

Minireview

Immune Exhaustion and Transplantation

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Exhaustion of lymphocyte function through chronic exposure to a high load of foreign antigen is well established for chronic viral infection and antitumor immunity and has been found to be associated with a distinct molecular program and characteristic cell surface phenotype. Although exhaustion has most commonly been studied in the context of CD8 viral responses, recent studies indicate that chronic antigen exposure may affect B cells, NK cells and CD4 T cells in a parallel manner. Limited information is available regarding the extent of lymphocyte exhaustion development in the transplant setting and its impact on anti-graft alloreactivity. By analogy to the persistence of a foreign virus, the large mass of alloantigen presented by an allograft in chronic residence could provide an ideal setting for exhausting donor-reactive T cells. The extent of T cell exhaustion occurring with various allografts, the kinetics of its development, whether exhaustion is influenced positively or negatively by different immunosuppressants, and the impact of exhaustion on graft survival and tolerance development remains a fertile area for investigation. Harnessing or encouraging the natural processes of exhaustion may provide a novel means to promote graft survival and transplantation tolerance.

Abbreviations: CTLA4, cytotoxic T lymphocyte-associated protein 4; KLRG1, killer-cell lectin like receptor G1; LCMV, lymphocytic choriomeningitis virus; PD1, programmed cell death 1; PDL1, programmed death ligand 1; TIM3, T cell immunoglobulin domain and mucin domain 3; Treg, regulatory T cell

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Introduction

The *in vivo* study of immune responses against viruses has shaped our understanding of essential immunological concepts such as memory, specificity and tolerance (1). The term *T cell exhaustion* was originally coined in the early 1990s to describe the peripheral deletion of antiviral CD8⁺ T cells following chronic infection with lymphocytic choriomeningitis virus (LCMV) in mice (2). The concept was later refined to describe the persistence of antiviral T cells capable of proliferating *in vivo* but unable to exert antiviral effector functions, followed by the physical deletion of these antigen-specific T cells. Since the original descriptions in the LCMV model in mice (3,4), T cell exhaustion has been reported in a variety of animal models, in humans with chronic viral infections, in bacterial and parasitic infections, in cancer, and more recently in situations of persistent severe inflammation due to sepsis, trauma, major surgery or burn injury (5–7). T cell exhaustion is considered to be a general mechanism through which chronic viral infections tamper or silence antiviral immune responses but also a T cell differentiation state that prevents immunopathology in situations of persistently high antigen load and inflammation. The possibility of reinvigorating exhausted T cells through “checkpoint blockade” therapies has substantially increased its clinical relevance, particularly in cancer. Mechanisms closely resembling those leading to T cell exhaustion during chronic infections and tumors are present during transplantation and likely contribute actively to the long-term effects of immunosuppression and tolerance-promoting strategies. Whether T cell exhaustion influences the outcome of alloimmune responses remains to be firmly established.

Differentiation Pathways in Exhausted T Cells

Although exhaustion affects both CD4⁺ and CD8⁺ T cells, the differentiation and fate of exhausted T cells has been most extensively studied for CD8⁺ T cells from hosts with chronic viral infections with high viral load, such as LCMV, hepatitis B virus, hepatitis C virus (HCV), HIV and simian immunodeficiency virus (Figure 1). In these settings, viral-specific CD8⁺ T cells initially expand and acquire effector functions but subsequently develop a progressive state of functional hyporesponsiveness characterized by

decreased proliferative capacity and loss of IL-2 production, followed by reduced capacity to secrete tumor necrosis factor α and interferon γ . This phenomenon is not an "all or none" differentiation state that occurs rapidly following T cell activation but rather a progressive hierarchical process that usually takes weeks or months of chronic antigen stimulation to develop (8,9). During an early phase, exhausted T cells may recover function and differentiate into functional memory T cells if transferred into naïve antigen-free hosts. In contrast, terminally differentiated exhausted T cells appear to require constant antigen stimulation to survive, a phenomenon termed *antigen addiction*, and eventually may be deleted. In the setting of chronic viral infections, exhausted T cells retain residual functionality and still play a role in the containment of the pathogen (7).

T cell exhaustion is associated with the surface expression of a number of inhibitory receptors, including programmed cell death 1 (PD1), T cell immunoglobulin domain and mucin domain 3 (TIM3), cytotoxic T lymphocyte-associated protein 4 (CTLA4), B and T lymphocyte associated (BATL), CD244, lymphocyte-activation gene 3 (LAG3), killer-cell lectin like receptor G1 (KLRG1), and CD160. Expression of some of these receptors, particularly PD1, is essential for the maintenance of the exhausted phenotype. This is illustrated by the observation that blocking the PD1 and programmed death ligand 1 (PDL1) pathway, alone or in combination with TIM3 or CTLA4 blockade, can restore exhausted T cell function (7). The functional and phenotypic properties of exhausted T cells may differ depending on the microenvironment and the eliciting antigen. Furthermore, in humans, viral-specific exhausted CD8⁺ T cells display substantial interindividual phenotypic heterogeneity (10). The following factors are known to promote and/or reinforce CD8⁺ T cell exhaustion: high antigenic load; lack of CD4⁺ T cell help; high availability of inhibitor receptor ligands; cytokines such as IL-10, TGF- β , and type I interferon; low levels of IL-6 and common- γ chain; T cell growth factors such as IL-2 and IL-7 (11); disruption of secondary lymphoid tissue architecture (5); and, potentially, CD4⁺Foxp3⁺ regulatory T cells (Tregs) (12,13).

PD1 signaling attenuates the stimulatory signals elicited by the T cell receptor and costimulatory receptors and has emerged as a key regulator of CD8⁺ T cell exhaustion. Recent data in the murine model of chronic LCMV infection indicate that CD8⁺ T cell exhaustion still occurs in the absence of PD1 signaling, although the long-term maintenance of exhausted T cells is impaired (14). The expression of PD1 and other inhibitory receptors is not exclusive to exhausted T cells and cannot be used as a surrogate for impaired T cell function. Inhibitory receptors are not "exhaustion markers" *per se* but rather are part of the normal T cell activation response and are involved in the differentiation of a variety of T cell subsets (15). PD1, for instance, is transiently expressed during CD8⁺

T cell activation and has been reported to be present on the surface of 60% of circulating memory T cells in healthy persons (16). During chronic infections, however, the expression of PD1 is higher than what is observed in memory CD8⁺ T cells from healthy individuals. Furthermore, coexpression of multiple inhibitory receptors is uncommon in memory T cells, and the number of inhibitory receptors expressed appear to positively correlate with the severity of exhaustion (17).

Although an exhaustion-determining transcriptional factor has not been identified, exhausted CD8⁺ and CD4⁺ T cells display distinct transcriptional signatures that differ from those present in memory or effector T cell subpopulations (18,19). The transcription factors T-box 21 (Tbet) and eomesodermin (EOMES) are essential to maintaining the exhausted CD8⁺ T cell repertoire, and their expression serves to identify 2 main exhausted CD8⁺ T cell subpopulations (Tbet^{high} PD1^{int} and the more prevalent EOMES^{high}PD1^{high}). Other transcription factors characteristic of exhausted CD8⁺ T cells are PR domain zinc finger protein 1 (Blimp1), basic ATF-like leucine zipper transcription factor (BATF), nuclear factor of activated T cells (NFAT), von Hippel-Lindau tumor suppressor (VHL), and forkhead box O1 (FOXO1). Exhausted CD4⁺ T cells, in contrast, are characterized by expression of GATA binding protein 3 (GATA3), B cell leukemia/lymphoma 6 (BCL6), Helios, IL-10, and IL-21 and tend to be biased toward a T follicular helper cell phenotype.

Pathways to T Cell Dysfunction: Exhaustion, Anergy, and Senescence

T cell exhaustion is just one of a number of pathways leading to T cell dysfunction that can alter the qualitative and quantitative nature of the immune response; others include T cell anergy and immune senescence. Each of these forms of lymphocyte dysfunction represent a distinct differentiation state that can be distinguished from each other and from memory T cells not only by the different processes leading to their development but also by characteristic molecular signatures and surface phenotypes (17,20). T cell anergy is generated through T cell receptor signaling in the absence of costimulation. Its hallmark is the loss of the capacity to produce IL-2 and to proliferate. Unlike exhaustion, which may require months of repeated antigenic stimulation, anergy may occur relatively quickly, within days, as seen in mixed lymphocyte reaction cultures in the presence of costimulatory blockade. Costimulatory blockade therapy is actively being pursued in the transplant arena as a means to prevent rejection and promote tolerance (21). How active pharmacological costimulatory blockade is compared with peripheral self-tolerance to tissue-specific antigens that lack costimulatory signals remains unclear, but some investigators have suggested the existence of different molecular programs (22). This may, however,

just be the consequence of highly selective signals being blocked in the former (e.g. B7) versus absence of multiple costimulatory signals in the latter. The balance, at a transcriptional level, between costimulatory signals and the inhibitory signals that define exhaustion was recently reported as a predictor of clinical relapse in both autoimmunity and persistent viral infections (23).

Replicative senescence was first defined by Hayflick as growth arrest following a finite number of divisions in fibroblasts in culture. The mechanistic underpinning of this phenomenon was later found to be related to repetitive shortening of telomeres on the ends of chromosomes (24). Ultimately, if repair of telomeres is not achieved by the telomerase enzyme, the telomere shortens to the point of rendering the cell incapable of further division. At least *in vitro*, this lack of replicative capacity does not necessarily confer lethality because irreversibly senescent cells may maintain metabolic activity and, in early senescence states, cells may maintain capacity for cytokine secretion. With end-stage irreversible senescence, however, effector functions tend to be lost and apoptosis may ensue. It has thus been suggested that exhaustion is an adaptive mechanism designed to avoid both an unrestrained response and an irreversible loss of cell replication and normal effector function in the setting of chronic viral infections and repetitive antigenic stimulation. Senescent and exhausted T cells may be distinguished by direct measure of telomere length, and these populations may differ by various cell surface markers, including increased relative KLRG1 expression and decreased PD1 expression in senescent compared with exhausted T cells (25). Lymphocyte senescence, as measured by telomere length, has been associated with the reduced propensity for rejection in elderly kidney transplant recipients. Establishing causality of senescence in the lack of rejection will require further dissection of other confounding age-related factors (26).

Exhaustion During Alloimmune Responses

Development of T cell exhaustion in the setting of transplantation and its impact on outcome has not been extensively assessed; however, conditions promoting T cell exhaustion, specifically chronic exposure to large quantities of foreign antigen, also occur in many experimental animal models of transplantation tolerance. When tolerance is induced using costimulation blockage-based strategies, following the initial phase in which T cell anergy seems to predominate, the long-term persistence of the allogeneic graft in a local microenvironment characterized by Treg infiltration and immunoregulatory cytokines should favor the gradual exhaustion of donor-reactive T cells. In the rodent model of spontaneous liver allograft tolerance, alloreactive recipient T cells are activated and proliferate vigorously within both the graft and the secondary lymphoid tissue but become partially inactivated and do not

elicit rejection, and their number rapidly wanes (27). The well-described tolerogenic bias of intrahepatic immune responses is probably involved in this phenomenon, but the mass of transplanted donor tissue by itself clearly contributes. A similar phenomenon is illustrated in studies showing that simultaneous transplantation of two kidneys and two hearts results in long-term graft acceptance, whereas single allogeneic heart and kidney graft are rejected acutely (28). This result suggests that excessive and continuous T cell stimulation favors donor-specific hyporesponsiveness and promotes successful engraftment. The extent to which T cell exhaustion is actually implicated has not been addressed thoroughly. The pathogenesis of these models needs to be reexamined and the role of T cell exhaustion delineated.

Direct evidence of lymphocyte exhaustion playing a protective role in transplantation was recently provided using the bm12 single MHC class II-mismatched heart transplant model of chronic rejection. Inhibition of selectin-dependent leukocyte recruitment by knocking out fucosyltransferase VII in the recipient resulted in the exhaustion of CD4⁺ T cells and prevention of chronic rejection (29).

In clinical transplantation, donor-reactive T cells from immunosuppressed transplanted patients are chronically exposed to the high alloantigen load of the transplanted graft, a key prerequisite for the development of T cell exhaustion. Furthermore, through a poorly understood process of host-to-graft adaptation, the magnitude of anti-donor T cell responses gradually decreases with time. This is illustrated by the rarity of T cell-mediated rejection in kidney transplantation after 10 years (30) and by the feasibility of intentionally withdrawing immunosuppression from late liver transplant recipients without eliciting rejection, which is also particularly prevalent beyond 10 years after transplant (31). This is reminiscent of the progressive T cell dysfunction elicited by chronic viral infections. In a recent trial of intentional immunosuppression withdrawal in HCV-infected liver transplant recipients, tolerance (i.e. successful drug discontinuation) was associated with an increased number of circulating virus-specific CD8⁺ T cells expressing multiple inhibitory receptors (32). Furthermore, the magnitude of the intrahepatic type I interferon response, known to regulate CD8⁺ T cell exhaustion in chronic viral infections (33), and the expression of immunoregulatory genes such as *PD1*, *PDL1*, *IL10*, *BATF*, and *Foxp3* were significantly higher in tolerant recipients than in those who rejected following immunosuppression withdrawal (32). This work suggests that in liver recipients with persistent HCV infection, the state of T cell exhaustion induced by HCV may also restrain alloimmune responses, by mechanisms that could include cross-reactivity, antigen cross-presentation on the same antigen-presenting cells or a bystander effect. This is consistent with data indicating that in chronic HCV infection, phenotypic features of immune exhaustion are not only acquired

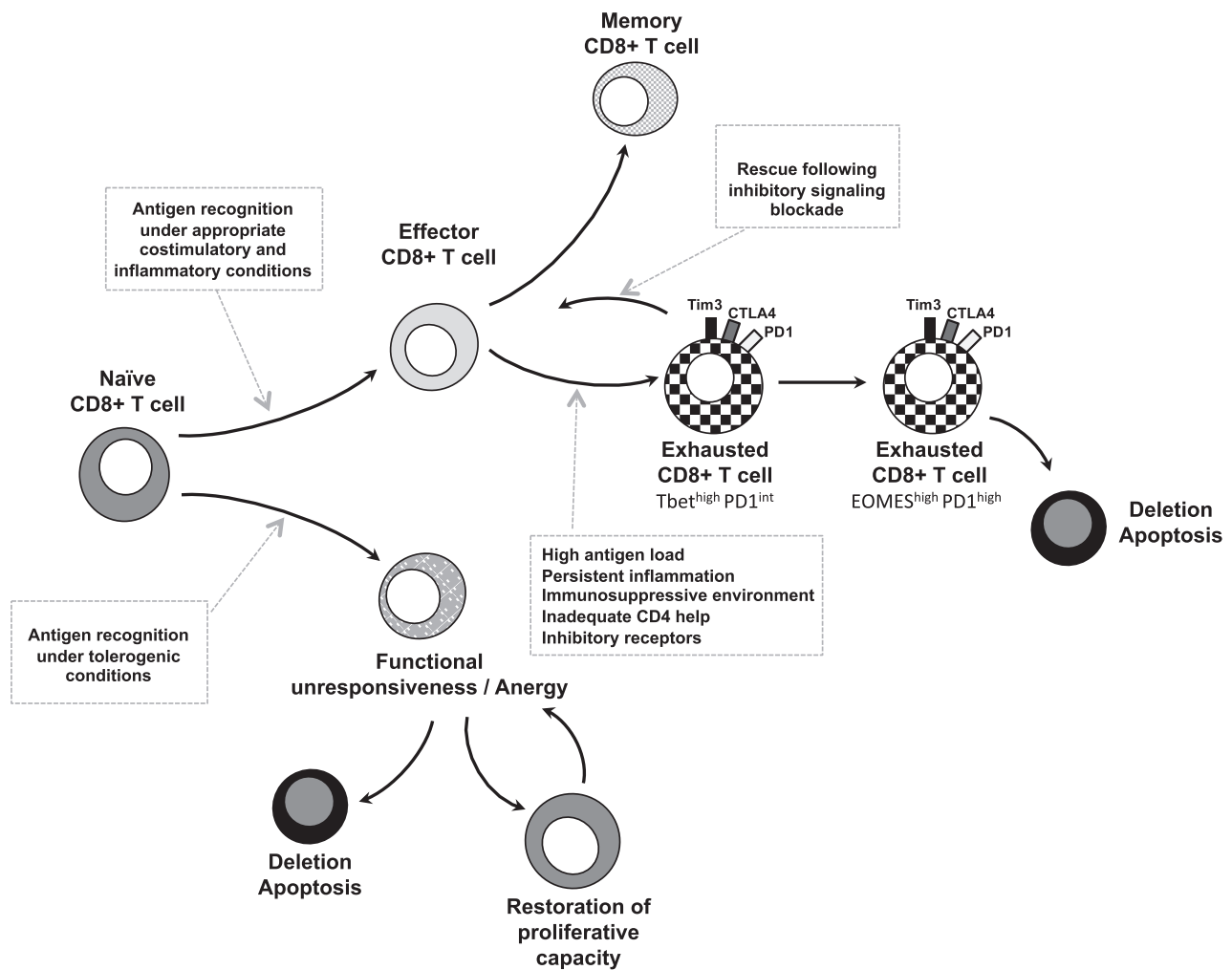


Figure 1: Following antigen recognition, naïve CD8⁺ T cells can differentiate into functional effector and memory T cells or dysfunctional unresponsive T cells, depending on the strength of the T cell receptor stimulus and the availability of costimulatory and growth factor signals. In addition, in situations of repeated antigen stimulation due to high antigenic load and persistent inflammation, effector CD8⁺ T cells gradually lose their capacity to proliferate and produce cytokines and increase the expression of multiple inhibitory receptors (e.g. TIM3, CTLA4, PD1). These exhausted CD8⁺ T cells can initially be rescued following blockade of the inhibitory signaling pathways but eventually become terminally differentiated and tend to die by apoptosis unless they are constantly stimulated by their cognate antigen. CTLA4, cytotoxic T lymphocyte-associated protein 4; PD1, programmed cell death 1; TIM3, T cell immunoglobulin domain and mucin domain 3.

by anti-HCV T cells, but also by anti-Epstein-Barr virus and anti-cytomegalovirus T cells (34).

Although the above results in HCV-positive recipients undergoing immunosuppression withdrawal provide suggestive evidence that exhaustion may contribute to the development of spontaneous tolerance to liver allografts, a missing element of the argument is evidence that donor antigen-specific T cells in the spontaneously tolerant host exhibit an exhaustion phenotype and characteristic alterations in function. A multicenter trial being conducted by the authors and sponsored by the Immune Tolerance Network (NCT02533180) has just commenced and will investigate this question directly. Donor antigen-specific and

nonspecific responding T cells in operationally tolerant and nontolerant recipients will be examined to determine whether only the tolerant patients possess an expanded population of exhausted donor antigen-specific T cells. If successful, the work may reveal new pathways that can be targeted to promote graft survival.

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References

- Zinkernagel RM. Immunology taught by viruses. *Science* 1996; 271(5246): 173–178.
- Moskophidis D, Lechner F, Pircher H, Zinkernagel RM. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. *Nature* 1993; 362: 758–761.
- Zajac AJ, Blattman JN, Murali-Krishna K, et al. Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* 1998; 188(12): 2205–2213.
- Gallimore A, Glithero A, Godkin A, et al. Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. *J Exp Med* 1998; 187(9): 1383–1393.
- Wherry EJ. T cell exhaustion. *Nat Immunol* 2011; 13(6): 492–499.
- Chang K, Svabek C, Vazquez-Guillamet C, et al. Targeting the programmed cell death 1: Programmed cell death ligand 1 pathway reverses T cell exhaustion in patients with sepsis. *Crit Care* 2014; 18(2): R3.
- Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol* 2015; 36(4): 1–12.
- Wherry EJ, Blattman JN, Murali-Krishna K, Van Der Most R, Ahmed R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J Virol* 2003; 77(8): 4911–4927.
- Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. *J Virol* 2004; 78(11): 5535–5545.
- Owusu Sekyere S, Suneetha PV, Kraft ARM, et al. A heterogeneous hierarchy of co-regulatory receptors regulates exhaustion of HCV-specific CD8 T cells in patients with chronic hepatitis C. *J Hepatol* 2015; 62(1): 31–40.
- Pellegrini M, Calzascia T, Toe JG, et al. IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. *Cell* 2011; 144(4): 601–613.
- Penaloza-MacMaster P, Kamphorst AO, Wieland A, et al. Interplay between regulatory T cells and PD-1 in modulating T cell exhaustion and viral control during chronic LCMV infection. *J Exp Med* 2014; 211(9): 1905–1918.
- Dietze KK, Zelinskyy G, Liu J, Kretzmer F, Schimmer S, Dittmer U. Combining regulatory T cell depletion and inhibitory receptor blockade improves reactivation of exhausted virus-specific CD8+ T cells and efficiently reduces chronic retroviral loads. *PLoS Pathog* 2013; 9(12): 1–7.
- Odorizzi PM, Pauken KE, Paley MA, Sharpe A, Wherry EJ. Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8+ T cells. *J Exp Med* 2015; 212(7): 1125–1137.
- Fuertes Marraco SA, Neubert NJ, Verdeil G, Speiser DE. Inhibitory receptors beyond T cell exhaustion. *Front Immunol* 2015; 6: 1–14.
- Duraiswamy J, Ibegbu CC, Masopust D, et al. Phenotype, function, and gene expression profiles of programmed death-1hi CD8 T cells in healthy human adults. *J Immunol* 2011; 186(7): 4200–4212.
- Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* 2015; 15(8): 486–499.
- Crawford A, Angelosanto JM, Kao C, et al. Molecular and transcriptional basis of CD4+ T cell dysfunction during chronic infection. *Immunity* 2014; 40(2): 289–302.
- Doering TA, Crawford A, Angelosanto JM, Paley MA, Ziegler CG, Wherry EJ. Network analysis reveals centrally connected genes and pathways involved in CD8+ T cell exhaustion versus memory. *Immunity* 2012; 37(6): 1130–1144.
- Schietinger A, Greenberg PD. Tolerance and exhaustion: defining mechanisms of T cell dysfunction. *Trends Immunol* 2014; 35(2): 51–60.
- Kirk AD, Guasch A, Xu H, et al. Renal transplantation using belatacept without maintenance steroids or calcineurin inhibitors. *Am J Transplant* 2014; 14(5): 1142–1151.
- Macián F, García-Cózar F, Im SH, Horton HF, Byrne MC, Rao A. Transcriptional mechanisms underlying lymphocyte tolerance. *Cell* 2002; 109(6): 719–731.
- McKinney EF, Lee JC, Jayne DR, Lyons PA, Smith KG. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* 2015; 523: 612–616.
- Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. *Exp Cell Res* 1965; 37(3): 614–636.
- Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? *Nat Rev Immunol* 2011; 11(4): 289–295.
- Trzonkowski P, Debska-Slizień A, Jankowska M, et al. Immunosenescence increases the rate of acceptance of kidney allotransplants in elderly recipients through exhaustion of CD4+ T-cells. *Mech Ageing Dev* 2010; 131(2): 96–104.
- Steger U, Denecke C, Sawitzki B, Karim M, Jones ND, Wood KJ. Exhaustive differentiation of alloreactive CD8+ T cells: Critical for determination of graft acceptance or rejection. *Transplantation* 2008; 85(9): 1339–1347.
- Sun J, Sheil AG, Wang C, et al. Tolerance to rat liver allografts: IV. Acceptance depends on the quantity of donor tissue and on donor leukocytes. *Transplantation* 1996; 62(12): 1725–1730.
- Sarraj B, Ye J, Akl AI, et al. Impaired selectin-dependent leukocyte recruitment induces T-cell exhaustion and prevents chronic allograft vasculopathy and rejection. *Proc Natl Acad Sci U S A* 2014; 111(33): 12145–12150.
- Halloran PF, Chang J, Famulski K, et al. Disappearance of T cell-mediated rejection despite continued antibody-mediated rejection in late kidney transplant recipients. *J Am Soc Nephrol* 2015; 26(7): 1711–1720.
- Benítez C, Londoño M-C, Miquel R, et al. Prospective multicenter clinical trial of immunosuppressive drug withdrawal in stable adult liver transplant recipients. *Hepatology* 2013; 58(5): 1824–1835.
- Bohne F, Londoño M-C, Benítez C, et al. HCV-induced immune responses influence the development of operational tolerance after liver transplantation in humans. *Sci Transl Med* 2014; 6(242): 242ra81.
- Wilson EB, Yamada DH, Elsaesser H, et al. Blockade of chronic type I IFN signaling to control persistent LCMV infection. *Science* 2013; 340: 202–207.
- Owusu Sekyere S, Suneetha PV, Hardtke S, et al. Type I interferon elevates co-regulatory receptor expression on CMV- and EBV-specific CD8 T cells in chronic hepatitis C. *Front Immunol* 2015; 6: 270.