (BCR). These naive B cells move into spleen and differentiate into follicular or marginal zone B cells.

3.5.1.11 B Cell Activation

B cells are activated by antigen stimulation. They undergo extrafollicular differentiation to plasma cells when the B cell has high affinity for that specific antigen. In contrary, if the affinity is low for the antigen, cells enter the germinal centers and undergo affinity maturation through a process of somatic hypermutation of the BCR. This ultimately leads to differentiation into either memory B cells or plasma cells. The purpose of this intense regulation is to ensure that the initial response against a specific antigen should be mounted by plasma cells which carry the highest binding capacity for that specific antigen. T helper cells facilitate B-cell activation either through intimate membrane contact involving a variety of receptors and ligands (such as CD40:CD154) or through the secreted soluble cytokines (such as IL-4). In a transplant, HLA

antibodies formed against donor HLA antigens is a major cause for allograft rejection and premature graft failure. Allograft injury is mediated either by activating the complement cascade [complement-dependent cytotoxicity (CDC)] or *via* Fc receptor on natural killer (NK) cells, neutrophils, and eosinophils (antibody-dependent cellular cytotoxicity).

3.5.1.12 B Cell Signaling

Differentiation of naïve B lymphocytes into effector cells (plasma and memory cells) starts with engagement of cytokine receptors by specific ligands. This activates Janus Kinase signal transducer and activator of transcription (STAT) signaling pathways [44, 45]. Four JAK and seven STAT proteins have been identified. IL-21 predominantly activates STAT 1 and STAT 3 in human B cells and has been identified as a potent regulator of B cell differentiation [46, 47]. In vitro and In vivo studies on inactivating STAT 3 mutations dramatically reduced the ability of IL-21 to induce differentiation of Naïve B cells into plasma and memory cells [48]. In contrast STAT 1 deficiency has no effect on the differentiation of naïve B cells. Tofacitinib (CP690550) a novel JAK 3 inhibitor, is an immunosuppressive agent that appears to selectively reduce natural killer- and T- cell subsets. This agent is FDA approved for treatment of Rheumatoid arthritis and psoriatic arthropathy. Unfortunately, clinical trials in kidney transplantation revealed efficacy and safety concerns halting development for this indication [49].

3.6 MHC: Major Histocompatibility Complex

The principal target of the alloimmune response are the major histocompatibility complex (MHC, described in more details in Chap. 2) molecules expressed on the surface of donor cells (allo-MHC). In humans, these MHC molecules are called human leukocyte antigens (HLA) and they are located on the short arm of chromosome 6. Each parent provides a haplotype (a linked set of MHC genes) to each offspring in Mendelian co-dominant inheritance. The protein products of the MHC have been classified into three classes; Class I, II and III Molecules. Class I and II proteins are integral components of the immune system whose primary role is the presentation of peptide antigen to T cell receptor.

Class I molecules (HLA-A, -B, and -C) are composed of a polymorphic heavy chain (α chain, 44 kDa) and a non-polymorphic light chain (β2 macroglobulin, 12 kDa). They are expressed on all nucleated cells and generally present endogenous small antigens (typically 9–11 amino acids), such as viruses and self-protein fragments to $CD8^+$ T cells. The CD8-positive cells then subsequently induce cell lysis (by inducing apoptosis or actively killing cells by cytotoxic proteins).

Class II molecules (HLA-DP, −DQ, and -DR) are composed of alpha and beta heterodimers. They are constitutively expressed only on professional antigen-presenting cells (APC), including dendritic cells, macrophages, and B-cells. Their expression is upregulated on epithelial and vascular endothelial cells after exposure to pro-inflammatory cytokines. Class II molecules present relatively larger antigens (12–28 amino acids), derived from extracellular proteins to $CD4^+$ T-cells. The degree of HLA mismatch between donor and recipient plays a role in determining the risk of chronic rejection and graft loss. HLA-A, -B, and -DR (3 pairs, 6 antigens) are traditionally used for typing and matching before kidney or pancreas transplant. HLA-Cw, -DP, and -DQ are now increasingly typed and used in many transplant centers. For kidney transplants, long-term graft survival is best in HLA-identical living related kidney transplants [50].

Class III molecules includes several components of complement system (i.e. C2, C4a, C4b, Bf) and inflammatory cytokines, tumor necrosis factor, two heat shock proteins (HSP) etc. They are not membrane proteins and have no role in Ag presentation. MHC Class III molecules are not structurally related to class I and class II molecules.

3.6.1 Minor Histocompatibility Antigens

Minor histocompatibility antigens (MiHA) are small endogenous peptides that occupy the antigen-binding site of donor MHC molecules.

Their importance in transplantation is best described when donor and recipients share identical MHC types, such as HLA matched, non-identical twin siblings, and yet still are at rejection risk in the absence of immunosuppression. The prototypic minor histocompatibility antigen, the male or H-Y antigen, is derived from a group of proteins encoded on the Y chromosome. Alloresponses to this antigen may explain reduced long-term graft survival observed in male-to-female donations. They are generally recognized by $CD8^+$ cytotoxic T-cells in the context of self-MHC, which leads to graft rejection. In bone marrow transplant, MiHA play an important role in graft-*vs*-host disease in patients who have received HLA-matched cells. MHC class 1 related chain A and B (MICA and MICB) antigens are surface glycoproteins with functions related to innate immunity. Antibodies against MICA and/or MICB can cause antibody-mediated rejection (AMR) and graft loss [51].

Other reported antibodies causing graft rejection include anti-angiotensin-II type 1(AT1) receptor antibodies (activating IgG antibodies) that have been implicated in causing allograft rejection and hypertension. Affected patients might benefit from removal of AT_1-receptor antibodies or from pharmacologic blockade of AT_1 receptors, anti-glutathione S-transferase T1, and anti-endothelial antibodies. Anti-endothelial antibody can be detected by using donor monocytes for crossmatch. Some minor transplant antigens may come from mitochondrial proteins and enzymes. As our knowledge in transplant immunology advances, there will likely be more alloreactive and autoreactive antibodies to uncover.

3.6.2 ABO Blood Group Antigens

ABO blood group antigens consist of oligosaccharides which is expressed on red blood cells, epithelial cells, lymphocytes, platelets and vascular endothelial cells. Patients with different blood groups differ with respect to their antigen density on erythrocytes. Compared to blood group A1 and blood group B individuals, blood group A2 recipients, (20% of blood group A Caucasians) have relatively low level expression (30–50%) on the surface of erythrocytes, thus explaining the reduced immunogenic potential of organs from blood group A2 donors. Of interest, anti-A/B antibodies are formed upon contact with gut bacteria during early infancy. Naturally occurring anti-A/B antibodies are predominantly of the IgM class but especially in blood group O individuals they also consist of IgG and IgA class. The pathogenic importance of anti-A/B antibodies in solid organ transplantation is well known. These preformed antibodies cause hyper acute rejection. Thus, ABO compatibility between donor and recipient are essential for organ transplantation. Desensitization protocols to remove the preformed hemagglutinin A and/or B from recipient circulation have been used for ABO incompatible kidney transplants [52, 53]. The rhesus factor and other red cell antigens are of minimal relevance to organ transplant, as they are not expressed on endothelium.

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Bristol Myers Squib and Astellas
There are no off-label discussions of any medications in this manuscript.
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