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Tumor-Induced Disruption of Proximal TCR-Mediated Signal Transduction in Tumor-Infiltrating CD8⁺ Lymphocytes Inactivates Antitumor Effector Phase

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Abstract

The presence in cancer tissue of Ag-specific, activated tumor infiltrating CD8⁺ T cells proves that tumors express Ags capable of eliciting immune response. Therefore, in general, tumor escape from immune-mediated clearance is not attributable to immunological ignorance. However, tumor-infiltrating lymphocytes are defective in effector phase function, demonstrating tumor-induced immune suppression that likely underlies tumor escape. Since exocytosis of lytic granules is dependent upon TCR-mediated signal transduction, it is a reasonable contention that tumors may induce defective signal transduction in tumor infiltrating T cells. In this review, we consider the biochemical basis for antitumor T cell dysfunction, focusing on the role of inhibitory signaling receptors in restricting TCR-mediated signaling in tumor-infiltrating lymphocytes.

Immune response to cancer is apparent; equally apparent is that tumors grow, implying escape from antitumor immunity (1) or defective antitumor immune responses (2). Multiple candidate mechanisms to account for failure of anti-tumor immunity have been described that involve a variety of cell types, factors, and mechanistic considerations (3). In murine models wherein tumor-bearing mice can be immunized with a variety of Ags (4), and patients in whom tumor-reactive Abs and T cells are commonly found (5), cancer does not cause defective systemic immune responses. Thus, tumor itself, or the host response, causes Ag-specific immune tolerance, almost certainly in the priming, and unequivocally in the effector phase of adaptive immunity, primarily in antitumor T cells resident in tumor tissue (6–8).

Priming of antitumor immune response is ineffectual to eliminate tumors

Detectable priming of antitumor T cells occurs during tumor growth but, because vaccination of patients can dramatically increase the frequency of antitumor T cells [in some cases resulting in a reduced rate of tumor growth (9)], either endogenous priming of antitumor immune response is insufficient to engender successful tumor elimination in patients receiving no therapy, or the effector phase is suppressed, or both. Analysis of APCs in murine tumors has shown that dendritic cells (DCs) are frequently defective in some aspect of priming: Ag capture, cytokine expression, costimulatory function, or migration to

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proximal lymph node (10). This results in diminished initiation of adaptive response to tumor Ags. In some cases, tumor DCs have been shown to be not only defective at priming but also tolerogenic (11, 12). Why tumor DCs do not function effectively as occurs in response to pathogens in which infection is resolved [e.g., *Listeria monocytogenes* (13)] is unclear but may be related to the kinetics of tumor growth (i.e., the dose of Ag available for priming, continual low amounts, as well as the lack of robust danger signals) (14). Similar observations have been made for DCs isolated from cancer patients (15, 16). An additional consideration is that, because many tumor Ags are closely related to self, cognate TCRs expressed in antitumor T cells that survive thymic selection are likely of low affinity and likely have enhanced activation requirements.

Furthermore, two immunosuppressive cell types, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), have been shown to accumulate in tumors, both of which are thought to restrict the priming (17) and effector (18, 19) phases of adaptive immune response. Depletion or inactivation of Tregs (20) or MDSCs (21) enhances experimental immunotherapy in preclinical models, although data from clinical trials are less robust. The basis by which either Tregs or MDSCs inhibit priming is not definitively known, but these cells can produce a variety of molecules that are known to inhibit both DCs and T cell function including: TGF β -1, IL-10, reactive oxygen and nitrogen species, and enzymes that are thought to either deplete the microenvironment of certain amino acids [arginine (22), tryptophan (23), and/or cysteine (24)] or produce toxic metabolites (25), therein leading either to a state of metabolic quiescence or induction of apoptosis in tumor-infiltrating lymphocytes (TILs) [although the notion of immune modulation by tryptophan metabolites has been questioned (26)]. Alternatively, or in addition, altered nitrogen metabolism in the context of enhanced production of reactive oxygen species is thought to produce highly reactive oxygen and nitrogen species that are capable of modifying both the cell surface (27) and enzyme activity within antitumor T cells (28). Postcoculture with T cells in vitro, MDSC-mediated production of reactive nitrogen has been shown to modify TCRs, resulting in diminished recognition by tetra-mer and reduced Ag-dependent lysis and cytokine release, a phenotype that may reflect inhibitory activity of those cells in tumor-draining lymph nodes (27, 29). Soluble bioactive molecules produced by Tregs and MDSCs that inhibit adaptive immunity, presumably by direct action on APCs in the priming phase, are also capable of potentially impacting other antitumor immune cell types in proximity, including NK cells and CD4⁺ and CD8⁺ T cells in the effector phase. In a mechanistic variation, purified MDSCs obtained from tumor (30) or peripheral lymphoid tissue (31) have been shown to cause apoptosis in activated T cells in vitro, implying a different role for MDSCs in dysregulation of the effector phase. However, TILs in situ are not appreciably apoptotic (32), thus arguing that the proapoptotic activity of either MDSCs or tumor cells is a function of in vitro analysis. Collectively considered, the extent to which MDSCs inhibit T cell signaling and function may reflect the tissue site of interaction with T cells (lymphoid or tumor) and as such may contribute to antitumor T cell dysfunction in addition to other candidate mechanisms, as described below.

The suppressed phenotype of TILs reflects the mechanism of the acquired functional defect and involves interference with the TCR-mediated signaling pathway

TILs have long been recognized as being deficient in cytokine release, proliferation, and lytic function (6). One potential mechanism for blockade of effector phase involves galectins, a family of carbohydrate binding proteins made in a variety of cells that have multiple functions but are thought to restrict the mobility of cell-surface T cell proteins important in Ag recognition and signaling (33). Such a mechanism has received support

from several lines of investigation but is as yet incompletely understood and undoubtedly complex because exposure of immune cells to some galectins induce tolerance or death (34); in contrast, other galectins change the phenotype of T cells (35), whereas yet others enhance DCs and CD8⁺ T cell numbers (36).

Our laboratory has pursued a murine model of TIL dysfunction wherein the defective phenotype was transient, being regained upon purification and culture in vitro (37). CD8⁺ TILs have hallmarks of proximal TCR-mediated signaling blockade, interpreted to be the basis of defective lytic function [in freshly isolated TILs assayed in vitro (38), as judged inferentially because tumor cells proximal to TILs in situ are not TUNEL⁺ (32) or evaluated by confocal microscopy wherein lytic granules and the microtubule organizing center fail to polarize to the immune synapse (38, 39)]. Considered with other TIL phenotypes [cell cycle arrest, lack of Ag-dependent cytokine release in vitro, and the inability to arrest migration in tumor tissue (40, 41)], TILs appear to be deficient in Ag-dependent TCR-mediated signal transduction. A detailed biochemical analysis of TCR signaling was investigated in a murine model (42) wherein nonlytic TILs were shown to be triggered in that p56^{lck} is activated upon recognition of cognate tumor cells (becoming phosphorylated on Y394) but the activation signal does not penetrate deeper into the signaling cascade because ZAP70 is not activated (37), calcium flux is extinguished (37) and activation of LFA-1-mediated TIL adhesion is deficient (43) [shown to require p56^{lck} activity (44)]. After initial p56^{lck} activation, Src homology region 2 domain-containing phosphatase (SHP)-1 was rapidly activated and localized to the immunological synapse coincident with dephosphorylation of p56^{lck} Y394. Reversal of both defective proximal signaling and effector function is rapidly achieved by purification of TILs and brief culture in vitro in the absence of tumor. In a similar manner, reintroduction of the signaling block is rapidly achieved by coculture of TILs with tumor [and not with MDSCs or syngeneic but antigenically distinct tumor cells (42)], observations that are consistent with a mechanism involving a fast-acting biochemical inhibitory switch, one that requires contact with cognate tumor for activation (42).

TILs express inhibitory receptors that mediate negative signaling and effector phase dysfunction

Considered collectively, the requirement for tumor cell contact to induce the signaling defect and the rapid kinetics of induction of the TIL signaling block (42) implies that tumor-induced inhibition of TIL signaling involves a ligand-receptor interaction, one similar to that characteristic of inhibitory receptors expressed on NK cells (45). Cell surface inhibitory signaling receptors (IRs) that contain cytoplasmic ITIMs are expressed in a wide variety of immune cells and function in homeostatic regulation of immune responses wherein they negatively regulate signaling mediated by Ag (activating) receptors. Most IRs function in concert with triggering of the Ag receptor in a manner analogous to that of costimulatory receptors (e.g., B7), with the distinction that the activating signal is dampened or abrogated, and function in the homeostasis of normal responses important during cell differentiation and activation (46).

As a consequence of ligand binding, typically IRs are activated by tyrosine phosphorylation on a consensus structure ([I/V/L/S]-X-Y-X-X-[L/V]) by a kinase that is associated with the Ag receptor. Upon ITIM phosphorylation, phosphatase(s) are recruited and likely activated by the kinase associated with the Ag receptor. The activated protein tyrosine phosphatase (PTP) rapidly dephosphorylates proximal substrates, typically the Src family kinase associated with the Ag receptor (e.g., p56^{lck}, but also additional proximal kinases [ZAP70] or adaptor proteins [TCR ζ , Vav-1]). Thus, coordinately with Ag-dependent activation of p56^{lck}, IRs are tyrosine phosphorylated, leading to recruitment of PTP and inactivation of the activation signal. In the course of induction of an activating immune response wherein a

sufficiently strong positive signal is provided to the T cell such that a sustained triggering event occurs (i.e., a high concentration of cognate Ag presented by an appropriately activated APC), PTP-mediated inactivation of proximal signaling occurs following cell activation, reflecting the downregulation of T cell activation during differentiation of naive cells into effector cells. It makes sense conceptually that IRs on effector T cells restrict inadvertent expression of effector phase functions until the T cell recognizes a target cell expressing cognate Ag (as discussed below). Thus, IRs function as part of a system that integrates positive (activating) and negative (inhibitory) signals, therein both maintaining tonic balance and influencing cell activation thresholds. We hypothesize that the activity of IRs can be considered as cell- or organ-specific rheostats that control the magnitude or extent of T cell activation, and, because there are >100 human genes containing ITIM sequences, it is likely that IRs play an important role in regulation of immune responses. A compilation of IRs that can be expressed in T cells is shown in Table I.

The majority of IRs are transmembrane plasma membrane proteins for which the extracellular portion contains recognition elements that govern ligand interaction. The variety of ligand–receptor interaction is considerable in terms of both the number of IRs and the number and type of ligands, leading to the consideration that T cell activation is under the constant influence, if not control, by this regulatory system. Because some IR ligands are widespread (e.g., MHC class I, sialic acid, collagen, and certain integrins), the notion of constant involvement of IR in the control of T cell activation seems plausible. Some IRs mediate homophilic interaction (e.g., CD31), therein restricting activation to a limited number of cell types; others interact with MHC Ag-presenting molecules both class I (CD85, CD158, Ly49) or class II (CD223), implying enhanced function during interaction with APCs.

Many IRs belong to families that share certain structural motifs, such as the Ig superfamily (e.g., CD31, CD66a, CD152) or the Siglec family (e.g., CD22, CD33, CD170). Because many IRs share related ligands (e.g., sialic acid), it is reasonable to consider that there exists overlap or redundancy in the types of target cells that can inhibit a given T cell. Almost all IRs contain at least one ITIM motif in their cytoplasmic domain, which, when phosphorylated, recruits SHP-1 (e.g., CD22), although some IRs contain sequence motif variants (e.g., immunoreceptor tyrosine-based switch motif, or ITSM), which are associated with recruitment of a related phosphatase SHP-2 (e.g., CD152 and CD279). In addition, some IRs have several closely related variants (e.g., CD66), some of which are restricted in terms of cell expression or lack a canonical ITIM sequence (e.g., CD152 or CD160). Variant IRs lacking ITIM can be activating rather than inhibitory in function, possibly due to contributions to T cell–target adhesion.

There are examples of IRs that contain no evident cytoplasmic motifs for recruitment of a PTP (e.g., β_1 integrins or CD160, which is also unusual in being GPI anchored that can be released in soluble form), but which nonetheless function to recruit SHP-1. In these cases, perhaps the IR interacts with a cytoplasmic adapter protein that in turn is responsible for binding and recruiting a PTP into proximity with regulatory proteins in the signaling cascade wherein cell activation can be inhibited. In addition to their major role as rheostats of Ag-dependent signaling, there may be other nonsignaling functions of IRs that affect the behavior or activity of immune cells. For example, several IRs are mediators of adhesion (e.g., CD22, CD66a, β_1 integrins), a function that may predominate in situations in which a T cell interacts with cells that express ligands for a given IR but do not express cognate Ag.

Functionally defective T cells in viral infection or cancer have been shown to express IRs (e.g., PD-1, LAG-3, or CTLA-4), and experimental therapeutic intervention based upon blocking IRs is extant. Tumor cells often express ligands (counterreceptors) for IRs and, as

such, when the tumor cell is recognized by Ag-specific CTL, deliver a negative signal that blocks (or partially blocks) the TIL activation signal, thus restricting effector phase functions, to the detriment of the host. The phenotype of suppression of T cell activation by IRs was shown in 1997 in a mouse model wherein cytokine release and cytotoxicity was blocked upon engagement of NKG2A/CD94 by tumor MHC class I (47). Biochemical analysis of signaling showed that TCR ζ was phosphorylated, demonstrating the cells were triggered, but ZAP70 was not activated, thus explaining how the functional defect was induced in that delivery of the activation signal deeper into the TCR signaling pathway was abrogated. [The detailed biochemical phenotype and functional defect was demonstrated more recently in a study of TILs (42)].

The factors that influence the basis of IR-mediated control of T cell activation are incompletely known but likely involve the following considerations. First, expression of IR ligands on the various cells that T cells contact—DCs during priming, endothelia during transit to the tissues, and ultimately target cells—is undoubtedly important in that receptor ligation is required for function and likely influenced by the differentiation and activation status of the cell. We hypothesize that interaction of IR with cognate ligands (on DCs, endothelia, or tumor cells) functions to recruit/stabilize IR into proximity with the Ag receptor/associated p56^{lck} so that, in turn, the recruited PTP will be in proximity to the target kinase (Fig. 1). During activation of a productive immune response, DCs receive appropriate danger signals leading to their state of full competency, and we hypothesize that this includes modest (or repressed) expression of IR ligands: either levels of a given ligand, or the type/number of IR ligands, or both. A testable corollary of this notion is that suboptimal DC activation, such as that leading to differentiation of DCs having an inhibitory or tolerogenic phenotype, may lead to enhanced expression of inhibitory receptor ligands. Putative involvement of IR ligands in suppression of DC function may be in addition to expression of other mediators of inhibition (e.g., IDO) and insufficient levels of costimulatory molecules and activation-associated factors (IL-12).

Secondly, in a similar manner, the differentiation and activation state of the T cell may control the type and number of IRs that are expressed. For example, perhaps naive T cells express a different repertoire of IR than do effector or memory T cells, reflecting differing activation requirements of T cells in different differentiation states. According to this notion, for example, a memory T cell may express an IR for which the cognate ligand is expressed on endothelial cells that the T cell must traverse en route to interaction with its cognate Ag-expressing tissue/target cell. Thus, a T cell may express multiple IRs, some or any of which may not function until interaction with its cognate ligand occurs. Expression and activity of IRs in this context can be considered to provide another level of safety against inadvertent cytokine release or degranulation of lytic cells that may occur during extravasation that is mediated by activated adhesion molecules that, if they use inside-out signaling, may stimulate inadvertent effector phase activity. In other words, IR activity may be protective against tissue damage by raising the T cell activation threshold, therein restraining effector phase function until an authentic target cell is engaged. Supporting this notion, it was recently reported that multiple IRs are coexpressed on CD8⁺ T cells in chronically infected mice (48). Both of the IRs in that example (PD-1 and LAG-3) were functional, as shown by *in vivo* blockade experiments.

In a murine tumor model (MCA38), lytic defective CD8⁺ antitumor TILs were shown to express several IRs (Fig. 2), further supporting the notion that tumor-induced blockade of CTL signaling suppresses the effector phase and thus abets tumor escape. The observation that multiple distinct IRs are expressed in TILs illustrates the apparent redundancy of their potential functional regulation (CD5, CD85, CD94-NKG2A, and CD279). Curiously, postrecovery of TIL signaling and lytic function, the level of those IRs remained unchanged

in purified lytic TILs, arguing against a role for any of those IRs in mediating TIL signaling defects. A similar observation was made in a pathogen model wherein multiple IRs were expressed in CD8⁺ T cells, but lytic function was not inhibited (49), suggesting that if any of the IRs detected by our flow analysis influence signaling in nonlytic TILs, perhaps the context of expression relative to target cell interaction may be important in their activity. This may be an important consideration because some IRs are expressed at elevated levels upon T cell activation. It is also possible that the IRs observed expressed in TILs do not function in the tumor environment; perhaps instead their ligands are expressed on endothelial cells where they can participate in restriction of the TIL lytic phase (or on other cells that contact the TIL during its transit into the tumor). Thus, it is possible that another as yet unidentified IR controls TIL function. Also of interest is the observation that multiple IRs are expressed in normal splenocytes (Fig. 2), raising the possibility that quiescent naive (or memory) cells depend on IRs to maintain tonic balance, possibly permitting a rapid response to a strong activation signal.

Several molecules are included in the list of IRs (Table I) even though they are not, strictly speaking, IRs, having very small extracellular domains unlikely to permit interaction with an extracellular ligand and no canonical ITIM. However, functionally, these proteins (LAT, PAG, LIME, SIT, and TSAd) may play a similar role as that of ITIM-bearing IRs in that by binding to p56^{lck} its kinase function may be repressed, therein having the equivalent inhibitory effect on TIL function as does recruitment and activation of a PTP.

Several unanswered questions arise in consideration of the role of IRs in tumor escape from antitumor immune response. For example, if tumors express ligands for IRs that are capable of mediating blockade of effector functions at the site of the tumor, why can adoptively transferred T cells eliminate tumor? Although not definitively known, we hypothesize that priming or culture conditions for T cells *in vitro* cause downregulation of IRs, thus permitting effector phase functions upon adoptive transfer. Another conundrum arises in consideration of why transplantable regressor tumors fail to grow. Again, although not known, we hypothesize that either regressor tumors lack expression of IR ligands (and thus do not impede the effector phase), or priming of antitumor immune response during early-stage growth of this class of tumor does not elicit expression of IRs.

Conclusions

To summarize the potential role of IRs in the regulation of antitumor immunity: 1) Different IRs can be expressed by a given T cell at any time during its activation or differentiation; 2) A given IR may function to inhibit T cell responses depending upon the levels and activity of PTP able to interact with the IR; 3) Multiple different IRs can be expressed in a given T cell simultaneously; therefore, activity of a given IR is influenced by interaction of the T cell with cells expressing IR ligands; 4) Tumors can subvert antitumor immunity by expressing IR ligands.

To fully investigate the possibility that experimental tumor therapy based upon the notion that interference with IR–ligand interaction may enable more vigorous antitumor T cell functions, future research efforts might involve the following considerations. Firstly, it will be important to know if different tumors use the same or different mechanisms to induce T cell defects. Therefore, categorization of TILs from different tumor types in terms of the biochemical basis of signaling defect is an objective. Because some candidate mechanisms of defective TIL function are controversial [loss of TIL TCR ζ (27, 42, 50, 51) or significant TIL apoptosis *in situ* due to Fas ligand expression by tumor cells (52)], it will be important to confirm in different laboratories any candidate mechanism of TIL defective signaling. In addition, it will be important to know the full panoply of IRs expressed in TILs. Therefore,

detailed understanding of IR expression in TILs will be imperative. Similarly, it will be important to know the identity of IR ligands expressed in tumor cells and also to understand induction of IR ligand expression in tumor. Once characterized in terms of candidate TIL IR and tumor ligand expression, rational design and testing of inhibitors of either IRs or ligands can be made and may include blocking Ab, small molecule inhibitors, or aptamers (53). Finally, design and testing of systems for delivery of IR/ligand inhibitors should be considered. Because many IRs are expressed on non-T cells and if inhibited may be deleterious, perhaps a linked combination of targeting molecules can be employed to enhance targeting specificity—for example, using tetramers or T cell activation Ags.

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Abbreviations used in this paper

Cbp	C-terminal Src kinase binding protein
Csk	C-terminal Src kinase
DC	dendritic cell
FasL	Fas ligand
HVEM	herpesvirus entry mediator
IR	inhibitory signaling receptor
ITSM	immunoreceptor tyrosine-based switch motif
KIR	killer Ig-related (or Ig-like) receptor
LAIR-1	leukocyte-associated Ig-like receptor-1
LAT	linker for activation of T cells
LILR	leukocyte Ig-like receptor
LIME	LCK-interacting molecule
LLT1	lectin-like transcript-1
MAFA-1	mast cell function-associated Ag-1
MDSC	myeloid-derived suppressor cell
MGL	macrophage galactose-type lectin
PAG	protein associated with glycosphingolipid-enriched micro-domains
PTP	protein tyrosine phosphatase
SHP	Src homology 2 domain-containing tyrosine phosphatase
TIL	tumor-infiltrating lymphocyte
TIM-3	T cell Ig and mucin domain-3
Treg	regulatory T cell

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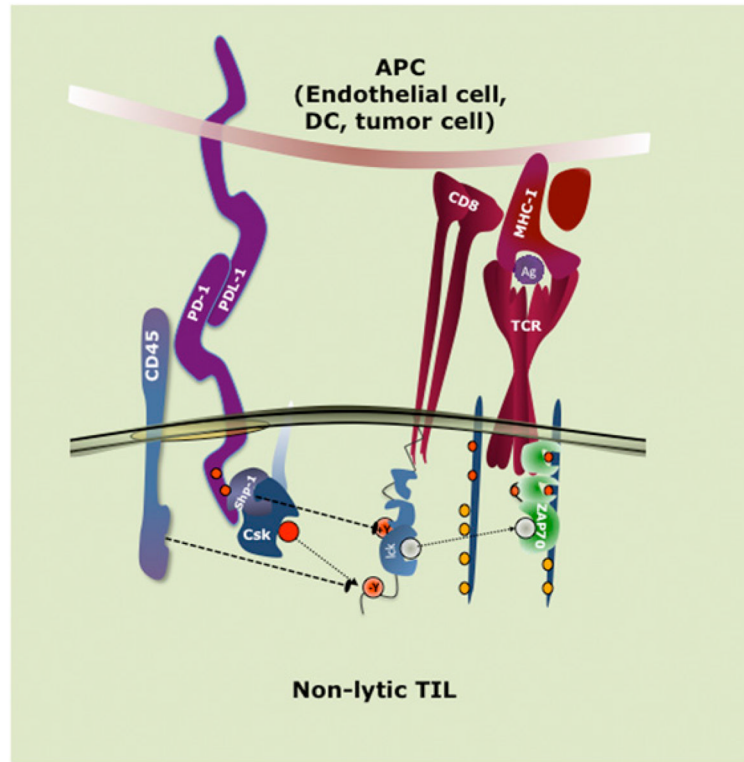
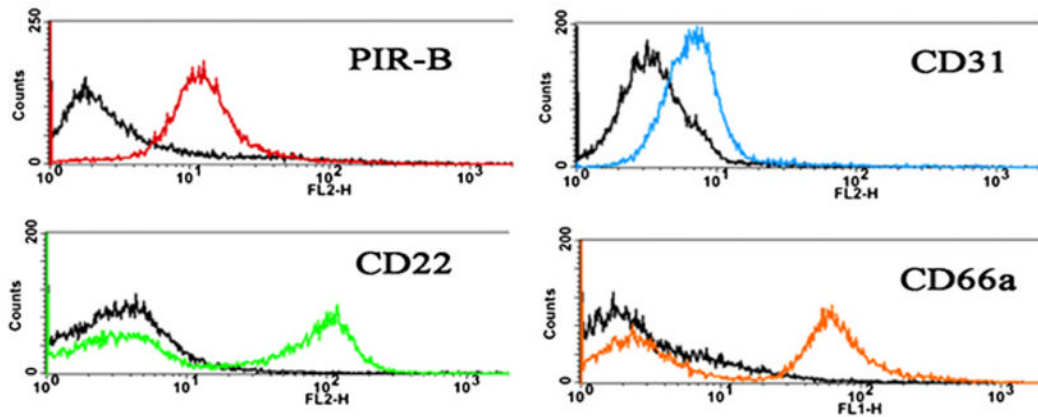


FIGURE 1.

Negative signaling in T cells mediated by IRs. T cells express a given inhibitory receptor (or multiple IRs, although only PD-1 is shown) for which the ligand can be expressed on DCs, endothelial cells, or tumor cells. When bound by its cognate ligand, IRs are phosphorylated on their ITIM and/or ITSM (which in turn reveals a PTP binding site, typically SHP-1 but possibly SHP-2) and brought into proximity to a Src kinase and become bound by PTP. PTP becomes activated by tyrosine phosphorylation, likely also by Src, and dephosphorylates proximal substrates, including the activating Src. Diminished p56^{lck}-mediated activation of downstream signaling raises the activation threshold for the T cell.

Inhibitory Receptor expression in splenocytes



Inhibitory Receptor expression in CD8⁺ TIL

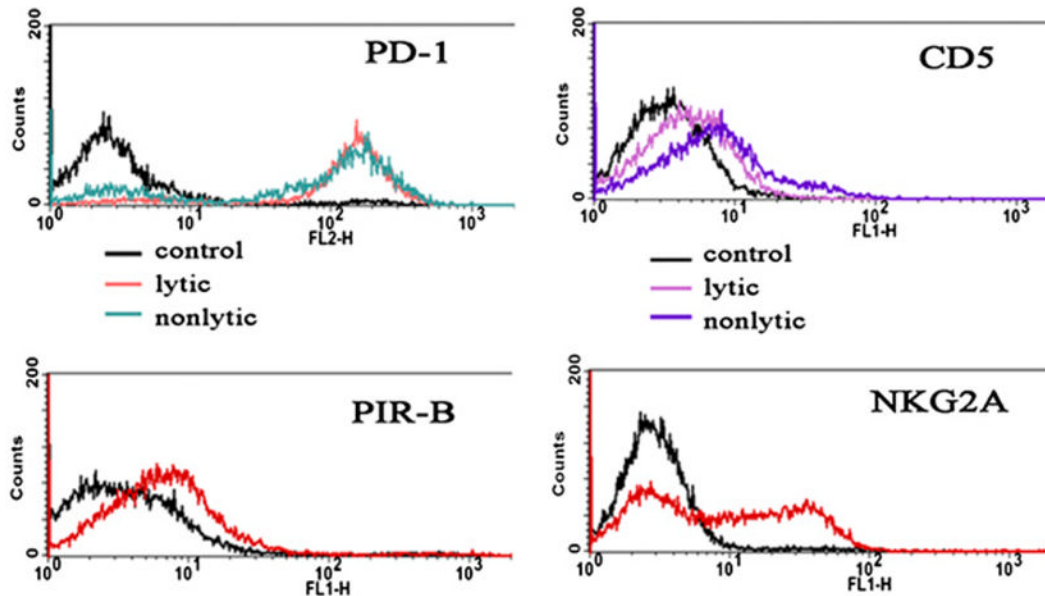


FIGURE 2.

Expression of IRs in nonlytic and lytic MCA38-derived TILs. Splenocytes or TILs were prepared from control or MCA38 tumor-bearing mice as described (42) and analyzed by flow cytometry for expression of the indicated IRs. Isotype-matched nonimmune Ig was used as labeling control, shown in each panel as the black tracing. Staining of nonlytic TILs for expression of additional IRs was made (anti-CD22, anti-CD31, anti-Ly49 [C/I, G2, A, and D], and anti-CD66a) and were consistently negative (dns).

Table I

IR expression in T cells

IR	Family	Ligand	Features	Reference
CD5	Scavenger (cysteine-rich)	gp150, CD72	Has a pseudo-ITAM; regulates FasL/caspase 8 activation; inhibits Ca flux and ERK activation; no PTP involvement	54–58
CD22 (Siglec-2)	Siglec	Sialic acid	Multiple ligands, ITIM	59, 60
CD31 (PECAM-e)	Ig superfamily	CD31, CD38, $\alpha\text{v}\beta_3$ integrin	CD31 is expressed widely, ITIM	61
CD33 (Siglec-3)	Siglec	Sialic acid	ITIM	62
CD38*	Cyclic ADP ribose hydrolase	CD31	No ITIM or PTP involvement, enzyme is soluble in serum, has both + and – function	63
CD66a (CEACAM-1)	Ig superfamily	Multiple	18 genes in family; is expressed widely; multiple splice variants; has both + and – function; those with ITIM are inhibitory; function depends on oligomerization	64–67
CD72	Cysteine-rich	CD5, CD100	ITIM	68
CD73*	Ecto-5'-nucleotidase		GPI-linked, adenosine production from tumor	69
CD85 (PIR-A/B in mice)	LILR	MHC class I	Some family members lack ITIM	70
CD94/NKG2A (KLRD1)	C-type lectin	HLA-E	CD94 has two forms: 39 kDa lacks ITIM, 43 kDa has ITIM (and recruits SHP-1); is expressed on NK cells, memory T cells, and DCs	45
CD152 (CTLA-4)	Ig superfamily	B7-1/2	Also is expressed on Tregs; no ITIM (has YVKM that mediates binding of PI3K, PP2A, and SHP-2); CD276-B7-H3 and CD276-B7-H4 are orphan ligands	23, 71–74
CD155 (TIGIT)	Ig superfamily	Poliovirus receptor	Enhances DC-mediated Treg production; ITIM	75
CD158a	KIR	MHC class I	14 genes in family; different members have + or – function; ITIM	76, 77
CD159a (NKG2A)	Ig superfamily	Pairs with CD94	Is a multigene family; some lack ITIM	78
CD160	Ig superfamily	HVEM	GPI-anchored, lacks ITIM; is expressed on CD44 ⁺ memory cells	79
CD161 (NKR-P1A)	C-type lectin	LLT1	Is expressed on NK and CD8 ⁺ T cells	80
CD169 (Siglec-1)	Siglec	Sialic acid	Lacks ITIM; expressed on macrophage and activated DCs (may be activating on macrophage but inhibitory on DCs)	81
CD170 (Siglec-5)	Siglec	Sialic acid	CD33 related; ITIM	82
CD172a (SIRP-A)	Ig superfamily	CD47	Recruits SHP-1/2; may interact with: Csk, SLAP-130, Grb2; is expressed in DCs and mast cells; some forms are activating; ITIM	83, 84
CD223 (LAG-3)	Ig superfamily	MHC class II	Widely expressed on lymphocytes; is related to CD4; effect is reversed by IL-2	85
CD244 (2B4)	CD2 family	CD48 and CD244	Is expressed on NK cells and T cells; has ITSM; binding to CD48 is inhibitory; binding to CD244 is activating	86, 87

IR	Family	Ligand	Features	Reference
CD272 (BTLA)	Ig superfamily	HVEM	ITIM	88, 89
CD279 (PD-1)	Ig superfamily	PDL-1 (CD274 = B7H1) and PDL-2 (CD273 = B7DC)	Recruits SHP-1/2, ITIM, and ITSM	90, 91
CD300a (IRp60)	Ig superfamily		Widely expressed; ITIM	92, 93
CD305 (LAIR-1)	Ig superfamily	Collagen	Recruits SHP-1/2; may interact with Csk; ITIM	94
CD328 (Siglec-7)	Siglec	Sialic acid	Expressed in myeloid, NK cells, subsets of T cells; ITIM	95
gp49B-1	Ig superfamily	$\alpha\text{v}\beta_3$ integrin	ITIM	96
KLRG1 (MAFA-1)	C-type lectin	Cadherin	Expressed on T cells and NK cells; ITIM	97–99
Ly49	C-type lectin	MHC class I	Part of a multigene family; some are activating; ITIM	100
MGL	C-type lectin	CD45	Expressed on immature DCs	101
Galectin-3	Galectin	β -galactoside	15-member family; intracellular but translocates to surface upon activation	102
β_1 integrin	Integrin	VCAM-1, soluble integrins	Recruits SHP-1; when bound to soluble integrin or ligated with monomeric Ab is inhibitory	103
TIM-3	Ig superfamily	Galectin-9	Multigene family; some activate (e.g., TIM-1); expressed on various immune cell types	104
T cell Src-binding proteins			May sequester p56 ^{lck} away from functional signaling complexes, therein reducing T cell responsiveness	
LAT			Modulates inhibitory feedback loop via Dok2/SHIP binding; homolog (LAB) induced upon activation	105, 106
PAG/Cbp			Ubiquitous; recruits Csk, ERM, RasGAP; also affects Ras	107
LIME			Deletion has only a modest effect on peripheral T and B cells	108, 109
SIT			Interacts with SHP-2	110
TSAd			Rapidly induced in primary T cells by activation	111–113

The common names of receptors are noted in parentheses. CD38 and CD73 (marked with *) are not true IRs per se but are involved in local production of adenosine thought to be immunosuppressive. Although not all IRs contain canonical ITIM elements (e.g., CTLA-4), an ITIM or a functional homolog on the prototypic IR functions to recruit SHP-1 to phosphorylated ITIM and thus into proximity with PTP targets.

Cbp, C-terminal Src kinase binding protein; Csk, C-terminal Src kinase; FasL, Fas ligand; HVEM, herpesvirus entry mediator; KIR, killer Ig-related (or Ig-like) receptor; KLRG1, killer cell lectin-like receptor G-1; LAIR-1, leukocyte-associated Ig-like receptor-1; LAT, linker for activation of T cells; LILR, leukocyte Ig-like receptor; LIME, LCK-interacting molecule; LLT1, lectin-like transcript-1; MAFA-1, mast cell function-associated Ag-1; MGL, macrophage galactose-type lectin; PAG, protein associated with glycosphingolipid-enriched microdomains; TIM-3, T cell Ig and mucin domain-3.