Review

Immunoregulatory Role of B7-H1 in Chronicity of Inflammatory Responses

Haidong Dong[1, 3] and Xianming Chen[2]

Pathogenesis of most chronic human diseases, including chronic infections, autoimmune diseases and cancers, often involves a persistent, unresolved inflammatory response. The molecular mechanisms that determine the conversion of an acute inflammatory response into a chronic process had puzzled researchers for many years. Recent studies reveal that B7-H1 (CD274, PD-L1), a newly identified co-stimulatory molecule, possesses dual functions of co-stimulation of naïve T cells and inhibition of activated effector T cells. The aberrant cellular expression and deregulated function of B7-H1 have been reported during chronic viral and intracellular bacterial infection, as well as in many autoimmune diseases and cancers. Importantly, the deregulation of B7-H1's dual functions appears to be associated with a prolonged and incomplete immune response by luring naïve T cells for activation and dampening activated effector T cells. Moreover, development of strategies targeting B7-H1 signals provides a new and promising approach to manipulate the devastating diseases associated with chronic inflammation. Thus, B7-H1 may play a critical immunoregulatory role in the chronicity of inflammatory responses. *Cellular & Molecular Immunology*. 2006;3(3):179-187.

Key Words: B7-H1, immune response, inflammation, chronization, infection, tumor

Introduction

Clinical and epidemiologic studies have implicated an association between infectious agents and chronic inflammatory disorders as well as cancers. However, a direct mechanistic link between pathogen-specific gene products and pathologic changes key to the pathogenesis of the disease is often missing (1, 2). Recent advances in the understanding of host immunity in response to microbial infection, have provided pieces for a more general model of chronic inflammatory diseases, in particular, those for which a specific pathogen yet to be identified (1). Emerging evidence has suggested that in many instances, aberrant host immune responses, rather than the pathogen-specific toxins or oncogene products, are the real pathogenetic factors that cause the illness or function as risk factors for these diseases (1).

Whereas most external pathogens provoke an acute inflammatory or immune response that results in complete clearance of the microorganisms, some immunogenic agents promote an immune response that does not lead to clearance but to a persistent inflammatory process. This process involves a dynamic procedure of host immune responses triggered by the interactions between the pathogen and host cells. Activation of host immune cells, especially antigen-presenting cells which directly control the strength and duration of an established immune response, initiates a series of anti-microbial defense responses against the pathogen. On the other hand, once the immune responses targeting the invaded or mutant self-antigens are established, the host cells usually upregulate a set of regulatory molecules that protect themselves from further or unwanted injury by those immune responses that are originally aimed at the immunogenic antigens. Recent studies have suggested that aberrant regulation of this self-protective mechanism may lead to

[1]Department of Immunology, and [2]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Mayo Clinic College of Medicine, Rochester, MN 55905, USA;

[3]Corresponding to: Dr. Haidong Dong, Department of Immunology, Mayo Clinic College of Medicine, 200 First Street, SW, Rochester, MN 55905, USA. E-mail: dong.haidong@mayo.edu.

Received Jun 14, 2006. Accepted Jun 27, 2006.

Abbreviations: PD-1, programmed death-1; IFN-γ, interferon-γ; TLR, Toll-like receptor; CTL, cytotoxic T lymphocyte; HIV, human immunodeficiency virus; NK, natural killer; DCs, dendritic cells; mDCs, myeloid dendritic cells; RSV, respiratory syncytial virus; LCMV, lymphocytic choriomeningitis virus; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; PI3K, phosphatidylinositol 3-kinase; ITIM, immunoreceptor tyrosine-based inhibition motif; ITSM, immunoreceptor tyrosine-based switch motif; SHP-2, Src homology region 2 domain-containing phosphatase-2; ERK, extracellular signal-regulated kinase; CoR, co-stimulaotry receptor.

insufficient clearance of pathogenic agents resulting in a chronic infection with devastating immunopathology. One of such regulatory molecules is B7-H1 (also named as CD274 or PD-L1), a newly identified member of the B7 family with important regulatory functions in cell-mediated immune responses (3, 4). In this review, we provide a concise summary of the most recent advances on B7-H1 in autoimmune diseases, tumors, viral and bacterial infectious diseases, including *in vivo* studies employing B7-H1 knockout mice. These studies support the concept that B7-H1 plays an important immunoregulatory role in the chronicity of inflammatory disorders.

B7-H1 is a co-stimulatory molecule with dual functions on T cell response

B7-H1 was identified by EST database searches based on its homology to the B7 family of co-stimulatory molecules (3). Although B7-H1 expression at the mRNA level is common in many tissues and cells, expression of B7-H1 at the protein level is not common in most cell types (3). Expression of B7-H1 protein is found constitutively on macrophages and dendritic cells (DCs) and is induced on activated T cells, B cells, endothelial cells and epithelial cells (5-7). The counter-receptor for B7-H1 is programmed death-1 (PD-1), a CD28/CTLA-4 like molecule expressed on activated T cells, B cells and macrophages (4, 8).

Using different *in vitro* and *in vivo* models and different forms of B7-H1 protein (fusion protein or cell-associated protein), several groups have independently revealed dual functions of B7-H1 in regulating T cell responses. First, B7-H1-mediated signals are able to co-stimulate early T cell priming and differentiation *in vivo* and *in vitro* (3, 9, 10). Second, B7-H1 signals negatively regulate activated T cell's function and survival (4, 5, 10). The current challenge in B7-H1 study is that the functional role of PD-1, the only unidentified B7-H1 receptor, could not confidently explain the dual functions of B7-H1 either *in vitro* or *in vivo*.

PD-1 deficiency causes a variety of autoimmune diseases (11-13), therefore, an inhibitory signaling through PD-1 receptor might be involved in the negative regulation of T cell responses. Engagement of B7-H1 with PD-1 on fully activated T cells actually inhibited the proliferation and cytokine production of these T cells (4, 14). Therefore, PD-1 signals may explain the B7-H1 mediated inhibition of T cell responses. This view is reflected in the signal transmission pathway of PD-1. PD-1 contains two tyrosine molecules within its cytoplasmic tail. The most membrane-proximal tyrosine is located in an immunoreceptor tyrosine-based inhibition motif (ITIM), and the distal tyrosine is located in an immunoreceptor tyrosine-based switch motif (ITSM). Upon engagement with its ligand, PD-1 recruited Src homology region 2 domain-containing phosphatase-2 (SHP-2) to the ITSM but not to ITIM (14, 15), suggesting that SHP-2 is a key downstream signaling molecule that mediates PD-1 signaling. PD-1 signaling inhibits AKT and ERK phosphorylation by preventing CD28-mediated activation of phosphatidylinositol 3-kinase (PI3K) (16, 17). Ligation of PD-1 in T cells also inhibits the Zap-70 engagement with CD3ζ chain and inhibits PKC and ERK activation (18). Thus, it becomes clear that B7-H1/PD-1 pathway negatively regulate T cells responses at their later stage of activation. This is further supported by the expression of PD-1 on activated T cells at later stages and broad distribution of B7-H1 in the peripheral tissues especially upon the stimulation of interferon-γ (IFN-γ) which is the main effector molecule released by activated T cells (5, 19).

The remained question is whether B7-H1-mediated co-stimulatory function or B7-H1-induced apoptosis of T cells is PD-1 dependent. It may be hard to study the function of PD-1 on naïve or resting T cells because the expression of PD-1 is not rapidly induced on the surface of these T cells (19, 20). Lack of agonistic antibody to PD-1 and the low affinity of ligand fusion protein in engagement of naïve T cells delayed the delineation of the co-stimulatory role of PD-1. However, a recent report revealed a co-stimulatory function of PD-1 in T cells using newly generated monoclonal antibodies (21). Anit-PD-1 mediated proliferation and cytokine production was observed in T cells *in vitro* and *in vivo*. Interestingly, PD-1's co-stimulatory role is dependent on CD28. Even though this outcome may need more confirmation by the identification of down stream signal pathways, this study actually indicated a cross-talk of two receptors in regulating T cells responses that needs our further attention. Because of the monomer structure of PD-1 on cell surface (22) and its inhibitory role is dependent on its close proximity with TCR (18), the engagement of the antibody with PD-1 may change the signal transmission of PD-1 by interfering the strand-to-strand binding mode of PD-1with its ligand, which is distinct from the loop-to-strand binding mode as observed in CTLA-4/B7 complexes (22).

Although the clear role of PD-1 in co-stimulatory function is still in debate, several studies have provided some evidence for an independent, non-PD-1 co-stimulaotry receptor (CoR) in induction of T cell proliferation (23, 24) and apoptosis (5, 25). Nevertheless, the rapidly upregulated B7-H1 on human T cells was found to be functional in transmitting outside-in signals (26). Cross-link of B7-H1 by an agonistic antibody increases the proliferation of resting T cells, and enhances the apoptosis of activated T cells through the activation of caspase-3 and expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (26). Taken together, B7-H1 possesses dual regulatory functions in T cell response: to co-stimulate naïve T cells and inhibit activated effector T cells (27) (Figure 1). In the following sections, we will explore the contribution of B7-H1 signals in the chronicity of inflammatory responses.

B7-H1 and autoimmune/chronic liver diseases

Besides its importance for the digestion system, the liver is an important organ in the regulation of T cell responses to pathogens or autoantigens. Immune responses in the liver often lead to tolerance that is reflected by the high acceptance

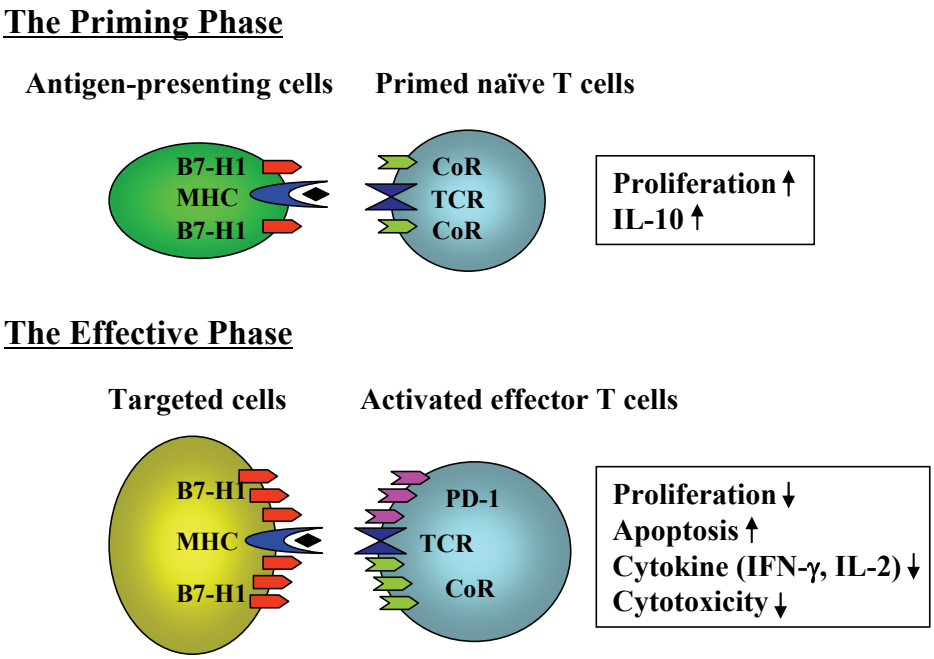

Figure 1. Dual regulatory functions of B7-H1 in T cell response. At the priming phase of immune response, T cells recognize the MHC/peptide complex from the antigen-presenting cells *via* T cell receptor (TCR). During this process, B7-H1 on the antigen-presenting cells engages with co-stimulatory receptor (CoR) on primed T cells and co-stimulates T-cell proliferation or IL-10 production that are leading to a T cell differentiation. At the later phase of immune response, both PD-1 and/or CoR are upregulated on fully activated effector T cells. The enhanced B7-H1 signals cause apoptosis or dysfunction of effector T cells resulting in inhibition/limitation of T cell responses.

rate of liver transplants without immunosuppression (28, 29). On the other hand, the tolerating feature of the liver is also a paradise for chronic viral infections, including hepatitis-B and hepatitis-C viruses. A potential mechanism of this tolerance could be the ability of the liver to control the apoptosis of activated T cells. It has been observed that systemically activated antigen-specific $CD8^+$ T cells subsequently accumulate and are deleted in the liver (30). The molecular mechanisms underlying these observations have not yet been elucidated. We and others have shown that Kupffer cells and monocyte-derived cells, as well as epithelial and endothelial cells in the liver, constitutively express B7-H1 protein on their surface (5, 31-33). Iwai et al. found that expression of B7-H1 protein was induced in liver nonparenchymal cells including sinusoidal endothelial cells and Kupffer cells during adenovirus infection (32). These cells could inhibit the proliferation and cell division of activated T cells expressing PD-1 from the wild type mice. We have also detected an increased accumulation of $CD8^+$ T cells in the liver of naïve B7-H1 knockout mice compared to wild-type mice (34). In addition, deletion of antigen-activated $CD8^+$ T cells was delayed in the liver of B7-H1 knockout mice, suggesting an important role for B7-H1 in controlling the deletion of activated intrahepatic $CD8^+$ T cells in the liver. Consequently, B7-H1 knockout mice are more vulnerable to the induction of experimental autoimmune hepatitis (34). Later on, the hepatic stellate cells were identified as the cellular source of B7-H1 in the mouse liver for the depletion of activated T cells. *In vitro* assay revealed that blocking B7-H1 reduced the hepatic stellate cells-mediated T cell apoptosis (35).

Similar results have also been obtained in studies using the PD-1 knockout mice. Using an adenovirus liver infection model, Iwai et al. observed increased percentage of proliferating $CD3^+$ T cells in the liver of PD-1 KO mice compared to wild type mice on day 7 after infection. In contrast, the percentage of $CD3^+$ T cells in the spleen of PD-1 KO mice was similar or even less than that of wild type mice. In the absence of PD-1, extensive hepatocellular injury and mononuclear cell infiltration were observed during adenovirus infection accompanied with a rapid clearance of the adenovirus from the liver (32). These results suggest that liver-associated B7-H1 negatively regulate the T cell responses by binding its receptor PD-1 on T cells.

The molecular basis to maintain the delicate balance of the dual immunoregulatory functions of B7-H1 in the liver appears to be associated with expression of IFN-γ, a strong inducer of B7-H1 expression (5). Because there is an intact inducible level of B7-H1 expression in the liver cells, the deletion or inhibition of activated T cells can be maintained at a normal level based on the endogenously produced IFN-γ. In contrast, high secretion of IFN-γ from activated T cells upon stimulation will increase B7-H1 expression in associated cells and cause a downregulation of T cell responses, resulting in a negative feedback loop in the liver. This view was elegantly demonstrated by the work of

Isogawa et al. (36). They found that when hepatitis B virus-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) were adoptively transferred into hepatitis B virus-transgenic mice, the CTLs not only secreted IFN-γ to inhibit viral replication in the liver but also killed their targeted liver cells, causing hepatitis. Surprisingly, the ability of these cells to produce IFN-γ decreased rapidly thereafter. Interestingly, the loss of IFN-γ expression coincided with the strong induction of PD-1 on viral-specific $CD8^+$ T cells. In contrast, they did not observe any CTLA-4 expression on the hepatitis B virus-specific T cells and any changes of intrahepatic regulatory ($CD4^+CD25^+$) T cells at any time point after adoptive transfer. Therefore, the downregulation of IFN-γ production by $CD8^+$ T cells after antigen recognition in the liver may be due to the suppressive influence of signaling *via* the PD-1 receptor on the activated T cells (4, 14). These results reveal that both the effector functions and the expansion-contraction kinetics of the CTLs are regulated in an oscillatory manner as a consequence of antigen recognition in the liver and in association with PD-1 upregulation.

Whereas the IFN-γ producing function of viral-specific $CD8^+$ T cells is related to the clearance of viral infection, the cytolytic function of T cells can also lead to the tissue injury. Therefore, the balance of stimulatory and inhibitory effects of antigen recognition and regulatory molecules on IFN-γ production and cytolytic activity is the key to limit the amount of tissue injury while keeping a strong antiviral T cell response. Any defects in this finely tuned process may lead to a persistent viral infection in the liver that lacks IFN-γ production but harbors more cytolytic T cells. Recent studies indicate that the IFN-γ production seems more sensitive to the PD-1 suppressive signals, while the cytolytic activity (Granzyme B-production) could be maintained even with high PD-1 on T cells (36). The asynchronous responses of T cell to PD-1 signal may reveal a potential pathogenetic role of B7-H1/PD-1 pathway in chronization of anti-viral immune response, because the prolonged signaling *via* B7-H1/PD-1 pathway may irreversibly damage the anti-viral T cell function resulting in an incomplete clearance of virus infection. Taken together, these results indicate that the B7-H1/PD-1 pathway plays an important role in T cell tolerance at the effector phase in the liver upon antigen-activation or viral infection.

B7-H1 and other chronic viral infections

An immunoregulatory role for B7-H1 in chronicity of inflammatory responses has also been reported in other chronic viral infections including a recent compelling study from the Ahmed's group (37). Chronic lymphocytic choriomeningitis virus (LCMV) infection is often characterized by varying degrees of functional impairment of virus-specific T cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen even the usage of their antigen-specific TCR does not change (38). Although functional effector T cells are initially generated during the early stages of infection, they gradually lose their functions during the course of the chronic infection. This exhaustion of virus-specific T cells was first shown during persistent LCMV infection in mice (39).

To identify the mechanism underlying the exhaustion of T cells, Barbar et al. performed a comparative genome-wide microarray analysis of genes expressed by exhausted LCMV-specific $CD8^+$ T cells. In their assay, PD-1 turned out to be the most upregulated gene by the exhausted LCMV-specific $CD8^+$ T cells. At the same time, they noticed that the PD-1 ligand 1 (PD-L1), B7-H1, was also highly expressed in splenocytes from persistently infected mice, especially on virally infected cells (37). In addition, blockade of B7-H1/PD-1 signal pathway by using neutralizing antibodies against B7-H1 or PD-1 resulted in a substantial reduction in virus levels and clearance of virus from infected organs in the treated mice.

In this LCMV infection model, the regulatory role of B7-H1 did not appear to dominate the priming stage of anti-viral $CD8^+$ T cells, because B7-H1 blockade did not increase virus-specific $CD8^+$ T-cell responses in the acute infection. In contrast, B7-H1 blockade restored all exhausted virus-specific $CD8^+$ T cells into a rapid proliferation stage, where these same $CD8^+$ T cells still expressed high levels of PD-1. These findings suggest the role of B7-H1/PD-1 signals in the chronization of LCMV infection by inhibiting proliferation of the viral-antigen experienced $CD8^+$ T cells.

Similar mechanisms may be extended to other chronic viral infection in human, in particular, infection by human immunodeficiency virus (HIV). HIV infection is associated with an increased IL-10 production (40), functional impairment and anergy of antigen-specific responses, as well as increased susceptibility of T cells to apoptosis (41). These clinical phenomena are remarkably similar to the effects induced by B7-H1 as reported by us (27). Trabattoni et al. reported that B7-H1 is upregulated in peripheral monocytes in AIDS patients and that the degree of upregulation is negatively associated with $CD4^+$ T cell counts and positively associated with HIV plasmavirusemia (42). Furthermore, parallel decreases in IL-10 production and in B7-H1 synthesis/expression are seen during antiretroviral treatment of HIV-infected patients. Engagement of B7-H1 expression in HIV-infected antigen presenting cells (like monocytes or macrophages) and subsequent IL-10 production would result in augmented susceptibility of antigen-specific T cells to apoptosis. Therefore, the HIV-infected cells will be preserved and continue their proliferation by upregulating B7-H1 expression that prevents them from the attack from antiviral T cells. These interesting observations indicate that B7-H1 expression may be a potential indicator for the chronic process of HIV infection.

The repeated infection of respiratory syncytial virus (RSV) could happen throughout life. Although the mechanisms for the deficient protective memory immune response to RSV infection are still obscure, it is noticed that RSV infection suppresses effector and memory $CD8^+$ T cell responses (43). Intriguingly, Stanciu et al. observed an increased expression of B7-H1 on the surface of the alevolar epithelial cells during RSV-infection (44). Treatment with

IFN-γ further increased B7-H1 expression. Because of the T cell inhibitory function of PD-1 signals, the upregulated PD-1 ligands on the surface of alevolar epithelial cells may inhibit T cell-mediated anti-viral immune responses to RSV (44). Therefore, unresolved RSV infection in the alveolar epithelial cells will persistent for a long time and increase the chance for repeated infection.

Thus, some viruses have developed a virulence capacity to evade host immune attack by upregulation of immune inhibitory molecules such as B7-H1 in the infected cells including spleen cells, monocytes and epithelial cells. Given the fact that these infected cells may contribute to the priming of T cells, the upregulation of B7-H1 may subsequently suppress the function of anti-viral T cells and consequently, causes the chronicity of the infection.

B7-H1 and chronic bacterial or parasite infections

Helicobacter pylori (*H. pylori*) infection of gastric epithelial cells is associated with chronic gastritis, peptic ulcers and appears to be a provocative factor in 60-90% of gastric carcinomas. However, most individuals whose stomachs host this bacterium develop no clinical symptoms. The mechanisms of the insufficiency of immune responses preventing the gastric mucous from injury by this pathogen are not clear. Gastric epithelial cells play an important role in gastric mucosal immunity against *H. pylori* infection. Expression of class II MHC and co-stimulatory molecules such as CD80 and CD86 in gastric epithelial cells suggests their role in local antigen presentation. Although T cells are recruited to the infected gastric mucosa, they have been reported to be hypo-responsive (45). Recent studies by Das et al. demonstrated a high level of B7-H1 expression on gastric epithelial cells during chronic *H. pylori* infection (46). The functional role of gastric epithelial cell-associated B7-H1 expression was assessed by co-culture of *H. pylori*-infected gastric epithelial cells with $CD4^+$ T cells. Suppression of proliferation and IL-2 synthesis in $CD4^+$ T cells was detected when they were co-cultured with infected gastric epithelial cells, a phenomenon inhibited by the blockage of the B7-H1 signals using a specific blocking antibody to B7-H1. These studies thus suggest a role for B7-H1 in gastric epithelial cells as a contributor in the chronicity of *H. pylori* infection.

A similar B7-H1-mediated insufficient immune response was also observed in the infection of macrophages by the parasitic helminthes. Helminthes are known to induce immune anergy and anti-inflammatory responses (47). Previous studies suggest that macrophages can be altered when hosts are chronically exposed to helminthes or their products. Recent investigations by Terrazas et al. demonstrated an increased expression of B7-H1 on macrophages obtained from *T. crassiceps*-infected mice (48). Blockade of B7-H1 or PD-1 significantly reduced the suppressive activity of infected macrophages on T cells proliferation. These results indicate that B7-H1 and PD-1 are directly involved in the cell-contact suppressive activity of macrophages in the host with chronic infection of parasitic helminthes.

In line with those results, a similar participation of B7-H1/PD-1 interactions was observed in experimental models of murine schistosomiasis. Utilizing both *in vivo* and *in vitro* infectious models, Smith et al. demonstrated that schistosome worms induced anergy of $CD4^+$ and $CD8^+$ T cells *via* a selective upregulation of B7-H1 on the surface of macrophages (49).

Taken together, the expression by antigen-presenting cells (like macrophages, and epithelial cells) of B7-H1, a co-inhibitory molecule, may diminish or dampen continuous T cell activation and limit potential T cell immune responses. Bacteria and worms seem to be able to manipulate this host immune regulatory mechanism to evade clearance by the host immune response. Another possibility is that this mechanism could also down-regulate immune surveillance mechanisms needed to clear infected cells that arise within the infection sites. Although the virulence factor responsible for the induction of B7-H1 expression is not yet fully characterize, B7-H1/PD-1 pathway may represent an important target in vaccine development against related, prevalent and significant human pathogens. Therefore, manipulation of B7-H1 expression in the infected host may not only improve the clearance of pathogens but also ameliorate the immunopathology caused by the chronic infection.

B7-H1 and cancer-related chronic inflammation

As we discussed early, upregulation of B7-H1 in host cells may contribute to the chronicity of inflammatory disorders caused by infectious microorganisms. These unresolved chronic inflammations also frequently precede the development of many human cancers including those most common and lethal ones such as lung, esophageal, gastric, pancreatic, cervical, bladder, prostate and colorectal cancers (50). A role for the chronic inflammatory microenvironment in the carcinogenesis is strongly supported by the finding that genetic deletion or antibody-mediated elimination of IL-23, a pro-inflammatory cytokine, renders mice resistant to chemically induced carcinogenesis and transplanted tumors (51). It is not surprising to see that an upregulated expression of B7-H1 protein has been reported in tumor cells, found in such cancers as lung, ovarian, colon and skin (melanoma) (5), glinoma cells (52), squamous cell carcinoma (53, 54), renal cell carcinoma (55), esophageal cancer (56), gastric carcinoma (57), and breast cancer (58). Histologically, all these cancers are in the presence of chronic inflammation. In addition, a significant positive correlation of the high level of B7-H1 protein with a poor prognosis of the diseases has been established in patients with renal cell carcinoma (55), esophageal cancer (56), and gastric carcinoma (57).

The molecular mechanisms by which upregulation of B7-H1 in tumor cells causes chronicity of inflammatory responses during tumorgenesis resulting in a poor prognosis of the disease are still obscure. For those tumors which are induced by viral or bacterial infections and repeated chemical exposure, expression of B7-H1 in tumor cells could inhibit activated T cells and thus cause a continuous inflammatory

process. Consequently, infectious microbes persistently present and hyperplasia, dysplasia and malignant neoplasia develop in infected individuals.

For other tumors, upregulation of B7-H1 on the *de novo* generated tumor cells will help them to escape immunosurveillance and to get a hold in metastatic tissues (59). Infiltration of leukocytes, either peritumorally or intratumorally, is a common pathological feature in the tissue sections from many of the tumor patients. Infiltration of leukocytes at the tumor area should lead an anti-tumor immune response and thus inhibit tumor growth. The intratumoral presence of infiltrating T cells, natural killer (NK) cells or myeloid dendritic cells (mDCs) is associated with a better prognosis in ovarian cancer patients (60) and gastric cancer (61). Nevertheless, the presence of heavy infiltrating leukocytes in other tumors such as renal cell carcinoma (RCC) is indeed a predictor of poor prognosis (62). In a recent long-term follow-up study, upregulation of B7-H1 expression was found in renal cell carcinoma cells and high levels of B7-H1 expression were associated with a higher mortality in patients with this disease (55). Theoretically, expression of B7-H1 in tumor cells could inhibit functions of infiltrated T cells and thus evade the anti-tumor responses. This could also explain the controversy between tumor infiltrating lymphocytes and corresponding clinical prognosis in various tumors as mentioned above.

Besides high expression in tumor cells, increased expression of B7-H1 was also detected in tumor-infiltrated T cells and tumor-associated dendritic cells (DCs) (59, 63). Zou's group found high expression of B7-H1 on the surface of myeloid dendritic cells (mDCs) infiltrating human ovarian cancers (63). These immature mDCs induced IL-10 production in T cells, and blockade of B7-H1 greatly improved the anti-tumor function of these T cells. Besides antigen-presenting cells, tumor infiltrating T cells also express B7-H1 and this expression is linked with poor prognosis (64).

However, we should not overlook the fact that B7-H1 also co-stimulates the priming of naïve T cells *in vivo* and *in vitro* (5, 9, 10). It could be speculated that tumor-associated B7-H1 may elicit the generation of type 1 regulatory T cells *via* its ability to co-stimulate IL-10 production in T cells. To this extend, upregulated B7-H1 expression may not only block the process of clearance of tumor cells by activated T cells, but also enhance the immunopathology by co-stimulating naïve T cells that recently recruited to tumor site.

Taken together, in the microenvironment of progressing tumors, upregulated B7-H1 expression is triggered on both antigen-presenting cells and effector cells (T cells). This self-protective mechanism is efficiently usurped by malignant cells to induce anergy or dysfunction of an established anti-tumor immunity. This prolonged inflammation inside tumors causes more tissue damages and may also open the way for the metastasis of primary tumors.

B7-H1 and chronic autoimmune diseases

The dual-functional role of B7-H1 also appears to be involved in the complicated and prolonged immune responses in some autoimmune diseases. If the main role of B7-H1 is to inhibit the ongoing immune responses, upregulation of B7-H1 should be inversely related to reduced inflammation or less immunopathology. However, some animal models do not support this expectation. Increased B7-H1 expression is frequently observed in ongoing active immune responses with more T cell infiltration in mouse diabetes and experimental autoimmune encephalomyelitis (EAE).

In the stage of active autoimmune response in diabetes, B7-H1 (PD-L1) was upregulated on islets upon insulitis in unmanipulated non-obesity diabetes (NOD) mice (65). Further studies identified that B7-H1 expression is high on β cells as well as dendritic cells (DCs), and moderate on α cells, whereas T cells and B cells do not express B7-H1. As the disease progressed, B7-H1 expression increased in islet cells. Interestingly, the expression of B7-H1 on β cells was stronger at the boundary with T cells than inside of islets in NOD mice, suggesting that β cells may suppress the activity of T cells at the boundary to prevent the invasion of T cells into islets. Using B7-H1 knockout mice, Sharp's group found that B7-H1 expression on these parenchymal cells plays an important role in inhibiting pathogenic self-reactive T cells-mediated tissue destruction and effector cytokine production (66). In addition, introducing PD-1 deficiency into NOD mice accelerated the onset and frequency of type 1 diabetes in NOD mice, in which a strong infiltration of T helper 1 T cells was observed in the islets (12).

During the course of overt disease in EAE model, an increased expression of B7-H1 is observed on resident astrocytes and microglial cells in the brains of animals with EAE (67, 68). B7-H1 knockout mice are more susceptible to EAE induction and develop severe EAE after adoptive transfer of pathogenic T cells (69). However, even early treatment with anti-B7-H1 antibody did not have effects on the progress of EAE (69). Nevertheless, it becomes clear that upregulation of B7-H1 after the onset of disease may have a protective role in the chronic process of autoimmune responses in the later stage of EAE. The stimulatory role of B7-H1 should be considered in the explanation of unfinished immune responses of EAE, if the negative regulatory arm of B7-H1 is not a strong indicator of good prognosis in this disease. The relation of the dynamic expression of B7-H1 on the brain cells and the relapse of EAE should be addressed by further studies.

The co-stimulatory role of B7-H1 in chronic inflammatory or autoimmune disease has also been reflected by several other studies. In a mouse chronic colitis model, B7-H1 expression markedly increased in the inflamed mucosa from colitic animals receiving naïve $CD4^+$ T cell transfers (9). Blockage of B7-H1 using an anti-B7-H1 antibody, shortly after the transfer of pathogenic T cells, prevented the development of colitis. Because the injected antibody did not deplete the B7-H1 positive T cells, those results suggest a co-stimulatory role of B7-H1 *in vivo* in priming the proliferation of naïve T cells. The co-stimulatory role of B7-H1 is also observed in transgenic mice that express

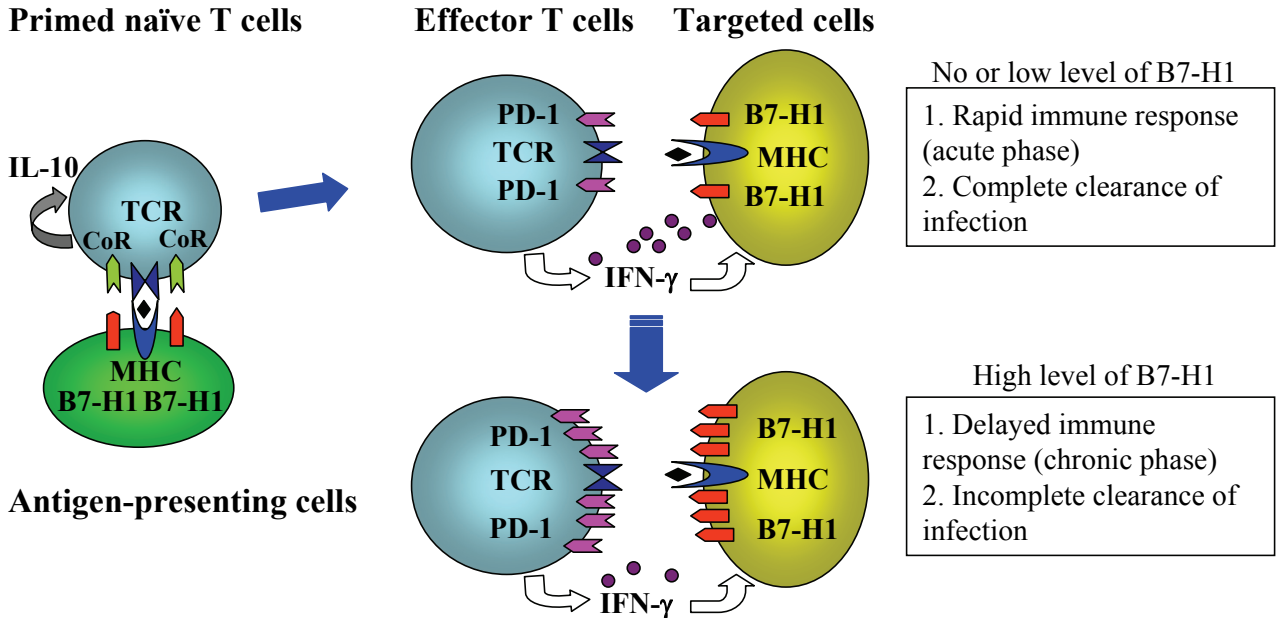

Figure 2. Immunoregulatory role of B7-H1 in the chronic T cell responses. During the process of infection, antigen-presenting cells (like dendritic cells) in the draining lymph nodes may use B7-H1 to co-stimulate the differentiation of effector cells. After migration into the infected tissues, activated effector T cells release more IFN-γ after encountering with target cells. At this early stage, the target cells may express no or less B7-H1, and a rapid immune response is warranted for a complete clearance of infection. As the infection continues, the accumulated IFN-γ induces high level of B7-H1 in the target cells and higher PD-1 expression on effector T cells. Thus, the inhibitory signal of B7-H1/PD-1 pathway may eventually delay the T cell response and results in a chronic immune response with an incomplete clearance of infection.

B7-H1 on pancreatic islet β cells. The transplantation of B7-H1-expressing islets resulted in accelerated allograft rejection that could be blocked by the anti-B7-H1 antibody (10). Furthermore, a significant fraction of B7-H1 transgenic mice developed T cell-dependent spontaneous autoimmune diabetes. In addition, B7-H1 expression in pancreatic islets promoted $CD8^+$ T cell priming and promoted autoimmunity induction. These studies imply that tissue-specific expression of B7-H1 co-stimulates T cell-mediated responses *in vivo* and may directly contribute to an autoimmune disease pathogenesis.

Summary

A possible link between infection and chronic illness provides a new model for the pathogenesis of chronic inflammatory diseases that contrasts with the traditional one. The traditional model envisions a battle between organism and host that produces acute, usually self-limited illnesses, and the main disease manifestations are due to the presence of the microorganisms or its toxic products in a specific organ system (1). Recent advances in our understanding of host immune responses during microbial infection suggest that in most cases, it may be the aberrant host responses rather than the pathogen-specific toxins or oncogenes induced by the pathogen's products that cause the disease. Emerging evidence implicates that the co-stimulatory and co-inhibitory role of B7-H1 at different stages of T cell activation and differentiation may contribute to the chronicity of inflammatory diseases, including autoimmune diseases, tumors, viral and bacterial infectious diseases. Upregualtion of B7-H1 may work in two ways to complicate the unsolved immune responses: naïve T cells will be primed or trapped by the co-stimulatory signals of B7-H1, and activated effector T cells will be diminished by the co-inhibitory signal of B7-H1 in the site of inflammation (Figure 2). Importantly, development of strategies targeting co-signal molecules, such as B7-H1, provides a new and promising approach to manipulate the devastating diseases associated with chronic inflammation. Future studies will be designed to manipulate the signal pathway of B7-H1 to improve the rapid resolving of immune responses.

Acknowledgements

This work was supported by Mayo Foundation FUTR Award (H.D.) and NIH AI071321 (X.C.).

References

1. Karin M, Lawrence T, Nizet V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell. 2006;124:823-835.
2. Lorber B. Are all diseases infectious? Ann Intern Med. 1996;

125:844-851.
3. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med. 1999;5:1365-1369.
4. Freeman GJ, Long A, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192:1027-1034.
5. Dong H, Strome S, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8:793-800.
6. Iwai Y, Okazaki T, Nishimura H, Kawasaki A, Yagita H, Honjo T. Microanatomical localization of PD-1 in human tonsils. Immunol Lett. 2002;83:215-220.
7. Yamazaki T, Akiba H, Iwai H, et al. Expression of programmed death 1 ligands by murine T cells and APC. J Immunol. 2002; 169:5538-5545.
8. Ishida Y, Aagata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11: 3887-3895.
9. Kanai T, Totsuka T, Uraushihara K, et al. Blockade of B7-H1 suppresses the development of chronic intestinal inflammation. J Immunol. 2003;171:4156-4163.
10. Subudhi SK, Zou P, Yerian LM, et al. Local expression of B7-H1 promotes organ-specific autoimmunity and transplant rejection. J Clin Invest. 2004;113:694-700.
11. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immuno-receptor. Immunity. 1999;11:141-151.
12. Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, Honjo T. Establishment of NOD-Pdcd1$^{-/-}$ mice as an efficient animal model of type I diabetes. Proc Natl Acad Sci U S A. 2005;102: 11823-11828.
13. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science. 2001; 29:319-322.
14. Latchman Y, Wood C, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol. 2001;2:261-268.
15. Okazaki T, Maeda A, Nishimura H, Kurosaki T, Honjo T. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. Proc Natl Acad Sci U S A. 2001;98: 13866-13871.
16. Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol. 2005;25:9543-6553.
17. Saunders PA, Hendrycks VR, Lidinsky WA, Woods ML. PD-L2: PD-1 involvement in T cell proliferation, cytokine production, and integrin-mediated adhesion. Eur J Immunol. 2005;35:3561-3569.
18. Sheppard KA, Fitz LJ, Lee JM, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3ζ signalosome and downstream signaling to PKCτ. FEBS Lett. 2004;574: 37-41.
19. Carter L, Fouser L, Jussif J, et al. PD-1:PD-L inhibitory pathway affects both CD4$^+$ and CD8$^+$ T cells and is overcome by IL-2. Eur J Immunol. 2002;32:634-643.
20. Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol. 2004;173:945-954.
21. del Rio ML, Penuelas-Rivas G, Dominguez-Perles R, Ramirez P, Parrilla P, Rodriguez-Barbosa JI. Antibody-mediated signaling through PD-1 costimulates T cells and enhances CD28-dependent proliferation. Eur J Immunol. 2005;35:3545-3560.
22. Zhang X, Schwartz JC, Guo X, et al. Structural and functional analysis of the costimulatory receptor programmed death-1. Immunity. 2004;20:337-347.
23. Wang S, Bajorath J, Flies DB, Dong H, Honjo T, Chen L. Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. J Exp Med. 2003;197:1083-1091.
24. Saudemont A, Jouy N, Hetuin D, Quesnel B. NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7-H1 that stimulates T cells. Blood. 2005;105:2428-2435.
25. Sandner SE, Clarkson MR, Salama AD, et al. Role of the programmed death-1 pathway in regulation of alloimmune responses *in vivo*. J Immunol. 2005;174:3408-3415.
26. Dong H, Strome S, Matteson EL, et al. Costimulating aberrant T cell responses by B7-H1 autoantibodies in rheumatoid arthritis. J Clin Invest. 2003;111:363-370.
27. Dong H, Chen L. B7-H1 pathway and its role in the evasion of tumor immunity. J Mol Med. 2003;81:281-287.
28. Wick MJ, Leithauser F, Reimann J. The hepatic immune system. Crit Rev Immunol. 2002;22:47-103.
29. Crispe I. Hepatic T cells and liver tolerance. Nat Rev Immunol. 2003;3:51-62.
30. Crispe IN, Dao T, Klugewitz K, Mehal WZ, Metz DP. The liver as a site of T-cell apoptosis: graveyard, or killing field? Immunol Rev. 2000;174:47-62.
31. Selenko-Gebauer N, Mejdic O, Szekeres A, et al. B7-H1 (programmed death-1 ligand) on dendritic cells is involved in the induction and maintenance of T cell anergy. J Immunol. 2003;170:3637-3644.
32. Iwai Y, Terawaki S, Ikegawa M, Okazaki T, Honjo T. PD-1 inhibits antiviral immunity at the effector phase in the liver. J Exp Med. 2003;198:39-50.
33. Brown JA, Dorfman D, Ma FR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol. 2003;170:1257-1266.
34. Dong H, Zhu G, Tamada K, Flies DB, van Deursen JM, Chen L. B7-H1 determines accumulation and deletion of intrahepatic CD8$^+$ T lymphocytes. Immunity. 2004;20:327-336.
35. Yu MC, Chen C, Liang X, et al. Inhibition of T-cell responses by hepatic stellate cells *via* B7-H1-mediated T-cell apoptosis in mice. Hepatology. 2004;40:1312-1321.
36. Isogawa M, Furuichi Y, Chisari FV. Oscillating CD8$^+$ T cell effector functions after antigen recognition in the liver. Immunity. 2005;23:53-63.
37. Barber DL, Wherry E, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439:682-687.
38. Fuller MJ, Khanolkar A, Tebo AE, Zajac AJ. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. J Immunol. 2004;172:4204-4214.
39. Zajac AJ, Blattman JM, Murali-Krishna K, et al. Viral immune evasion due to persistence of activated T cells without effector function. J Exp Med. 1998;188:2205-2213.
40. Clerici M, Wynn TA, Berzofsky JA, et al. Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus. J Clin Invest. 1994;93:768-775.
41. Shearer GM. HIV-induced immunopathogenesis. Immunity.

1998;9:587-593.
42. Trabattoni D, Saresella M, Biasin M, et al. B7-H1 is up-regulated in HIV infection and is a novel surrogate marker of disease progression. Blood. 2003;101:2514-2520.
43. Chang J, Braciale TJ. Respiratory syncytial virus infection suppresses lung $CD8^+$ T-cell effector activity and peripheral $CD8^+$ T-cell memory in the respiratory tract. Nat Med. 2002;8: 54-60.
44. Stanciu LA, Bellettato CM, Laza-Stanca V, Coyle AJ, Papi A, Johnston SL. Expression of programmed death-1 ligand (PD-L) 1, PD-L2, B7-H3, and inducible costimulator ligand on human respiratory tract epithelial cells and regulation by respiratory syncytial virus and type 1 and 2 cytokines. J Infect Dis. 2006; 193:404-412.
45. Fan XJ, Chua A, Shahi CN, McDevitt J, Keeling PW, Kelleher D. Gastric T lymphocyte responses to Helicobacter pylori in patients with H pylori colonisation. Gut. 1994;35:1379-1384.
46. Das S, Suarez G, Beswick EJ, Sierra JC, Graham DY, Reyes VE. Expression of B7-H1 on gastric epithelial cells: its potential role in regulating T cells during Helicobacter pylori infection. J Immunol. 2006;176:3000-3009.
47. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol. 2003;3:733-744.
48. Terrazas LI, Montero D, Terrazas CA, Reyes JL, Rodriguez-Sosa M. Role of the programmed death-1 pathway in the suppressive activity of alternatively activated macrophages in experimental cysticercosis. Int J Parasitol. 2005;35:1349-1358.
49. Smith P, Walsh CM, Mangan NE, et al. Schistosoma mansoni worms induce anergy of T cells *via* selective up-regulation of programmed death ligand 1 on macrophages. J Immunol. 2004; 173:1240-1248.
50. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nat Rev Immunol. 2004;4:641-648.
51. Langowski JL, Zhang X, Wu L, et al. IL-23 promotes tumour incidence and growth. Nature. 2006; in press.
52. Wintterle S, Schreiner B, Mitsdoerffer M, et al. Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. Cancer Res. 2003;63:7462-7467.
53. Strome SE, Dong H, Tamura H, et al. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer Res. 2003;63:6501-6505.
54. Tsushima F, Tanaka K, Otsuki N, et al. Predominant expression of B7-H1 and its immunoregulatory roles in oral squamous cell carcinoma. Oral Oncol. 2006;42:268-274.
55. Thompson RH, Kuntz SM, Leibovich BC, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow up. Cancer Res. 2006;66:3381-3385.
56. Ohigashi Y, Shao M, Yamada Y, et al. Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res. 2005; 11:2947-2953.
57. Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. Acta Histochem. 2006;108:19-24.
58. Ghebeh H, Mohammed S, Al-Omair A, et al. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia. 2006;8: 190-198.
59. Thompson RH, Gillett MD, Cheville JC, et al. Costimulatory molecule B7-H1 in primary and metastatic clear cell renal cell carcinoma. Cancer. 2005;104:2084-2091.
60. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348:203-213.
61. Ishigami S, Natsugoe S, Tokuda K, et al. Clinical impact of intratumoral natural killer cell and dendritic cell infiltration in gastric cancer. Cancer Lett. 2000;159:103-108.
62. Webster WS, Lohse CM, Thompson RH, et al. Mononuclear cell infiltration in clear-cell renal cell carcinoma independently predicts patient survival. Cancer. 2006;107:46-53.
63. Curiel TJ, Wei S, Dong H, et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med. 2003;9:562-567.
64. Thompson RH, Gillett MD, Cheville JC, et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A. 2004;101:17174-17179.
65. Ansari MJ, Salama AD, Chitnis T, et al. The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. J Exp Med. 2003;198:63-69.
66. Keir ME, Liang SC, Guleria I, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. J Exp Med. 2006;203: 883-895.
67. Salama AD, Chitnis T, Imitola J, et al. Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. J Exp Med. 2003; 198:71-78.
68. Liang SC, Latchman YE, Buhlmann JE, et al. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. Eur J Immunol. 2003;33:2706-2716.
69. Latchman YE, Liang S, Wu Y, et al. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. Proc Natl Acad Sci U S A. 2004; 101:10691-10696.