REVIEW

Immune Inhibitory Receptors

Jeffrey V. Ravetch[1] and Lewis L. Lanier[2]

With the detailed description and analysis of several inhibitory receptor systems on lymphoid and myeloid cells, a central paradigm has emerged in which the pairing of activation and inhibition is necessary to initiate, amplify, and then terminate immune responses. In some cases, the activating and inhibitory receptors recognize similar ligands, and the net outcome is determined by the relative strength of these opposing signals. The importance of this modulation is demonstrated by the sometimes fatal autoimmune disorders observed in mice with targeted disruption of inhibitory receptors. The significance of these receptors is further evidenced by the conservation of immunoreceptor tyrosine-based inhibitory motifs during their evolution.

A hallmark of the vertebrate immune system is its ability to maintain a precarious equilibrium between the extremes of reactivity and quiescence—at any moment poised to unleash an arsenal of cytotoxic cells and molecules, yet capable of maintaining control over these potentially lethal responses. A key aspect of this ability is encoded in the specificity of the response that can target molecules alien to the organism while sparing the organism itself. Equally critical is the capacity to limit and ultimately terminate a response, inactivating or eliminating the relevant pathways when they are no longer required.

Although many aspects of positive signaling in the generation of effective immunity have been clarified, our knowledge about the counterbalancing inhibitory pathways has remained quite limited until recently. From the detailed analysis of some inhibitory receptor systems, a central paradigm has emerged in which the pairing of activation and inhibition is necessary to modulate immune responses (*1–3*). Loss of inhibitory signaling is often associated with autoreactivity and unchecked inflammatory responses, illustrating the essential role these systems play in immune regulation (*4–6*).

The common feature that identifies receptors as members of the inhibitory class is their ability to attenuate activation signals initiated by other receptors that are often of the immunoreceptor tyrosine-based activation motif (ITAM) class (*7, 8*). Inhibitory receptors mediate this function only upon their clustering with an activating counterpart on the cell surface. In some cases, the activating and inhibitory receptors recognize different ligands, whereas in other circumstances cells simultaneously express pairs of activating and inhibitory receptors with closely related extracellular domains binding similar ligands. When both activating and inhibitory receptors are coengaged by their respective ligands, the net outcome is determined by the relative strength of these opposing signals.

An expanding family of immune inhibitory receptors can be identified by a consensus amino acid sequence, the immunoreceptor tyrosine-based inhibitory motif (ITIM), present in the cytoplasmic domain of these molecules (*2, 8*). The prototype 6–amino acid ITIM sequence is (Ile/Val/Leu/Ser)-X-Tyr-X-X-(Leu/Val), where X denotes any amino acid. Ligand-induced clustering of these inhibitory receptors results in tyrosine phosphorylation, often by a Src family kinase, which provides a docking site for the recruitment of cytoplasmic phosphatases having a Src homology 2 (SH2) domain (*9–11*). Two classes of SH2-containing inhibitory signaling effector molecules have been identified: the tyrosine phosphatase SHP-1 and the inositol phosphatase SHIP (SH2-containing inositol polyphosphate 5-phosphatase) (*12, 13*). The ITIM can also bind to another cytoplasmic tyrosine phosphatase, SHP-2; however, the role of this molecule in inhibitory function is less well defined.

SHP-1 and SHIP interact with the ITIMs of nonoverlapping subsets of inhibitory receptors and couple to distinct signaling pathways (Fig. 1), resulting in subtle but important differences in the final state of the inhibited cells (*14*). In addition to their role in transducing signals from immune inhibitory receptors, SHIP and SHP-1 have a broader role in cellular inhibition and can couple to both immune and nonimmune activation receptors by undefined sequences other than the ITIM (*12, 13*). As an illustration of the SHP-1–dependent inhibition pathway, the B cell inhibitory receptor PIR-B is shown (Fig. 1B). Recruitment of SHP-1 to PIR-B attenuates B cell antigen receptor (BCR)–triggered activation responses through the dephosphorylation of a number of intracellular substrates. These substrates include the BCR itself, tyrosine kinases activated by BCR engagement (such as Syk), and the targets of these kinases, including the adaptor BLNK and the effector phospholipase C (PLC)–γ1 (*11, 15, 16*). Inhibition by SHP-1 recruitment occurs at the earliest steps of the activation response, shutting down all subsequent events, including calcium mobilization, cytokine release, transcriptional activation, and cellular proliferation. In contrast, SHIP, shown to be recruited to the tyrosine-phosphorylated ITIM of the inhibitory immunoglobulin G (IgG) Fc receptor FcγRIIB (*17*), leads to the abrogation of BCR activation signaling by hydrolysis of the membrane inositol phosphate PIP_3 (phosphatidylinositol 3,4,5-trisphosphate), itself the product of receptor activation (Fig. 1A). In the absence of PIP_3, binding proteins of the PH domain class (e.g., Btk and PLCγ1) are released from the membrane and a sustained calcium signal is blocked by preventing influx of extracellular calcium through the capacitance-coupled channel (*13, 18, 19*). FcγRIIB phosphorylation also leads to an arrest of BCR-triggered proliferation by perturbing the activation of mitogen-activated protein (MAP) kinases and the recruitment of the antiapoptotic protein kinase Akt (*20, 21*).

This review emphasizes the common aspects of inhibitory receptors and their in vivo consequences for signaling by immunoreceptors. Prototypic examples of this class of molecules serve as a framework for incorporating newly discovered molecules in this rapidly expanding class of receptors.

Immunoglobulin G Fc Receptors: A Paradigm of Activation-Inhibition Coupling

Receptors for IgG provide the best characterized and most detailed examples of the coordinate and opposing roles displayed by activating and inhibitory receptors (*22, 23*). Studies on these receptors have defined several general paradigms for the class of inhibitory receptors as a whole and pointed to the physiological relevance of these pathways in the immune response. IgG immune complexes were recognized as potent inhibitory ligands more than 30 years ago with the observation that B cell activation could be attenuated by immune complexes (*24*). A molecular basis for this activity was suggested with the cloning of two genes for murine low-affinity IgG Fc receptors, now referred to as FcγRIIB and FcγRIII (*25*). The extracellular domains were found to be 95% identical in their primary amino acid sequence and to mediate low-affinity binding to IgG immune complexes with similar specificity. However, these near-

[1]Laboratory of Molecular Genetics and Immunology, Rockefeller University, New York, NY 10021, USA. [2]Department of Microbiology and Immunology and Cancer Research Institute, University of California, San Francisco, CA 94143, USA.

ly identical domains were coupled to distinctly different intracytoplasmic domains, which suggested that different signaling pathways might be triggered by their engagement. Later studies defined FcγRIII as an activating receptor capable of triggering calcium mobilization, degranulation, antibody-dependent cellular toxicity (ADCC), phagocytosis, cytokine release, and inflammatory responses by macrophages, natural killer (NK) cells, neutrophils, and mast cells (*26, 27*). The activating sequence was found on a noncovalently associated accessory subunit, the γ chain, and contained a sequence common to antigen receptors of B and T cells, the ITAM (*28*). The γ subunit is shared by a variety of activation receptors, including FcεRI, T cell antigen receptor, PIR-A, ILT1, and ILT7 (see below). In contrast, the inhibitory motif, embedded in the cytoplasmic domain of the single-chain FcγRIIB molecule, was defined as a 13–amino acid sequence (Ala-Glu-Asn-Thr-Ile-Thr-Tyr-Ser-Leu-Leu-Lys-His-Pro) that has been shown to be both necessary and sufficient to mediate the inhibition of BCR-generated calcium mobilization and cellular proliferation (*29, 30*).

Phosphorylation of the tyrosine of this motif was shown to occur upon BCR coligation and was required for its inhibitory activity. This modification generated an SH2 recognition domain that is the binding site for the inhibitory signaling molecule SHIP (*30*). In addition to its expression on B cells, where it is the only IgG Fc receptor, FcγRIIB is expressed on macrophages, neutrophils, and mast cells, missing only from T and NK cells (*22*). These studies provided the impetus to identify similar sequences in other surface molecules that mediated cellular inhibition and resulted in the description of the ITIM, now recognized as a general feature of inhibitory receptors.

FcγRIIB displays three separable inhibitory activities, two of which are dependent on the ITIM motif and one that is independent of this motif. Coengagement of FcγRIIB to an ITAM-containing receptor leads to tyrosine phosphorylation of the ITIM by the Lyn kinase, recruitment of SHIP, and the inhibition of ITAM-triggered calcium mobilization and cellular proliferation (*7, 9, 17*). These two inhibitory activities result from different signaling pathways, with calcium inhibition requiring the phosphatase activity of SHIP to hydrolyze PIP_3 and the ensuing dissociation of PH domain–containing proteins like Btk and PLCγ (*17, 18*) (Fig. 1A). The net effect is to block calcium influx and prevent sustained calcium signaling, which prevents calcium-dependent processes such as degranulation, phagocytosis, ADCC, and cytokine release. Arrest of proliferation in B cells is also dependent on the ITIM pathway, through the activation of the adaptor protein Dok and subsequent inactivation of MAP kinases (*31, 32*). The role of SHIP in this process has not been fully defined, because it can affect proliferation in several ways. Through its catalytic phosphatase domain, SHIP can prevent recruitment of the PH domain survival factor Akt by hydrolysis of PIP_3 (*20, 21, 33*). SHIP also contains PTB domains that could act to recruit Dok to the membrane and provide access to the Lyn kinase that is involved in its activation. Dok-deficient B cells are unable to mediate FcγRIIB-triggered arrest of BCR-induced proliferation but retain their ability to inhibit a calcium influx, demonstrating the dissociation of these two ITIM-dependent pathways (*31*).

The third inhibitory activity displayed by FcγRIIB is independent of the ITIM sequence and is displayed upon homoaggregation of the receptor. Under these conditions of FcγRIIB clustering, a proapoptotic signal is generated through the transmembrane sequence (Fig. 2). This proapoptotic signal is blocked by recruitment of SHIP, which occurs upon coligation of FcγRIIB to the BCR, because Btk is required for this apoptotic pathway (*34*). This activity has only been reported in B cells and has been proposed to act as a means of maintaining peripheral tolerance for B cells that have undergone somatic hypermutation. Discrimination of those somatically hypermutated germinal center B cells that have high-affinity cognate antigen binding from those with low affinity and potentially cross-reactive specificities is proposed to result from engagement of antigen. Antigen is retained in the form of immune complexes on follicular dendritic cells. In this form it either interacts with B cells through FcγRIIB alone, which results in apoptosis, or is coengaged with BCR, favoring survival (Fig. 2). Support for this model comes from the B cell autonomous loss of peripheral tolerance in FcγRIIB knockout mice on the C57Bl/6 background (*6*). These animals develop antibodies to DNA and chromatin, and they succumb to a fatal, autoimmune glomerulonephritis at 8 months of age. This phenotype is strain-dependent and is not seen in Balb/c or 129 strains of mice. FcγRIIB thus appears to act as a genetic susceptibility factor for autoimmune disease, under the control

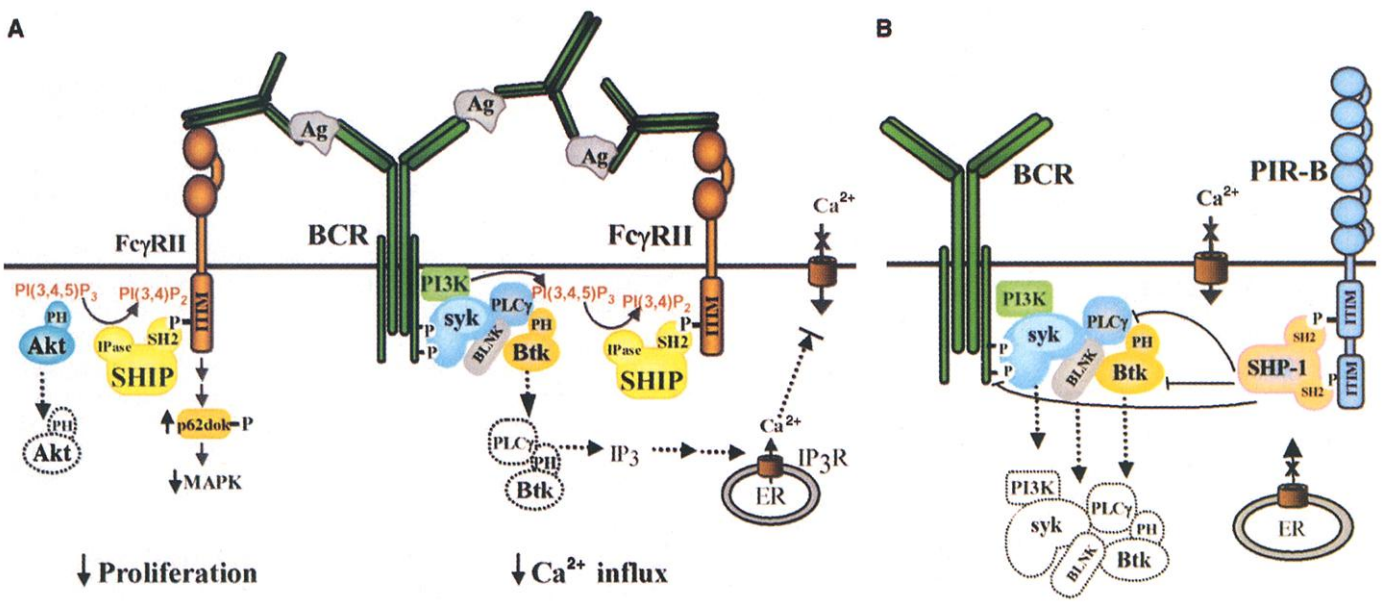

Fig. 1. (**A**) SHIP-mediated inhibition of cellular activation. FcγRIIB is shown as an example of this class of inhibitory receptor. (**B**) Inhibition of BCR activation by SHP-1. PIR-B is displayed as an example of a SHP-1–dependent inhibitory receptor. PI3K, phosphatidylinositol 3-kinase; IP_3, 1,4,5-inositol trisphosphate; ER, endoplasmic reticulum.

of epistatic modifiers.

The phenotype of animals deficient in the inhibitory FcγRIIB further illustrates the critical role this receptor plays in maintaining a balanced immune response. FcγRIIB-deficient mice are also susceptible to induced autoimmune disease, as demonstrated in models of collagen-induced arthritis and Goodpasture's syndrome (*35, 36*). This susceptibility results from the ability of FcγRIIB-deficient mice to develop autoantibodies to type II and IV collagen upon immunization with bovine antigens, presumably as a consequence of failing to maintain peripheral tolerance to potentially cross-reactive autoantigens. No changes in B cell development were observed in FcγRIIB-deficient mice, and B cell responses to BCR cross-linking were not perturbed. Thus, the defects are attributable to the inability of this inhibitory receptor to control ligand-mediated engagement of its activating counterpart. The efferent response to pathogenic IgG antibodies is also enhanced in FcγRIIB-deficient mice, as demonstrated in models of antiglomerular basement membrane glomerulonephritis and tumor protection by antitumor antibodies (*37, 38*). Immune complexes, long known to be potentially inflammatory triggers, are enhanced in their activity in FcγRIIB-deficient mice in the reverse passive Arthus reactions in the skin and lung (*4, 39*). Previous results have indicated that these reactions are FcγRIII-dependent and complement C3–independent (*40–42*). The response to the IgG immune complex is thus the net result of activation and inhibitory pathways working in concert, a theme that is central to the function of inhibitory receptors.

Modulation of the IgG effector response can be accomplished by regulating the ratio of FcγRIII to FcγRIIB and by controlling the levels and activity of SHIP. Activated macrophages and mast cells have high RIII/RIIB ratios, favoring inflammatory responses upon engagement by immune complexes, whereas quiescent effector cells have low RIII/RIIB ratios, providing a high threshold for activation of those cells by immune complexes and thus preventing inappropriate inflammatory responses. SHIP-deficient mice have diminished FcγRIIB inhibitory responses, as expected (*43*). Because SHIP is a pleiotropic inhibitory molecule, it is not surprising that these mice display a complex phenotype, resulting from the interaction of SHIP with cytokine and growth factor receptors in addition to immune receptors. $SHIP^{-/-}$ mice are generally hyperresponsive, displaying a phenotype with increased myeloid proliferation and decreased apoptosis (*21, 44*). Some abnormalities in B cell differentiation are found, presumably resulting from increased BCR signaling.

B Cell Inhibitory Receptors

In addition to FcγRIIB, B cells express a number of ITIM-containing inhibitory receptors that function to regulate the amplitude of the B cell antigen receptor response. These receptors are often constitutively phosphorylated on their ITIM sequence and have broad ligand specificities (*2, 3*). Perturbations in these receptors can result in hyperresponsiveness to BCR stimulation and developmental abnormalities in B cells. B cell inhibitory receptors include CD22, CD5, CD72, CD66a, ILT, PIR-B, PD-1, and LAIR-1.

CD22 is an ITIM-containing surface molecule of the sialoadhesin class in the Ig superfamily interacting with sialic acid–bearing ligands (*45*). The ubiquitous distribution of sialic acid–bearing ligands may account for the generalized inhibitory property of CD22 on B cells, regulating the activation of those cells by the BCR. The ITIMs in CD22 interact with the tyrosine phosphatase SHP-1, thus opposing the activation mediated by the BCR (*46*). Interaction with SHIP has also been described and involves Shc and Grb2 in the resulting complex (*47*). Mice deficient in CD22 display a B cell–hyperresponsive phenotype, autoantibody production (without autoimmune disease), and increased numbers of peritoneal $CD5^+$ B cells (B-1 cells) (*5, 48, 49*). In contrast, SHP-1–deficient mice have a more severe phenotype, with aspects of both severe combined immunodeficiency and autoimmunity, and succumb to pulmonary complications in the first weeks of life (*50*). As also seen in the SHIP-deficient mice, myeloproliferation and infiltration of macrophages and granulocytes is prominent. B-1 cells are expanded and are hyperresponsive to BCR aggregation. However, and in contrast to the $SHIP^{-/-}$ mice, FcγRIIB signaling is not perturbed, confirming the discrimination of inhibitory signaling by SHIP- and SHP-1–dependent inhibitory receptors (*14, 17, 51*). The pleiotropic phenotype of SHP-1 results from the role of this phosphatase in regulating activation by a number of other inhibitory receptors (as well as cytokine and growth factor receptors) and is likely the consequence of the accumulation of multiple primary cell defects.

CD5 belongs to the scavenger receptor superfamily and is expressed on T cells and B-1 cells. Generation of mice deficient in CD5 revealed the inhibitory role of this molecule (*52*). B-1 cells derived from CD5-deficient mice proliferate in response to BCR aggregation; under similar conditions, wild-type B-1 cells are nonproliferative. CD5 has an ITIM that is constitutively phosphorylated in B-1 cells and is associated with SHP-1, which may account for its inhibitory activity (*53*). However, receptor reconstitution studies have challenged this simple model, because inhibitory activity is retained in CD5 constructs lacking the ITIM sequence (*54*). Presumably other sequence elements are involved together with other inhibitory signaling molecules to prevent proliferation of these cells upon BCR aggregation.

ITIM motifs have also been defined in CD72, a member of the calcium-dependent lectin superfamily (*55, 56*). B cells from CD72-deficient mice are hyperresponsive to lipopolysaccharide stimulation and BCR aggregation (*57*). There is a growing list of ITIM-bearing receptors expressed on B cells (Table 1); however, in the absence of data on the physiological ligands for these receptors and their in vivo contribution to B cell development and function, conclusions about their role in normal immune responses are necessarily limited.

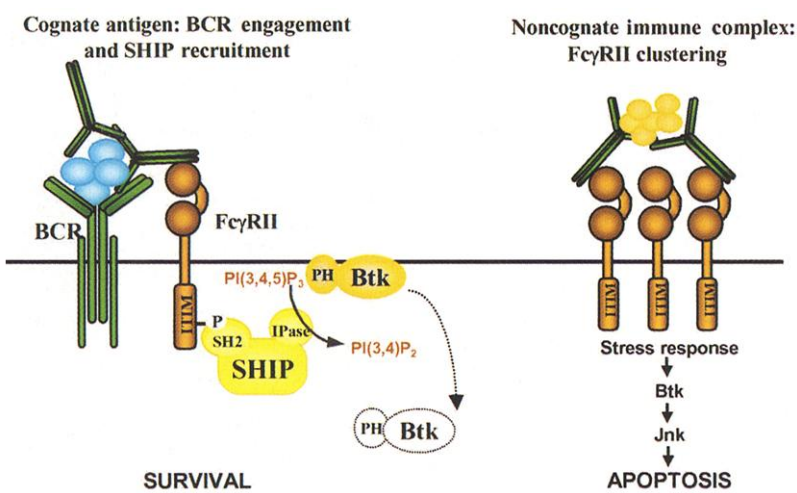

Fig. 2. FcγRIIB can determine B cell fate. A model for the role of FcγRIIB in affinity maturation of antibodies is shown. See text for details.

Inhibitory NK Cell Receptors for MHC Class I

The existence of inhibitory receptors on NK cells was predicted by the observation that these lymphocytes preferentially killed cells

lacking major histocompatibility complex (MHC) class I antigens. This implied the existence of receptors for class I that suppressed their cytolytic activity (*58*). Three families of cell surface receptors (Ly49, KIR, and CD94/NKG2) expressed on NK cells and memory T cells have been identified that bind MHC class I on potential target cells and dampen or prevent NK cell and T cell activation [reviewed in (*3*)]. The mouse Ly49 receptors, encoded by a small family of genes, recognize polymorphic epitopes on the H-2D and H-2K class I molecules (*59*). The *Ly49* genes are themselves polymorphic and are further diversified by alternative RNA splicing. In addition, the expression of each *Ly49* locus is independently controlled, allowing for monoallelic expression of these receptors on overlapping subsets of NK cells and memory T cells. Ly49 proteins are type II membrane glycoproteins of the C-type lectin-like family, expressed on the cell surface as disulfide-bonded homodimers. Seven of the nine *Ly49* genes expressed in C57BL/6 mice contain an ITIM in their cytoplasmic domains, allowing for inhibitory function. In contrast, Ly49D and Ly49H lack ITIM and function as activating receptors because of their noncovalent association with the ITAM-bearing membrane adaptor protein DAP12 (*60*). Presumably, binding of an inhibitory Ly49 receptor to its MHC class I ligand results in tyrosine phosphorylation of the ITIM, with subsequent recruitment of SHP-1 or SHP-2 (*61*).

In primates, NK cells are regulated by the killer cell Ig-like receptors (KIRs, also collectively termed CD158) [reviewed in (*3*)]. Like Ly49, KIRs are encoded by about 12 polymorphic genes, recognize polymorphic epitopes on human leukocyte antigen classes HLA-A, HLA-B, and HLA-C, and are expressed on overlapping subsets of NK cells and memory T cells. About half of the KIR molecules have two ITIMs in their cytoplasmic domains that bind predominantly SHP-1 and inhibit cell-mediated cytotoxicity and cytokine secretion (*61–63*). Activating isoforms in the KIR family, lacking ITIM, associate with the ITAM-bearing DAP12 adaptor protein (*60*). Simultaneous expression of activating and inhibitory KIRs that recognize different HLA class I ligands would provide for elimination of cells that had lost the expression of the ligand for the inhibitory receptor but retained expression of the ligand for the activating receptor (Fig. 3).

The CD94/NKG2A receptor, present and conserved in rodents and primates, is a disulfide-bonded heterodimer composed of subunits encoded by the *CD94* and *NKG2A* genes [reviewed in (*3*)]. The CD94/NKG2A receptor is typically expressed on about half of all NK cells and a subset of memory $CD8^+$ T cells. Whereas CD94 has a short cytoplasmic domain lacking signaling function, NKG2A contains two ITIMs, which upon tyrosine phosphorylation can recruit SHP-1 or SHP-2 (*64, 65*). This heterodimeric receptor recognizes human HLA-E and the homologous mouse $Qa1^b$ molecule. Of note, the peptide-binding groove of HLA-E and $Qa1^b$ is often occupied by 9–amino acid peptides derived from the leader segments of other MHC class I molecules (i.e., H-2D, H-2K, HLA-A, HLA-B, HLA-C, HLA-G) (*66, 67*). In the absence of these peptides, HLA-E and $Qa1^b$ are retained in the cytoplasm of the host cells and degraded. This provides a mechanism for the NK cells or T cells expressing CD94/NKG2A to globally monitor the expression of MHC class I on tissues. The CD94/NKG2C heterodimer also binds HLA-E/$Qa1^b$, but NKG2C lacks an ITIM and associates with DAP12 to form an activating receptor complex (*60*).

Table 1. Immune inhibitory receptors. Homolog identified refers to mouse for human receptors and human for mouse receptors.

Receptor	Chromosomal location	Predominant cell distribution	Homolog identified	Putative number of ITIMs	Putative activating receptor within family	Ligands
Human						
FcγRIIB	1q23-24	B, myeloid, mast	Yes	1	FcγRIII (FcεRIγ, CD3ζ), FcγRIIA	IgG complexes
CTLA-4	2q33	T	Yes	None	CD28	CD80, CD86
PD-1	2q37.3	T, B, NK	Yes	1		PD-1 ligand
PILRα	7q22	Myeloid	Yes	2	PILRβ?	?
CD72	9p	B	Yes	2		?
CD5	11q13	T, subset B	Yes	1		?
MAFA	12p12-13	Myeloid, mast, NK	Yes	1		?
NKG2A	12p13.1-13.2	NK, T	Yes	2	NKG2C, NKG2E (DAP12)	HLA-E
CD31	17q23	Myeloid, platelet, endothelial cells, subset T and NK	Yes	1		CD31
CMRF35H	17q24	Leukocytes	No	3	CMRF35A?	?
CD22	19q13.1	B	Yes	4		Sialic acid
CD66a	19q13.2	Granulocytes, T, subset, NK, B, epithelial cells		2		CD66, CD62E
CD66d	19q13.2	Granulocytes		1		?
CD33	19q13.3	Myeloid	Yes (no ITIM)	1		Sialic acid
SIGLEC5	19q13.3	Myeloid	No	1		Sialic acid
SIGLEC6	19q13.3	B cells, myeloid, cytotrophoblast	No	1		Sialic acid
SIGLEC7	19q13.3	NK, myeloid, subset T	No	1		Sialic acid
ILT2, 3, 4, 5; LIR8	19q13.4	Myeloid, B, subset T, NK	Mouse PIR?	4	ILT1, ILT7, ILT8, LIR6a (FcεRIγ)	MHC class I for ILT2, ILT4; others unknown
LAIR-1	19q13.4	Leukocytes	No	2		?
KIR2DL	19q13.4	NK, T	No	2	KIR2DS	HLA-C
KIR3DL	19q13.4	NK, T	No	2	KIR3DS	HLA-B, -A
SIRPα	20p13	Myeloid, nonhematopoietic cells	Yes	2	SIRPβ (DAP12)	CD47
Mouse						
gp49B1	10 (band 4)	Myeloid, mast, NK	No	2		?
Ly49A-I	6	NK, T	Human pseudogene	1	Ly49D, Ly49H (DAP12)	MHC class I
PIR-B	7	Myeloid, B	Human ILT?	3	PIR-A (FcεRIγ)	?

In summary, the Ly49, KIR, and CD94/NKG2 receptors all recognize MHC class I ligands, contain both activating and inhibitory isoforms within the families, and are expressed exclusively on overlapping subsets of NK cells and memory T cells. The inhibitory receptors associate with SHP-1 or SHP-2. As yet, however, there is little information about the substrates for these phosphatases during NK cell or T cell activation, although SLP-76, LAT, CD3ζ, and FcεRIγ have been suggested as targets (*68, 69*). Inhibitory NK cell receptors have been implicated in the rejection of allogeneic bone marrow allografts, in the elimination of tumors lacking MHC class I, and in protection against viral infection [reviewed in (*3*)].

Inhibitory Receptors on Myeloid and Other Hematopoietic Cell Types

The *ILT* (also known as *LIR*, *MIR*, or *CD85*) and *LAIR-1* genes are immediately centromeric of the *KIR* loci on human chromosome 19q13.4 (*70, 71*). The LAIR-1 and ILT receptors contain Ig-like domains, and 8 of the 10 ILT receptors have ITIM in their cytoplasmic domains. ILT1 and ILT7 lack ITIM, and they associate with the ITAM-containing FcεRIγ membrane adaptor protein, forming an activating receptor complex (*72*). Although certain ILT receptors are present on minor subsets of NK cells and memory T cells, these molecules are predominantly expressed on monocytes, macrophages, dendritic cells, and B lymphocytes. The inhibitory ILT2 and ILT4 receptors bind MHC class I, but (unlike KIRs) they appear to recognize epitopes shared by HLA-A, -B, -C, -G, and -E (*73, 74*). In addition, ILT2 binds with high affinity to a MHC class I homolog present in human cytomegalovirus, possibly to suppress macrophage activation during viral infection (*71*). Ligands for other members of the ILT family have not as yet been identified. The ITIM-containing ILT and LAIR-1 receptors associate with SHP-1 and SHP-2. The mouse *PIR* genes are quite similar to the human *ILT* genes (*75, 76*). The PIR-B receptors contain ITIM in their cytoplasmic domains, whereas PIR-A receptors lack ITIM and associate with FcεRIγ (*77, 78*).

Several other ITIM-bearing receptors expressed on myeloid and other hematopoietic cells are summarized in Table 1, including CD33, SIGLEC5 (*79*), SIGLEC6 (*80*), SIGLEC7 (AIRM1/p75) (*81, 82*), CD66a (*83*), MAFA (*84*), PD-1 (*85*), CD31 (*86*), PILRα (*87*), CMRF35H (*88*), gp49B1 (*89*), and SIRPα (BIT and SHPS-1) (*90, 91*). Of note, the ligand of SIRPα, CD47, is broadly distributed on many tissues including erythrocytes (*92*). When red blood cells from mice expressing a disrupted *CD47* gene are transfused into wild-type animals, macrophages rapidly engulf and clear these cells (*93*). Because CD47 is expressed on erythrocytes in a complex with Rh blood group antigens, this may explain why Rh^{null} individuals have hemolytic anemia, reticulocytosis, and stomatocytosis. Homozygous mice with disrupted *PD-1* genes suffer an autoimmune disease involving lupus-like proliferative arthritis and glomerulonephritis with predominant IgG3 deposition (*94*). These findings suggest an important physiological role for this inhibitory receptor in immune regulation in vivo.

Inhibition of T Cell Activation by CTLA4

Like many of the NK cell receptors, the CD28 and CTLA4 receptors on T cells bind the same ligands (i.e., CD80 and CD86) yet deliver opposing activating and inhibitory signals, respectively [reviewed in (*95*)]. The cytoplasmic domain of CTLA4 lacks an ITIM sequence. Although CTLA4 associates with SHP-2 (*96*), it is uncertain whether this phosphatase is responsible for the suppressive activity of this receptor. Studies in vitro indicate that mutation of the tyrosine residues in the cytoplasmic domain of CTLA4 or removal of the cytoplasmic domain altogether does not prevent the receptor from suppressing T cell activation (*97, 98*). Thus, the molecular basis for CTLA4's activity is not resolved. Nonetheless, the inhibitory function of CTLA4 is clearly revealed by mice with a homozygous disruption of this gene. These animals die of a T cell–mediated autoimmune disease that is lethal within 1 month of birth, indicating the critical role of this molecule in controlling T cell activation (*99*).

Conclusions

Inhibition is as important as activation of the immune system, as shown by the sometimes fatal autoimmune disorders observed in mice with targeted disruption of inhibitory receptors. The significance of these receptors is further evidenced by the conservation of ITIM in immune receptors during their evolution. The inhibitory IgG Fc receptor FcγRIIB is highly conserved in many species; two ITIM-containing NK cell receptors are encoded by genes within the chicken MHC, and membrane receptors of the Ig superfamily with ITIM in their cytoplasmic domain are expressed in pufferfish. Although the function of ITIM-containing sequences in proteins expressed in nonhematopoietic cells is not yet understood, molecules bearing this motif are widely distributed in various tissues, implying a broader role for these structures in physiological regulation.

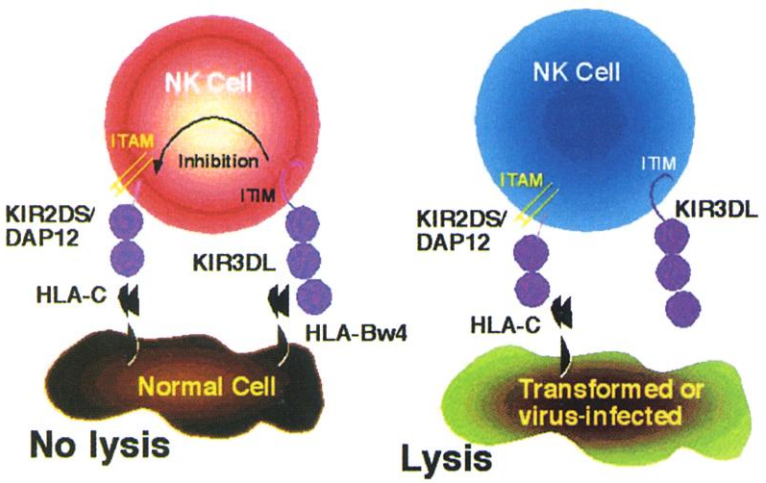

Fig. 3. Paired activating/inhibitory NK cell receptors provide surveillance for MHC allele-specific loss. DAP12-associated activating KIR and ITIM-bearing inhibitory KIR molecules are arrayed in overlapping subsets on human NK cells. NK cells simultaneously expressing an activating KIR2DS-DAP12 receptor for HLA-C and an inhibitory KIR3DL1 receptor for HLA-Bw4 would not harm normal tissues, which will coexpress both HLA-C and HLA-Bw4, because the inhibitory KIR would inactivate positive signals induced by the activating KIR molecule. However, virus-infected or transformed cells that had preferentially lost expression of HLA-Bw4, but not HLA-C, would be recognized as abnormal and attacked.

References and Notes

1. J. I. Healy and C. C. Goodnow, *Annu. Rev. Immunol.* **16**, 645 (1998).
2. S. Bolland and J. V. Ravetch, *Adv. Immunol.* **72**, 149 (1999).
3. E. O. Long, *Annu. Rev. Immunol.* **17**, 875 (1999).
4. R. Clynes *et al.*, *J. Exp. Med.* **189**, 179 (1999).
5. T. L. O'Keefe, G. T. Williams, F. D. Batista, M. S. Neuberger, *J. Exp. Med.* **189**, 1307 (1999).
6. S. Bolland and J. V. Ravetch, *Immunity* **13**, 277 (2000).
7. M. Daeron *et al.*, *Immunity* **3**, 635 (1995).
8. F. Vely and E. Vivier, *J. Immunol.* **159**, 2075 (1997).
9. O. Malbec *et al.*, *J. Immunol.* **160**, 1647 (1998).
10. K. G. C. Smith, D. M. Tarlinton, G. M. Doody, M. L. Hibbs, D. T. Fearon, *J. Exp. Med.* **187**, 807 (1998).
11. A. Maeda *et al.*, *Oncogene* **18**, 2291 (1999).
12. I. Tamir, J. M. Dal Porto, J. C. Cambier, *Curr. Opin. Immunol.* **12**, 307 (2000).
13. L. R. Rohrschneider, J. F. Fuller, I. Wolf, Y. Liu, D. M. Lucas, *Genes Dev.* **14**, 505 (2000).
14. M. Ono *et al.*, *Cell* **90**, 293 (1997).
15. A. Maeda, M. Kurosaki, M. Ono, T. Takai, T. Kurosaki, *J. Exp. Med.* **187**, 1355 (1998).
16. M. Blery *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 2446 (1998).
17. M. Ono, S. Bolland, P. Tempst, J. V. Ravetch, *Nature* **383**, 263 (1996).

18. S. Bolland, R. N. Pearse, T. Kurosaki, J. V. Ravetch, *Immunity* **8**, 509 (1998).
19. A. M. Scharenberg *et al.*, *EMBO J.* **17**, 1961 (1998).
20. M. J. Aman, T. D. Lamkin, H. Okada, T. Kurosaki, K. S. Ravichandran, *J. Biol. Chem.* **273**, 33922 (1998).
21. Q. Liu *et al.*, *Genes Dev.* **13**, 786 (1999).
22. J. V. Ravetch and J. P. Kinet, *Annu. Rev. Immunol.* **9**, 457 (1991).
23. M. Daeron, *Annu. Rev. Immunol.* **15**, 203 (1997).
24. P. L. Chan and N. R. S. C. Sinclair, *Immunology* **21**, 967 (1971).
25. J. V. Ravetch *et al.*, *Science* **234**, 718 (1986).
26. J. D. Young, S. S. Ko, Z. A. Cohn, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5430 (1984).
27. J. A. Titus, P. Perez, A. Kaubisch, M. A. Garrido, D. M. Segal, *J. Immunol.* **139**, 3153 (1987).
28. M. Reth, *Nature* **338**, 383 (1989).
29. S. Amigorena *et al.*, *Science* **256**, 1808 (1992).
30. T. Muta *et al.*, *Nature* **368**, 70 (1994).
31. Y. Yamanashi *et al.*, *Genes Dev.* **14**, 11 (2000).
32. I. Tamir *et al.*, *Immunity* **12**, 347 (2000).
33. C. D. Helgason *et al.*, *J. Exp. Med.* **191**, 781 (2000).
34. R. N. Pearse *et al.*, *Immunity* **10**, 753 (1999).
35. T. Yuasa *et al.*, *J. Exp. Med.* **189**, 187 (1999).
36. A. Nakamura *et al.*, *J. Exp. Med.* **191**, 899 (2000).
37. Y. Suzuki *et al.*, *Kidney Int.* **54**, 1166 (1998).
38. R. A. Clynes, T. L. Towers, L. G. Presta, J. V. Ravetch, *Nature Med.* **6**, 443 (2000).
39. T. Takai, M. Ono, M. Hikida, H. Ohmori, J. V. Ravetch, *Nature* **379**, 346 (1996).
40. D. L. Sylvestre and J. V. Ravetch, *Science* **265**, 1095 (1994).
41. W. L. Hazenbos *et al.*, *Immunity* **5**, 181 (1996).
42. D. Sylvestre *et al.*, *J. Exp. Med.* **184**, 2385 (1996).
43. Q. Liu *et al.*, *J. Exp. Med.* **188**, 1333 (1998).
44. C. D. Helgason *et al.*, *Genes Dev.* **12**, 1610 (1998).
45. T. F. Tedder, J. Tuscano, S. Sato, J. H. Kehrl, *Annu. Rev. Immunol.* **15**, 481 (1997).
46. G. M. Doody *et al.*, *Science* **269**, 242 (1995).
47. J. C. Poe, M. Fujimoto, P. J. Jansen, A. S. Miller, T. F. Tedder, *J. Biol. Chem.* **275**, 17420 (2000).
48. T. L. O'Keefe, G. T. Williams, S. L. Davies, M. S. Neuberger, *Science* **274**, 798 (1996).
49. K. L. Otipoby *et al.*, *Nature* **384**, 634 (1996).
50. L. D. Shultz, T. V. Rajan, D. L. Greiner, *Trends Biotechnol.* **15**, 302 (1997).
51. M. S. Nadler, B. Chen, J. S. Anderson, H. H. Wortis, B. G. Neel, *J. Biol. Chem.* **272**, 20038 (1997).
52. G. Bikah, J. Carey, J. R. Ciallella, A. Tarakhovsky, S. Bondada, *Science* **274**, 1906 (1996).
53. G. Sen, G. Bikah, C. Venkataraman, S. Bondada, *Eur. J. Immunol.* **29**, 3319 (1999).
54. H. Gary-Gouy *et al.*, *J. Biol. Chem.* **275**, 548 (2000).
55. T. Adachi, H. Flaswinkel, H. Yakura, M. Reth, T. Tsubata, *J. Immunol.* **160**, 4662 (1998).
56. Y. Wu *et al.*, *Curr. Biol.* **8**, 1009 (1998).
57. C. Pan, N. Baumgarth, J. R. Parnes, *Immunity* **11**, 495 (1999).
58. K. Karre, H. G. Ljunggren, G. Piontek, R. Kiessling, *Nature* **319**, 675 (1986).
59. F. M. Karlhofer, R. K. Ribaudo, W. M. Yokoyama, *Nature* **358**, 66 (1992).
60. A. B. H. Bakker and L. L. Lanier, *Immunol. Today*, in press.
61. L. Olcese *et al.*, *J. Immunol.* **156**, 4531 (1996).
62. D. N. Burshtyn *et al.*, *Immunity* **4**, 77 (1996).
63. A. M. Fry, L. L. Lanier, A. Weiss, *J. Exp. Med.* **184**, 295 (1996).
64. M. Carretero *et al.*, *Eur. J. Immunol.* **28**, 1280 (1998).
65. E. Le Drean *et al.*, *Eur. J. Immunol.* **28**, 264 (1998).
66. C. J. Aldrich *et al.*, *Cell* **79**, 649 (1994).
67. V. Braud, E. Y. Jones, A. McMichael, *Eur. J. Immunol.* **27**, 1164 (1997).
68. N. M. Valiante, J. H. Phillips, L. L. Lanier, P. Parham, *J. Exp. Med.* **184**, 2243 (1996).
69. B. A. Binstadt *et al.*, *Immunity* **5**, 629 (1996).
70. N. Wagtmann, S. Rojo, E. Eichler, H. Mohrenweiser, E. O. Long, *Curr. Biol.* **7**, 615 (1997).
71. D. Cosman *et al.*, *Immunity* **7**, 273 (1997).
72. H. Nakajima, J. Samaridis, L. Angman, M. Colonna, *J. Immunol.* **162**, 5 (1999).
73. M. Colonna *et al.*, *J. Immunol.* **160**, 3096 (1998).
74. T. L. Chapman, A. P. Heikeman, P. J. Bjorkman, *Immunity* **11**, 603 (1999).
75. K. Hayami *et al.*, *J. Biol. Chem.* **272**, 7320 (1997).
76. H. Kubagawa, P. D. Burrows, M. D. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5261 (1997).
77. H. Kubagawa *et al.*, *J. Exp. Med.* **189**, 309 (1999).
78. A. Maeda, M. Kurosaki, T. Kurosaki, *J. Exp. Med.* **188**, 991 (1998).
79. A. L. Cornish *et al.*, *Blood* **92**, 2123 (1998).
80. N. Patel *et al.*, *J. Biol. Chem.* **274**, 22729 (1999).
81. M. Falco *et al.*, *J. Exp. Med.* **190**, 793 (1999).
82. C. Vitale *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 15091 (1999).
83. M. J. Moller, R. Kammerer, F. Grunert, S. von Kleist, *Int. J. Cancer* **65**, 740 (1996).
84. M. D. Guthmann, M. Tal, I. Pecht, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9397 (1995).
85. Y. Ishida, Y. Agata, K. Shibahara, T. Honjo, *EMBO J.* **11**, 3887 (1992).
86. C. T. Hua, J. R. Gamble, M. A. Vadas, D. E. Jackson, *J. Biol. Chem.* **273**, 28332 (1998).
87. D. D. Mousseau, D. Banville, D. L'Abbe, P. Bouchard, S. H. Shen, *J. Biol. Chem.* **275**, 4467 (2000).
88. G. J. Clark, B. J. Green, D. N. Hart, *Tissue Antigens* **55**, 101 (2000).
89. H. R. Katz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10809 (1996).
90. A. Kharitonenkov *et al.*, *Nature* **386**, 181 (1997).
91. Y. Fujioka *et al.*, *Mol. Cell. Biol.* **16**, 6887 (1996).
92. P. Jiang, C. F. Lagenaur, V. Narayanan, *J. Biol. Chem.* **274**, 559 (1999).
93. P. A. Oldenborg *et al.*, *Science* **288**, 2051 (2000).
94. H. Nishimura, M. Nose, H. Hiai, N. Minato, T. Honjo, *Immunity* **11**, 141 (1999).
95. C. A. Chambers and J. P. Allison, *Curr. Opin. Cell Biol.* **11**, 203 (1999).
96. L. E. Marengere *et al.*, *Science* **272**, 1170 (1996).
97. M. L. Baroja *et al.*, *J. Immunol.* **164**, 49 (2000).
98. T. Cinek, A. Sadra, J. B. Imboden, *J. Immunol.* **164**, 5 (2000).
99. E. A. Tivol *et al.*, *Immunity* **3**, 541 (1995).

100. Supported in part by the Sandler Family Supporting Fund (L.L.L.) and by grants from NIH (J.V.R.). We thank B. Seaman, S. Tangye, J. Katheiser, S. Bolland, and R. Guinamard for helpful discussions and assistance in preparing this review.

VIEWPOINT

T-Independent Immune Response: New Aspects of B Cell Biology

Sidonia Fagarasan and Tasuku Honjo*

Recent results emphasize the roles of T-independent antibody response in humoral defenses, for which B1 cells and marginal zone B cells are mostly responsible. We discuss how these cells are activated, migrate, and differentiate into antibody-producing cells in various lymphoid tissues. Based on recent findings in each of these areas of B cell biology, we propose a possible mechanism for peripheral tolerance of autoreactive B cells at target organs.

T and B lymphocytes are two principal players in the immune response, with T cells controlling much of the activity of B cells. B cell activation by protein antigens requires binding of the antigen to the B cell surface immunoglobulin (Ig) and also requires costimulation by antigen-specific T cells through CD40-CD40 ligand interaction and the secretion of cytokines. Appropriately activated B cells proliferate and differentiate to plasma cells or to long-lived memory cells, and it is during this process of differentiation that B cells manifest unique strategies for further diversifying the repertoire of antigen-specific B cell receptors (BCR). They achieve this by altering the genetic information that encodes the BCR through somatic hypermutation and class-switch recombination. Yet another mechanism for genetic alteration, RNA editing, can now be added to the list of remarkable strategies of the B cell for this amplification of genetic information. Activation-induced cytidine deaminase (AID) is a potential RNA-editing enzyme, and AID deficiency in mice and humans causes a complete defect in class switching and hypermutation (*1*, *2*). One may wonder why B cells use such sophisticated genetic strategies for diversification of their antigen receptor molecules, whereas T cells use only VDJ recombination to achieve the same ends. The use of additional strategies for increasing repertoire formation in B cells seems somewhat at odds with the ability of T cells to control all the activities of B cells. In fact, recent development in B cell biology indicates that B cell activities can be regulated in a T-independent manner. In this Viewpoint, we summarize the recent progress in this field and propose a possible mechanism for T-independent regulation of autoreactive B cells.

Department of Medical Chemistry, Faculty of Medicine, Kyoto University, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan.

*To whom correspondence should be addressed. E-mail: honjo@mfour.med.kyoto-u.ac.jp