Inhibitory Receptors on Lymphocytes: Insights from Infections

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Inhibitory Receptors on Lymphocytes: Insights from Infections

Pamela M. Odorizzi and E. John Wherry

Costimulatory and inhibitory receptors are critical regulators of adaptive immune cell function. These pathways regulate the initiation and termination of effective immune responses to infections while limiting autoimmunity and/or immunopathology. This review focuses on recent advances in our understanding of inhibitory receptor pathways and their roles in different diseases and/or infections, emphasizing potential clinical applications and important unanswered mechanistic questions. Although significant progress has been made in defining the influence of inhibitory receptors at the cellular level, relatively little is known about the underlying molecular pathways. We discuss our current understanding of the molecular mechanisms for key inhibitory receptor pathways, highlight major gaps in knowledge, and explore current and future clinical applications. The Journal of Immunology, 2012, 188: 2957–2965.

A major function, and perhaps a driver for evolutionary development of inhibitory receptors in the immune system, is regulating autoreactivity. Not surprisingly, therefore, inhibitory receptor pathways in T and B cells, including CTLA-4, PD-1, Lag-3, and others, have been implicated in autoimmunity in mice. Importantly, polymorphisms in inhibitory receptor genes are associated with susceptibility to several human autoimmune diseases, including diabetes, multiple sclerosis, and rheumatoid arthritis (1). This regulatory system has also been co-opted, and perhaps diversified, to help temper overzealous immune responses. Many studies have shown that inhibitory receptors are critical negative regulators of the immune response to allografts (2), tumors (3), infections (4), and perhaps even allergens (5).

In some settings, efficient negative regulation by inhibitory receptors may help to restrain detrimental immune responses (6, 7). However, inhibitory receptors can also hinder the effective immune responses needed to clear pathogens and tumors (4). Several studies demonstrated the benefit of both positive and negative manipulation of inhibitory receptor pathways (1–4). In fact, mAbs targeting inhibitory receptor pathways are currently in clinical trials and several have been approved by the U.S. Food and Drug Administration (FDA) in settings of autoimmunity and cancer (1, 2). With the growing clinical significance of these approaches, better mechanistic insight into these pathways may provide safer and more robust therapeutic opportunities.

Acute infections

Inhibitory receptors and their ligands play crucial roles in shaping the immune response to pathogenic microbes. The opposing functions of inhibitory and activating pathways provide the immune system with a mechanism to fine-tune innate and adaptive immune responses, ensuring pathogen control without excessive immune-mediated damage. The cascade of events involved in T and B cell responses during acute infection provides multiple points where inhibitory receptors could have an impact, such as opposing positive costimulation during priming, curbing effector functions to limit immunopathology, or slowing the response at later stages of infection. In addition, there are clearly ways that inhibitory receptors could influence T and B cell responses during acute infections that are cell extrinsic, such as a role for many inhibitory receptor pathways on NK cells, dendritic cells (DCs), macrophages, and regulatory T cells (8). Although we still understand relatively little about how and where inhibitory receptors act during acute infections, there are clear examples of the importance of these pathways.

Modulating the PD-1 pathway during acute infection can, in some cases, increase the effectiveness of antipathogen immune responses. For example, knocking out or blocking the PD-1 pathway in mice increases immune responses and survival following infection with Histoplasma capsulatum, rabies virus, or respiratory syncytial virus (9–11). In addition, Lag-3 was shown to negatively regulate the development of CD8+ T cell memory following Sendai virus infection (12). These studies suggest that inhibitory receptors may hinder effective immune responses during some acute infections and that blockade of negative regulatory pathways might improve immunity to pathogens. In contrast, disrupting inhibitory receptor pathways can also be harmful. For example, although PD-1-deficient mice can more efficiently clear adenovirus from the liver than wild-type mice, they also develop more severe hepatocellular injury, likely due to an overaggressive

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Abbreviations used in this article: DC, dendritic cell; IgSF, Ig superfamily; ITSM, immunoreceptor tyrosine-based switch motif; LCMV, lymphocytic choriomeningitis virus.
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defects in proliferation, IL-2 production, and cytotoxicity, T cells during chronic infection is hierarchical, with early are initially activated and gain effector functions but pro-

Inhibitory receptors clearly play a role during some acute infections, but exactly how different inhibitory receptors regu-
late these responses remains poorly understood. Although PD-1 (18), CTLA-4 (19), Lag-3 (12), CD200:CD200R (20), and some Ly49 family members (21) have been examined on T cells during acute infection, the functions of many other inhibitory receptors have yet to be investigated. Several other important unanswered questions remain. Whether additional inhibitory receptors are acting individually or synergistically to regulate adaptive immune cells during acute infections is not known. There is also evidence for distinct functions of inhibitory receptors on different cell types, emphasizing the importance of studying these negative regulators in multiple adaptive and innate immune cells. Given the role of inhibi-

Topical transcriptional profiling of exhausted CD8+ T cells led to the discovery of other inhibitory receptors that are also upregulated on T cells during chronic infection, including Lag-3, 2B4, CD160, CTLA-4, PIR-B, and GP49b (36). Many of these inhibitory receptors are coexpressed on exhausted CD8+ T cells, and the pattern of this coexpression has im-
portant functional implications (36). In general, the severity of exhaustion correlates with the number of different receptors expressed, as well as the level of expression of each individual receptor. Patterns of coexpression or cooperative negative reg-
ulation by these receptors on CD8+ and CD4+ T cells exist for PD-1, Lag-3, CTLA-4, Tim-3, and others during infections in mice and humans (36–39). These studies identified func-
tionally distinct subpopulations of exhausted CD8+ T cells that express unique combinations of inhibitory receptors and that respond differently to inhibitory receptor blockade.

High expression of inhibitory receptors was also docu-
mented on B cells during HIV and malaria infections in humans (40, 41). In the context of HIV infection, CD20Hi

Adaptive immune response (13). Other studies showed that the absence or blockade of PD-L1 reduces early CD8+ T cell responses to influenza virus or Listeria monocytogenes in mice. In these examples, inhibitory receptors on innate immune cells, such as DCs and macrophages, play important roles in T cell activation and survival (14–17). Thus, a beneficial as-

Chronic infections

Inhibitory receptors play a major role during persisting infec-
tions. During many chronic infections, Ag-specific T cells are initially activated and gain effector functions but pro-
gressively lose functionality over time. Exhaustion of CD8+ T cells during chronic infection is hierarchical, with early defects in proliferation, IL-2 production, and cytotoxicity, followed by the loss of TNF, IFN-γ, and β-chemokine production at late stages (4). Additional alterations also occur in the development of memory properties, such as Ag-independent maintenance and responsiveness to IL-7 and IL-15 (4). T cell exhaustion occurs in many animal models of infection, as well as during human chronic infections (24). Exhausted CD8+ T cells express high levels of inhibitory receptors, whereas their ligands are upregulated on APCs (24, 25). Consequently, exhausted CD8+ T cells are more likely to receive inhibitory signals, resulting in decreased effector function.

PD-1 was one of the first inhibitory receptors implicated in T cell exhaustion (26). Unlike functional effector T cells

The importance of PD-1 in exhaustion was highlighted by the ability to partially reverse CD8+ T cell dysfunction and lower viral load with in vivo blockade of the PD-1/PD-L1 pathway during chronic lymphocytic choriomeningitis virus (LCMV) infection (26). Soon thereafter, several groups showed upreg-
ulation of PD-1 expression on exhausted CD8+ T cells during human viral infections, such as HIV, hepatitis C virus, and hepatitis B virus, and demonstrated improved function of T cells following in vitro PD-1/PD-L1 blockade or in vivo blockade of the PD-1 pathway in SIV-infected primates (27–30). Increased expression of PD-1 and its ligands also impairs the effector responses against persisting pathogens, such as Helicobacter pylori, Mycobacterium tuberculosis, Schistosoma mansoni, Leishmania donovani, and Toxoplasma gondii (31–35). Thus, the PD-1/PD-L pathway is a central negative regulator of immune responses during persisting infections.

Global transcriptional profiling of exhausted CD8+ T cells revealed the discovery of other inhibitory receptors that are also upregulated on T cells during chronic infection, including Lag-3, 2B4, CD160, CTLA-4, PIR-B, and GP49b (36). Many of these inhibitory receptors are coexpressed on exhausted CD8+ T cells, and the pattern of this coexpression has im-
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CD27-CD21lo B cells have a shortened replication history, altered expression of homing receptors, and decreased Ig di-
versity. These B cells also express the inhibitory receptors FCRL4, CD22, CD72, and Lair-1, consistent with some functional deficiencies (40). Dysfunction in B cells is poorly understood, but inhibitory receptors could influence the ex-
haustion of HIV- or malaria-specific B cells and subsequent inefficient Ab responses.

The expression and coexpression of many inhibitory re-
ceptors has now been demonstrated on exhausted T and B cells in many chronic infections, revealing complexity and diversity in inhibitory receptor expression (Table I). Although access to distinct ligands is a clear reason for differences in inhibitory receptor expression on various lymphocyte populations, it remains unclear how these patterns of expression are regu-
lated. It is also unclear whether this diversity in inhibitory receptor expression is necessary to regulate unique functions of T and B cells or is required for other reasons.

One factor that is associated with the diversity of inhibitory receptor expression is the type and severity of infection. In-

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Table I. The role of inhibitory receptors on lymphocytes in infection

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<td>Improves (4, 27, 40)</td>
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<td>SIV</td>
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<td>CD8+ T cells, CD4+ T cells</td>
<td>PD-1</td>
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<td></td>
<td>Hepatitis C virus</td>
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<td>CD8+ T cells, CD4+ T cells</td>
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<td>Improves (29, 38)</td>
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<td>Hepatitis B virus</td>
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<td>PD-1, CTLA-4</td>
<td>Improves (28)</td>
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<td>PD-1, PD-L1, PD-L1+Lag-3, Tim-3</td>
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<td>PD-1</td>
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<td>H. pylori</td>
<td>PD-L1</td>
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<td>PD-L1, CTLA-4</td>
<td>Improves (32)</td>
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<td>L. donosani/mexicana</td>
<td>PD-L1</td>
<td>CD8+ T cells</td>
<td>PD-L1</td>
<td>Improves (34)</td>
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<td>S. mansoni</td>
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<td>PD-L1</td>
<td>Improves (35)</td>
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<td>Trypanosoma cruzi</td>
<td>CTLA-4</td>
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<td>CTLA-4</td>
<td>Improves (25)</td>
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<td></td>
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<td>PD-1</td>
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<td>Improves (33)</td>
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<tr>
<td></td>
<td>Plasmodium falciparum/chabaudi/yolii</td>
<td>PD-1, Lag-3, FCyRIIb</td>
<td>CD8+ T cells, CD4+ T cells, B cells</td>
<td>PD-L1+Lag-3</td>
<td>Improves (39, 41)</td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>Rabies virus</td>
<td>PD-L1</td>
<td>CD8+ T cells</td>
<td></td>
<td>Improves (10)</td>
<td></td>
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<td></td>
<td>Respiratory syncytial virus</td>
<td>PD-1</td>
<td>CD8+ T cells</td>
<td></td>
<td>Improves (9)</td>
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<td></td>
<td>Influenza virus</td>
<td>PD-L1</td>
<td>CD8+ T cells</td>
<td></td>
<td>Reduces (14)</td>
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<td>Sendai virus</td>
<td>Lag-3</td>
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<td>Lag-3</td>
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<td></td>
<td>Vaccinia virus</td>
<td>PD-L1</td>
<td>CD8+ T cells</td>
<td></td>
<td>Improves (18)</td>
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<td></td>
<td>Adenovirus</td>
<td>PD-1</td>
<td>CD8+ T cells</td>
<td>PD-1</td>
<td>Improves (13)</td>
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<td></td>
<td>HSV</td>
<td>PD-1, Tim-3</td>
<td>CD4+ T cells, CD8+ T cells</td>
<td>Tim-3</td>
<td>Improves (80)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H. capsulatum</td>
<td>PD-1</td>
<td>CD4+ T cells, CD8+ T cells</td>
<td>PD-1</td>
<td>Improves (11)</td>
<td></td>
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<tr>
<td></td>
<td>L. monocytogenes</td>
<td>PD-L1</td>
<td>CD4+ T cells, CD8+ T cells</td>
<td></td>
<td>Improves/reduces (15–17)</td>
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</table>
deed, a positive correlation between severity of infection and inhibitory receptor expression has been demonstrated for chronic LCMV, HIV, and hepatitis C virus infections (27, 29, 36). Because AgR signaling is a key factor for expression of many inhibitory receptors, pathogen burden likely has a major influence in these settings. Other factors, including inflammation (42), γ-chain cytokines (43), CD4+ T cell help (44), and availability of ligands for inhibitory or costimulatory pathways, might also influence inhibitory receptor expression patterns. For example, lack of CD4+ T cell help during priming leads to higher expression of PD-1 on virus-specific CD8+ T cells upon re-exposure to Ag (45), suggesting that epigenetic changes in the Pdcd1 regulatory regions might retain information about environmental encounters for a T cell during infection. Indeed, methylation of the Pdcd1 gene appears to be a major mechanism of regulating PD-1 expression, and this methylation status can be influenced by the type of infection (46). Consequently, it is likely that the design of therapies to target inhibitory receptor pathways will have to be tailored to the type and severity of the infection. Therefore, a major goal in the field is to better understand how these, as well as other factors, influence exhaustion and the regulation of inhibitory receptors.

Inhibitory receptors play critical immunoregulatory roles in many settings of disease. Similar to chronic infection, CD8+ T cell responses to tumors also become dysfunctional and express high levels of inhibitory receptors, including CTLA-4, PD-1, and Lag-3 (3). In humans, high expression of PD-L1 by tumor cells correlates with poor prognosis in pancreatic cancer, renal cancer, ovarian cancer, and several others (3). In mouse tumor models, knocking out or blocking PD-1 expression has been demonstrated for chronic LCMV, HIV, and hepatitis C virus infections (27, 29, 36). Because AgR signaling is a key factor for expression of many inhibitory receptors, pathogen burden likely has a major influence in these settings. Other factors, including inflammation (42), γ-chain cytokines (43), CD4+ T cell help (44), and availability of ligands for inhibitory or costimulatory pathways, might also influence inhibitory receptor expression patterns. For example, lack of CD4+ T cell help during priming leads to higher expression of PD-1 on virus-specific CD8+ T cells upon re-exposure to Ag (45), suggesting that epigenetic changes in the Pdcd1 regulatory regions might retain information about environmental encounters for a T cell during infection. Indeed, methylation of the Pdcd1 gene appears to be a major mechanism of regulating PD-1 expression, and this methylation status can be influenced by the type of infection (46). Consequently, it is likely that the design of therapies to target inhibitory receptor pathways will have to be tailored to the type and severity of the infection. Therefore, a major goal in the field is to better understand how these, as well as other factors, influence exhaustion and the regulation of inhibitory receptors.

Inhibitory receptor mechanisms of action

The first opportunity for inhibitory receptors to negatively regulate immune cell function is at the cell surface, where competition for costimulatory ligands can prevent proper activation signals. For example, one proposed mechanism for how CTLA-4 exerts inhibitory effects is by competing with CD28 for their shared ligands, B7.1 (CD80) and B7.2 (CD86), thus preventing proper costimulatory signaling. CTLA-4 binds with 10-fold greater affinity to the B7 ligands, and this inhibitory receptor was shown to form lattice-like networks that physically prevent CD28 from interacting with CD80 and CD86 (48, 49). Similarly, PD-L1 also binds CD80 in addition to PD-1 and sequesters CD80 away from CD28 (49). By preventing initial activation of adaptive immune cells, inhibitory receptors that use this mechanism can have a profound effect on the generation of immune responses.

The most well-described inhibitory receptor mechanism of action is the local and transient intracellular attenuation of positive signals from activating receptors, including AgRs and costimulatory receptors. Many inhibitory receptors attenuate TCR or BCR signaling events by targeting these activating receptor complexes directly or their downstream signaling molecules (50). As a consequence, inhibitory receptors cause a broad, quantitative reduction in activation-induced signal transduction and downstream gene expression. In addition, inhibitory receptors also interfere with costimulatory signaling pathways, resulting in qualitative effects on cell survival, proliferation, and metabolism.

To mediate this negative regulation, many inhibitory receptors exploit sequence motifs in their cytoplasmic tails to recruit effector molecules (50). Perhaps the most widely used of these is ITIM. Inhibitory receptor ligation results in ITIM tyrosine phosphorylation and recruitment of cytosolic phosphatases containing Src homology-2 domains, including SHP-1, SHP-2, and SHIP-1 (51, 52). SHIP and SHP molecules have been shown in vitro to dephosphorylate a variety of molecules involved in TCR, BCR, and costimulatory-signaling cascades (53, 54). However, because these phosphatases can theoretically act on many substrates, their direct in vivo targets remain largely elusive. In addition to ITIMs, many inhibitory receptor cytoplasmic domains contain immunoreceptor tyrosine-based switch motifs (ITSMs). These six amino acid motifs are able to recruit both inhibitory and activating effector molecules in different contexts (55). Although the widespread mechanism of ITIM- and/or ITSM-mediated inhibition does not prevent the activation of adaptive immune cells, disrupting early signaling events allows inhibitory receptors to fine-tune the activation status of the cell.

Recent work by Quigley et al. demonstrated that inhibitory receptors are also capable of upregulating genes involved in T cell dysfunction, suggesting a novel mechanism for inhibition (56). Using integrated genomic approaches, PD-1 signaling was shown to upregulate the expression of basic leucine zipper transcription factor, activating transcription factor-like (BATF), in exhausted T cells from humans and mice. Overexpression of BATF in primary human T cells reduced proliferation and IL-2 secretion upon stimulation. Conversely, effector functions could be rescued in exhausted T cells by short hairpin RNA-mediated silencing of BATF, further supporting an inhibitory function of BATF in T cells (56). These studies suggest that inhibitory receptors not only blunt positive signaling but are also capable of inducing transcriptional pathways that actively regulate immune cell function. While BATF acts as a negative regulator of normal AP-1 activity, it may also have unique transcriptional activity (57), suggesting that this inhibitory receptor mechanism has the potential to influence cellular differentiation. It will be interesting to determine whether other inhibitory receptors use this mechanism of qualitatively altering gene expression.

Overall, these data support the existence of three major mechanisms by which inhibitory receptors negatively regulate adaptive immune cell function (Fig. 1). First, inhibitory receptors can sequester ligands for costimulatory molecules, preventing the cell from receiving the proper activation signals (Fig. 1, mechanism #1). Second, inhibitory receptors can use intracellular motifs to disrupt the signaling cascades of activating receptors, such as the TCR, BCR, or costimulatory...
however, the pathways leading to this gene upregulation are not known. Recently demonstrated to upregulate genes that inhibit immune cell function; induction-induced gene expression. Mechanism #3: Inhibitory receptors were re-
costimulatory molecules, causing a broad, quantitative reduction in activa-
phorylate signaling molecules downstream of the TCR (or BCR) and 
cytoplasmic tail of inhibitory receptors are phosphorylated upon cellular ac-
Mechanism #2: Inhibitory sequence motifs, such as ITIMs or ITSMs, on the 
activation signals by sequestering the ligands for costimulatory receptors. 
#1: Inhibitory receptors prevent T cells (or B cells) from receiving complete 
inhibitory and activating roles have been demonstrated for 
inhibitory motifs, specifically ITIMs and ITSMs. Many inhibitory 
receptors. This inhibitory mechanism causes a global reduc-
tion in activation-induced gene expression (Fig. 1, mechanism #2). Finally, recent work indicated that inhibitory receptors 
can upregulate genes that inhibit immune cell function (Fig. 1, mechanism #3). Whether this occurs by a direct or indirect 
molecular pathway remains unknown. Inhibitory receptors could use any combination of these mechanisms to inhibit 
T cell function, and it is possible that other unknown mechanisms still exist. As we begin to understand inhibitory 
receptor pathways in more detail, new opportunities to therapeutically target these pathways are likely to emerge.

Ig superfamily

Of the three mechanisms described above, by far the most 
well-studied is the use of ITIMs and ITSMs. Many inhibitory receptors possess ITIMs or ITSMs and exert their inhibitory 
effects by recruiting SHP or SHIP molecules (Table II), in-
cluding a large number in the Ig superfamily (IgSF). For example, two well-described inhibitory receptors, PD-1 and 
BTLA, attenuate TCR and/or costimulatory signaling via an 
ITIM followed by an ITSM in their cytoplasmic domains. 
SHP-1 and SHP-2 were shown to bind PD-1 and BTLA; however, in the case of PD-1, SHP-1 may be recruited to the 
ITSM (58).

In some cases, the presence of an ITSM does not necessarily 
indicate an inhibitory function. The IgSF family member 2B4 harbors four ITSMs in its cytoplasmic tail; however, both 
inhibitory and activating roles have been demonstrated for 
2B4 in NK cells and T cells (23, 59). Three major factors 
were shown to contribute to 2B4 dual functionality, including 
the level of surface expression, degree of cross-linking, and 
abundance of different effector molecules (23). Thus, many 
IgSF family members use intracellular ITIMs or ITSMs as a 
widespread mechanism to attenuate adaptive immune cell 
activation. However, diversity in the number of inhibitory 
motifs expressed, as well as the intracellular effector proteins 
recruited, may indicate important differences in functions 
within this family of receptors.

C-type lectin family

The C-type lectin family of inhibitory receptors, like the 
IgSF, can mediate inhibition of cellular function via ITIM-
mediated phosphatase recruitment. Two ITIM-containing 
members of the C-type lectin family, CD94/NKG2 and 
KLRG1, inhibit CD8+ T cell cytotoxicity and cytokine pro-
duction (60, 61). Recent work in primary CD8+ T cells dem-
onstrated that a mechanism for KLRG1 inhibition is reduced 
Akt phosphorylation by SHIP-1 recruitment (61).

The mouse Ly49 family of inhibitory receptors has been well 
characterized in NK cells as ITIM-containing molecules that 
recruit SHP-1 to inhibit cytotoxicity. Although Ly49 family 
members are expressed by T cells, their role in this setting 
remains less well understood (62). The functional equivalents 
of Ly49 receptors in humans are inhibitory killer cell Ig-like 
receptors and leukocyte Ig-like receptors. These inhibitory re-
ceptors are present primarily on NK cells and CD8+ T cells 
and were shown to negatively regulate cytokine production 
and cytotoxicity through SHP-1 recruitment to ITIMs (63). 
Thus, both IgSF and C-type lectin family members use ITIMs 
and ITSMs as a mechanism to inhibit adaptive immune cell 
function. These families of inhibitory receptors, with distinct 
ligand specificities and expression patterns, might diversify 
how inhibitory receptors can be used to regulate T cell 
responses.

Since the first description of an ITIM in FcγRIIB >15 y ago, many inhibitory receptors have been discovered by the 
presence of intracellular inhibitory motifs. Recent advances in 
genomic and proteomic informatics allowed for the identifi-
cation of a large number of novel ITIM-containing molecules 
(64, 65). These complementary studies revealed >800 pre-
viously unidentified ITIM-bearing molecules in the human 
genome. It is likely that these databases contain some false 
positives, emphasizing the need for functional and biological 
analysis of these molecules. However, these ITIM databases 
provide the field with a basic foundation for future work on 
the functional roles of novel ITIM-containing molecules. It 
will be interesting to extend these types of approaches to other 
motifs, such as ITSMs, in the future.

Inhibitory receptors without ITIMs/ITSMs

Much of what is known about inhibitory receptor mechanisms 
of action has been obtained through studies of cytoplasmic 
inhibitory motifs, specifically ITIMs and ITSMs.

However, several well-known inhibitory receptors, includ-
ing CTLA-4, Tim-3, Lag-3, and CD160, do not mediate their 
inhibitory effects through classical ITIMs or ITSMs. For ex-
ample, the well-studied inhibitory receptor CTLA-4 lacks an 
ITIM or ITSM. Some work suggested that a phosphorylated 
YxxM motif indirectly recruits SHP-2 to the intracellular tail 
of CTLA-4. However, this issue remains controversial because 
CTLA-4 was shown to mediate inhibitory effects without
SHP-2 recruitment (66). It is, of course, possible that CTLA-4 uses more than one mechanism of T cell inhibition. Nevertheless, these observations clearly demonstrate inhibitory receptor function in the absence of an ITIM or ITSM.

Although the Tim gene family members do not contain classical ITIMs, these molecules contain intracellular tyrosine-phosphorylation motifs (67). Tim-3, which has recently acquired a GPI-anchored receptor, inhibits T cell activation by reducing phosphorylation of CD3ε (68). Inhibitory motifs other than ITIMs and ITSMs have also been identified in some inhibitory receptors. For example, the IgSF member Lag-3 negatively regulates the homeostatic expansion of T cells via a KIEELE motif in its cytoplasmic tail (12, 69). Relatively little is known about how Lag-3 mediates these inhibitory effects through its KIEELE motif. Finally, CD160, a GPI-anchored receptor, inhibits T cell activation by reducing phosphorylation of CD3ε (70). However, the signaling cascade that leads to this inhibitory effect remains elusive, given the absence of an intracellular tail (70).

The ability of many inhibitory receptors to disrupt proximal TCR/costimulatory signaling events emphasizes the importance of this widespread mechanism of cell inhibition. However, the intricacies of these inhibitory pathways continue to be defined. Many studies on inhibitory receptor signaling have been performed in limited cell types and often under nonphysiological conditions. As a result, a major gap in knowledge is the cell-specific effects of inhibitory receptor ligation. This issue is especially important for those inhibitory receptors known to recruit SHP and SHIP molecules, because it has been proposed that baseline expression of these phosphatases can vary in different cell types and at different stages of immune responses (58, 71).

Targeting inhibitory receptor pathways therapeutically

Enhancing inhibitory receptor activity might help to prevent the activation or function of autoreactive and allospecific immune cells, whereas blockade of inhibitory receptor pathways has shown promise in partially reversing T cell exhaustion during chronic infections and cancer. However, it is not yet clear why blockade of some inhibitory receptors is beneficial, whereas disrupting others has little effect. Moreover, although some combined blockades are beginning to show promise, it remains unclear which combinations of inhibitory receptor blockades can synergize for the most optimal benefit or how this synergy works mechanistically. For example, blockade of...
PD-1/PD-L1 alone, but not Lag-3 alone, restores function of exhausted CD8+ T cells during chronic LCMV infection, whereas blockade of both the PD-1 and Lag-3 pathways provides synergistic recovery of effector T cell functions and viral control (36). Similarly, blockade of PD-1 and Lag-3 dramatically improves T and B cell responses during Plasmodium infection in mice, which results in accelerated parasite clearance (39). Combined blockade of PD-1 with Tim-3 also synergistically increases the function of exhausted CD8+ T cells during chronic infection and cancer (37, 72).

In addition to targeting multiple inhibitory receptor pathways, recent work demonstrated the potential of coordinately targeting inhibitory receptors and other types of immune regulatory pathways. For example, PD-1 blockade has been successfully combined with IL-10 blockade or low-dose anti-4-1BB agonistic Ab to enhance antiviral T cell responses and decrease viral load (73, 74). Thus, combining blockade of inhibitory receptors with blockade of suppressive cytokines or stimulation of positive regulatory pathways may be a promising approach for enhancing immunity to chronic infections or cancers.

Targeting of multiple inhibitory receptor pathways may be a potent way to boost immune responses during infection and cancer, but the potential risk for autoimmunity is a concern when considering these treatments for clinical use. CTLA-4 blockade has been associated with autoimmune events in some human subjects, although most of these side effects subside with appropriate clinical management (75). Similar concerns will exist for most inhibitory receptor blockades in humans. In addition to modulating self-tolerance, one must consider potential effects on immunopathology. Although PD-1 and PD-L1–deficient mice tolerate many acute infections quite well, these mice succumb to chronic LCMV infection within 7–10 d, and PD-1/PD-L1–deficient mice also have reduced survival after infection within 10 d, and PD-1–deficient mice also have also synergistically increases the function of exhausted CD8+ T cells during chronic infection and cancer (37, 72).

In addition to targeting multiple inhibitory receptor pathways, recent work in mice demonstrated the beneficial effects of treatment with agonistic inhibitory receptor Abs during autoimmune (1). To successfully translate these types of therapies into human use, the field exploited an important mechanism of CTLA-4 inhibition: binding and sequestration of CD80 and CD86. This knowledge led to the design of CTLA-4-Ig, a fusion protein that blocks the engagement of CD28 with its ligands and prevents autoreactive T cell activation. In 2005, the FDA approved the use of CTLA-4-Ig (abatacept) for the treatment of moderate-to-severe rheumatoid arthritis (79). The development of abatacept is a prime example of how the design of effective therapeutics can be aided by our understanding of inhibitory receptor mechanisms of action.

Conclusions

The successes of CTLA-4 and PD-1 mAbs are at the forefront of inhibitory receptor clinical applications. Given the important roles of inhibitory receptors in autoimmunity and the prevention of immunopathology, the effects that administration of blocking Abs have on other cell types and healthy tissues must also be carefully examined. As the field begins to better grasp how inhibitory receptors function individually and synergistically on distinct cell types, it may be possible to design more effective and specific therapeutic and prophylactic treatment strategies.

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