Integration of the Innate and Adaptive Immunity by CD137-CD137L Bidirectional Signals: Implications in Allograft Rejection

Two-signal models are useful in explaining various types of immune responses. In particular, secondary, so-called costimulatory, signals are critically required for the process of T-cell activation, survival, differentiation, and memory formation. Early studies in rodent models showed that targeting T-cell costimulatory pathways elicits immunological tolerance, providing a basis for development of costimulatory therapeutics in allograft rejection. However, as the classic definition of T-cell costimulation continues to evolve, simple blockade of costimulatory pathways has limitations in prevention of allograft rejection. Furthermore, functions of costimulatory molecules are much more diverse than initially anticipated and beyond T cells. In this mini-review, we will discuss CD137-CD137L bidirectional signals as examples showing that two-signals can be applicable to multiple phases of immune responses.

Key Words: Costimulation, CD137, CD137L, Immune response

INTRODUCTION

Two-signal models have helped shape our understanding of T-cell activation for over 40 years(1). T cells specifically recognize antigens through their surface T-cell receptor (TCR) in the context of MHC-peptide complexes. This signal (signal 1) can fully activate T cells only when signal 2, also known as a costimulatory signal, is provided. This paradigm of T cell activation shed light on the area of immunotherapy, since blocking costimulatory signaling pathways at the time of antigen encounter was shown to result in an antigen-specific T-cell tolerance. Consequently great efforts were exerted to reduce alloreactive or autoreactive T cells during autoimmunity or transplantation using this approach. Now, one fruit of these efforts, a blocker of a prototype of costimulatory molecules called CTLA-4-Ig, was translated into the clinic(2). In the mean, as our understanding of costimulation has evolved considerably, its concept has been more broadly used and simple blockade of costimulatory pathways has had its limitations in clinical applications. First, it now become clear that costimulation blockade acts on multiple phases of immune response and its mechanisms of action involve not only clonal deletion but also regulation(3,4). Second, costimulation blockade has shown to be insufficient to induce allograft tolerance in non-human primates due to high frequency of memory T cells that are less dependent upon conventional costimulatory pathways.
ulation for their activation(5-10). Finally, the classical two-signal model is now being modified due to existence of numerous costimulatory molecules with a diversity of functions(11,12). To make the story more complex, spatio-temporal expression of costimulatory receptor and their ligand(s) are continuously influenced by dynamic environmental conditions, making combinations of cell surface interaction more complex(12). In addition multiple pairing of receptor and ligand molecules and the existence of bidirectional signaling indicate that more complex combinations of signaling events occur after receptor-ligand interactions.

Two-signal models exist in a variety of forms and provide a frame for activation of innate immunity cells as well as T cells(12,13). In this case, signal 1 is pattern recognition receptors (PRRs), antibody receptors, and molecules expressed in stressed cells. Signal 2 is provided by molecules belonging to many molecular families, including T-cell costimulatory receptors or ligands. The requirement for two signals is thought to be evolutionally conserved in the immune system because it acts as a safeguard that regulates the powerful and potentially harmful immune reaction and prevents the accidental triggering of responses against the host’s own tissues(13). In this mini-review, we will discuss costimulatory network during immune responses, focusing on CD137-CD137L bidirectional signals as an example.

**T-CELL COSTIMULATION**

CD137 is a member of the TNF receptor family and its expression is inducible in T cells(14,15). Induction of CD137 can be triggered by TCR signaling and CD28 signaling further increases TCR-mediated upregulation of CD137 expression, suggesting that CD137 signaling is dependent upon CD28 signaling(16). Nonetheless, CD137 is thought to have distinct roles in T-cell-mediated immune responses. Either blocking the CD137 signaling pathway using gene deletion/blockers or stimulating it using agonist antibodies is widely adopted to exploit CD137 functions. Even though it is believed that CD137 cooperates with so many other costimulatory receptors to regulate T-cell activities, numerous studies have demonstrated that CD137 delivers a potent costimulatory signal in CD8+ T cells. There is evidence supporting this interpretation in a transplantation setting in mice. Rejection of intestinal allografts mediated by CD8+ T cells is effectively blocked by CD137-Fc fusion protein(17). In this case, proliferation of CD8+ T cells is not influenced by CD137-Fc fusion protein but its effect is correlated with downregulation of TNF-α (17). These results may suggest that CD137 signaling is critical in the generation of effector CD8+ T cells. In the parent-into-unirradiated F1 acute graft-versus-host disease (GVHD) or chronic GVHD model, blockade of the CD137 costimulatory pathway, using anti-CD137L monoclonal antibody, inhibits acute GVHD, while exacerbating chronic GVHD(18). The effect of anti-CD137L monoclonal antibody on GVHD is due to its inhibitory effect on donor CD8+ T-cell activity in both models. Such an inactivation of donor CD8+ T cells results in elevation of autoantibody levels in chronic GVHD, since they cannot delete host B cells, including autoreactive B cells, compared with intact donor CD8+ T cells. It is well known that donor CD8+ T cells are transiently activated early after transfer into the host and that host B cells are very susceptible to the attack by activated donor CD8+ T cells(19,20). In this model, anti-CD137L monoclonal antibody has a minimal effect on donor CD4+ T cells. However, blockade of CD137 in conditioned recipients reduces either CD8+ T cell-mediated or CD4+ T cell-mediated GVHD lethality(21). Consistently, in a MHC-disparity acute GVHD model, depletion of alloreactive donor CD137+ T cells does not induce the development of GVHD(22). It also has been shown that cardiac allograft rejection is inhibited by anti-CD137L monoclonal antibody in T-cell-replete mice(23).

Investigations of the effects of agonist anti-CD137 monoclonal antibody have provided an insight into CD137 signals in CD4+ T cells. An explosion of literature have demonstrated that stimulation of CD137 results in strong suppression of a variety of autoimmune or inflammatory diseases that are believed to be mediated mainly by CD4+ T cells(15,24). Mechanisms of action behind anti-CD137-mediated immunosuppression still remain to be fully elucidated but seem to depend upon the context of inflammatory processes involved in the development of diseases(24). For example, agonistic anti-CD137 monoclonal antibody inhibits autoantibody production almost without exception, subsequently blocking autoantibody-mediated autoimmune diseases such as systemic lupus erythematosus and rheumatoid
TWO-SIGNAL ACTIVATION OF INNATE RESPONSES

Observations showing that lower extents of inflammatory diseases occur in CD137-deficient mice or mice treated with CD137 blockers are considered to be evidence for the involvement of CD137 signaling in CD4+ T-cell-mediated inflammation. For example, there is a milder disease severity in the absence of CD137 signaling in various types of immunological diseases, including rheumatoid arthritis, atherosclerosis, acute coronary syndrome, autoimmune myocarditis, herpetic stromal keratitis, allograft rejection, and GVHD(24,29). CD137 signaling is also involved in inflammatory responses mediated by other lymphoid cells, including NK and NKT cells and myeloid cells. Furthermore, reverse signaling through CD137L is critical in inflammation induced by various insults(30,31).

The first evidence for CD137’s costimulatory roles in cells other than T cells was provided by Nishimoto et al.(32). They have shown that CD137 signaling costimulates mast cell activation induced by high-affinity FcεRI stimulation. However, this in vitro observation was not confirmed in in vivo experimental settings. There is evidence that CD137 signaling in neutrophils is indispensable for defense mechanisms for Listeria monocytogenes(33,34). CD137 signaling directly enhances neutrophil activities related to bacterial clearance (phagocytosis, ROS production, calcium influx, and cytokine/chemokine production). Neutrophil activation by L. monocytogenes and other Gram-positive bacteria is mediated through TLR2 as a primary signal(35). However, CD137 signaling shows an opposite effect on infection with Gram-negative bacteria: it exacerbates bacteremia and subsequent mortality(36). Even though CD137 signaling negatively regulates TLR2 signaling leading to bacterial killing of neutrophils for Gram-negative bacteria, production of proinflammatory cytokines by neutrophils is nothing to do with CD137 signaling(35). As CD137L signaling seems to be critical in sepsis induced by Gram-negative bacteria or other pathogens (our unpublished data), resistance of CD37-/- mice to sepsis is likely to be due to blockade of the CD137L costimulatory pathway (discussed below).

Mesenteric dendritic cells (DCs) play a pivotal role in oral tolerance. These DCs generate regulatory CD4+ T cells by producing retinoic acids. CD137 signaling costimulates mesenteric DCs after TLR2 or GM-CSF receptor signaling to upregulate retinal dehydrogenase, an enzyme important for retinoic acid production(37). This case provides a typical example where the primary signal determines the cellular fate with the costimulatory signal enforcing the primary signal.

Recent studies have identified expression of CD137 on non-hematopoietic cells under disease conditions (e.g., endothelial cells, smooth muscle cells, and cardiomyocytes). CD137 expression is increased in endothelial cells in athero-
sclerotic plaque and tumor(38,39). Engagement of CD137 in endothelial cells using anti-CD137 monoclonal antibody results in the production of cytokines and chemokines and upregulation of cell adhesion molecules(40). Macrophages not only produce these inflammatory mediators in response to CD137 signaling but also secrete MMP-3 and MMP-9, thus regulating the stability of atherosclerotic plaque(40). In isolated cells, CD137 stimulation alone can activate endothelial cells and macrophages to some extent but the primary signal is likely to be provided to them in in vivo situations. Thus, two-signal models are useful in explaining activation of non-immune cells as well as immune cells by a primary signal and a costimulatory signal.

CD137L is expressed in antigen-presenting cells (APCs) and other myeloid cells (B cells, macrophages, DCs, mast cells, and eosinophils) and non-hematopoietic cells (endothelial cells, fibroblasts, and epithelial cells)(15). Evidence supporting that CD137L signals play an in vivo physiological role in inflammation is just being emerged, even though accumulating evidence has demonstrated the existence of CD137L signals at molecular and cellular levels. For example, CD137L signaling mediates cellular functions ranging from cell differentiation, proliferation, and survival to the production of inflammatory mediators in a variety of cells(41).

It is now becoming clear that CD137L signaling is critical in multiple phases of inflammation. Inflamed vessels express CD137L as well as CD137 and CD137L signaling in endothelial cells leads to the production of proinflammatory cytokines and chemokines(38). Further, CD137L signaling may facilitate trans-endothelial migration of leukocytes through upregulation of cell adhesion molecules on endothelial cells(37). On the other hand, CD137L signaling increases the expression of cell adhesion molecules on monocytes and promotes their extravasation(39). Since endothelial cells express both CD137 and CD137L, CD137-CD137L interactions between endothelial cells and various types of leukocytes may amplify inflammation such a way that endothelial cells induce sustained production of inflammatory mediators and prime leukocytes before they arrive at inflamed tissue territories. In the tissues, it seems that CD137L signaling in recruited leukocytes, resident cells, and parenchymal cells is also critical in the amplification of inflammation. Macrophages express CD137L on exposure to an inflammatory environment and produce high levels of pro-inflammatory cytokines and chemokines in response to CD137L signaling(41). In collaboration with other inflammatory inducers, CD137L signaling results in the production of inflammatory mediators by macrophages in a synergistic manner (our unpublished data), indicating that CD137L signaling is a central amplifier of inflammation. It is noteworthy that CD137L can sustain TLR signaling by binding to TLRs without engagement of CD137(42). Recently, we have identified a novel inflammatory pathway involving CD137L signaling in epithelial cells(43). In ischemia-reperfusion kidney injury, CD137L signaling in infiltrated NK cells stimulates CD137L in tubular epithelial cells to produce CXCL1 and CXCL2 that are required for recruitment of neutrophils in the kidney. Since ischemia-reperfusion kidney injury does not occur without NK cells or neutrophils, it is thought that the axis of NK cell-tubular epithelial cell-neutrophil is the major pathogenic pathway for ischemia-reperfusion kidney injury(31,43). Interestingly, tubular epithelial cells function as a master regulator for immune cell recruitment during ischemia-reperfusion injury(44). In the early phase of ischemia and reperfusion, damaged tubular epithelial cells secrete damage-associated molecular patterns (DAMPs) such as HMGB-1, which induces production of CCR5 chemokines required for NK cell recruitment. It is believed that TLR2 signaling alone in tubular epithelial cells also results in a small wave of neutrophil as well as NK cell recruitment. Even though TLR2 and CD137L signaling in tubular epithelial cells is independent signaling events that sequentially occurs, the two signaling events act together for a full wave of neutrophil recruitments. Thus, we believe that CD137L costimulatory signaling is required for TLR2 and presumably other TLR receptors to fully amplify ischemia-reperfusion-induced renal inflammation.

There are few reports on roles of CD137L signaling in disease context. As mentioned above, CD137L signaling is indispensable for ischemia-reperfusion kidney injury(43). Considering that CD137L signaling is critical in inflammatory responses, it is predicted that milder inflammatory diseases will occur in the absence of CD137L signals. Indeed, we showed that blocking of CD137L can inhibit in-
flammary responses and prevent mortality in *Candida albicans*-induced sepsis (our unpublished data). In this model, CD137 signaling enhances the phagocytic activity of neutrophils, whereas CD137L signaling induces massive cytokine production by macrophages following *C. albicans* infection, which is associated with sepsis-induced mortality. In this model, TLR2 and Dectin-1 are the primary signals for macrophage activation. However, renal inflammation cannot be fulminant without CD137L signaling. All these data suggest that CD137L delivers a costimulatory signal mainly for PPRs in macrophages and parenchymal cells.

**CD137-CD137L BIDIRECTIONAL SIGNALS AS AN INTEGRATOR OF INNATE AND ADAPTIVE IMMUNITY**

Now it is clear that CD137 and CD137L provide a costimulatory signal bidirectionally to both innate and adaptive immune cells. Two facts have to be considered to understand the physiological roles of CD137-CD137L bidirectional signaling. First, as they are cell surface molecules, their signaling is triggered by cell-cell contact. Second, a microenvironment governing a primary signaling is critical in determining the characteristics of immune responses. The primary signal may be required for an initial recruitment of immune cells and induction or upregulation of CD137 or CD137L on their cell surface or other stromal or parenchymal cells during immune responses. During ischemia-reperfusion kidney injury, tubular epithelial cells produce chemokines to recruit immune cells in response to DAMPs in an autocrine fashion (Fig. 1A)(43,44). Later, CD137-expressing NK cells stimulate tubular epithelial cells together with DAMPs to produce higher levels of chemokines(43,44). On the other hand, NK cells can be activated by NKG2D, which is engaged by Rae-1 of tubular epithelial cells(45). NKG2D signaling is the primary signal during ischemia-reperfusion kidney injury with CD137 functioning as a costimulatory signal, without which NK cells are impaired in the cytotoxic activity against damaged tubular epithelial cells(43). It is thought that, if damage in tubular epithelial cells is beyond a threshold for tissue repair, they choose to be killed by NK cells and inflammatory processes where CD137-CD137L bidirectional signaling plays a pivotal role(44).

**Fig. 1.** A schematic diagram showing two representative in vivo functions of CD137-CD137L bidirectional signaling. (A) CD137-CD137L interactions occur between NK cells and tubular epithelial cells during ischemia-reperfusion kidney injury. TLR2 and CD137L signaling in tubular epithelial cells induces the production of chemokines required for immune cell recruitment. NKG2D and presumably CD137 signaling enhances the cytotoxicity of NK cells. (B) In atherosclerotic lesions, PRR and CD137L signaling together results in the production of proinflammatory cytokines and chemokines, while TCR and CD137 signaling in activated T cells leads to IFN-γ production. These immune mediators contribute to chronic inflammation in atherosclerotic lesions. Abbreviations: DAMP, damage-associated molecular patterns; IFN-γ, Interferon-γ; HMGB1, high-mobility group box 1; NK cell, Natural Killer cell; NKG2D, Natural killer group 2, member D; PRR, pattern recognition receptor; TCR, T cell receptor; TLR2, Toll-like receptor 2.
Even though numerous combinations of cell-cell interactions can be envisioned for CD137-CD137L bidirectional signaling, T cell-monocyte/macrophage interactions seem to be the most important for CD137-CD137L bidirectional signaling. For example, activated T cells and macrophages express CD137 and CD137L, respectively, in atherosclerotic lesions. CD137 signaling in activated T cells results in IFN-γ, whereas CD137L signaling in macrophages induces proinflammatory cytokines and chemokines (Fig. 1B). These inflammatory mediators activate endothelial cells together with CD137 or CD137L signaling, subsequently contributing to establishing a vicious inflammatory cycle in atherosclerosis. Similarly, interactions of CD137 on regulatory CD4+ T cells and CD137L on monocytes/macrophages are required for renal inflammation caused by *C. albicans* infection (our unpublished data). In this case, CD137L signaling in monocytes/macrophages is responsible for proinflammatory responses. On the other hand, CD137 signaling drives regulatory CD4+ T cells to immunostimulatory cells by decreasing their immunosuppressive activity in a TLR2-dependent manner and thus CD137 on these cells effectively stimulates CD137L on monocytes/macrophages in the presence of *C. albicans* without inflicting immunosuppressive activity to these cells. By marked contrast, CD137 on regulatory CD4+ T cells interacts with CD137L on myeloid-derived suppressive cells in a tumor microenvironment and promotes M2 macrophage polarization (our unpublished data). CD137L-driven M2 macrophage polarization is critical in the generation of CD8+ T-cell tolerance.

There is a consensus that CD137 signaling is involved in the generation of effector and memory T cells. Currently, it is not clear whether CD137L signaling in APCs can influence this process. As APCs express molecules belonging to PRR families, it is possible that CD137L functions as a costimulatory signal in these cells which can affect T-cell differentiation. It also is possible that CD137L signaling can influence T-cell responses during the evolution of immune responses in a context-dependent way. In particular, CD137L signaling is likely to be important for effector mechanisms. For example, this signaling may be involved in tissue remodeling and vasculopathy during chronic allograft rejection, considering that CD137L is highly expressed on fibroblasts and endothelial cells. To define CD137-CD137L bidirectional signaling in various types of cells, we need to use cell-specific conditional knockout mice for CD137 or CD137L gene.

**CONCLUSION**

In various clinical settings, blockade of the CD37 signaling pathway was expected to inhibit the full activation of T cells. However, effects of CD137 blockers were evident in a few disease models. Rather, CD137 agonists have been shown to be potent in inhibiting inflammatory and autoimmune diseases. The existence of the CD137L signaling pathway at least partially provides an adequate explanation for why CD137 blockers or agonists have opposite effects as expected on therapeutic outcome. It should be appreciated that reagents thought to be CD137 blockers may stimulate CD137L signaling and thus may increase inflammation in some situations. In a similar context, CD137 agonists can function as blockers for CD137L signaling. Therefore, an ideal blocker for CD137-CD137L bidirectional signaling is neutralizing anti-CD137 or anti-CD137L antibody.

Accumulating is evidence indicating that CD137L signaling is a convergence point for inflammation. CD137L signaling is particularly a good target for ischemia-reperfusion renal injury. Blocking the CD137L signaling pathway may turn out to be effective in chronic allograft rejection. However, it should be clearly defined before clinical applications of CD137 or CD137L-related reagents how immune networks involving CD137-CD137L bidirectional signaling determine pathophysiological environments.

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