Cellular & Molecular Immunology www.nature.com/cmi

REVIEW ARTICLE OPEN

Novel human immunomodulatory T cell receptors and their double-edged potential in autoimmunity, cardiovascular disease and cancer

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In the last decade, approaches based on T cells and their immunomodulatory receptors have emerged as a solid improvement in treatments for various types of cancer. However, the roles of these molecules in the therapeutic context of autoimmune and cardiovascular diseases are still relatively unexplored. Here, we review the best known and most commonly used immunomodulatory T cell receptors in clinical practice (PD-1 and CTLA-4), along with the rest of the receptors with known functions in animal models, which have great potential as modulators in human pathologies in the medium term. Among these other receptors is the receptor CD69, which has recently been described to be expressed in mouse and human T cells in autoimmune and cardiovascular diseases and cancer. However, inhibition of these receptors individually or in combination by drugs or monoclonal antibodies generates a loss of immunological tolerance and can trigger multiple autoimmune disorders in different organs and immune-related adverse effects. In the coming decades, knowledge on the functions of different immunomodulatory receptors will be pivotal for the development of new and better therapies with less harmful side effects. In this review, we discuss the roles of these receptors in the control of immunity from a perspective focused on therapeutic potential in not only cancer but also autoimmune diseases, such as systemic lupus erythematosus, autoimmune diabetes and rheumatoid arthritis, and cardiovascular diseases, such as atherosclerosis, acute myocardial infarction, and myocarditis.

Keywords: Immunomodulatory receptors; Autoimmune diseases; T cells; Immunotherapy

Cellular & Molecular Immunology (2021) 18:919–935; https://doi.org/10.1038/s41423-020-00586-4

INTRODUCTION

Targeting immunomodulatory T cell receptors has emerged as a successful approach to manipulate the immune system, generating spectacular outcomes in certain diseases, such as cancer. Advances in the knowledge on the mechanisms by which tumors affect the balance between effector and regulatory T cells have revealed new therapeutic strategies for the treatment of autoimmune diseases[1] and are also very promising for cardiovascular diseases.[2] In this review article, we focus on receptors with the main function of triggering immunosuppressive signaling pathways in T cells, mainly programmed cell death protein (PD1), cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), B- and T-lymphocyte attenuator (BTLA), *Lymphocyte-activation gene 3* (LAG-3), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif [ITIM] domain (TIGIT), 2B4 (CD244), and V-domain Ig suppressor of T cell activation (VISTA), all of which belong to the immunoglobulin (Ig) superfamily;[3] CD5, a scavenger-receptor cysteine-rich (SRCR) superfamily receptor; and CD69, a C-type lectin with a role as an immunomodulatory T cell receptor that has been demonstrated in recent years.[4] We summarize the main characteristics of each of the above immunomodulatory T cell receptors, their expression in different T cell subsets, the mechanism of suppression, or negative regulation of T cell activation and associated positive and negative repercussions in the contexts of human autoimmunity, cardiovascular disease, and cancer (Table 1). Moreover, we also discuss the role of the new generation of targeted immunotherapies (immune checkpoint inhibitors, ICIs) in cancer and how checkpoint inhibition is also being adapted to treat autoimmune diseases.[1]

OVERVIEW OF THE IMMUNOMODULATORY RECEPTORS EXPRESSED BY T CELLS

PD1, CTLA-4, and BTLA belong to the CD28 Ig superfamily and share similar protein structures.[5] PD-1 (CD279) is expressed by all T cells during activation, B cells, natural killer, and myeloid cells.[6] PD-L1 and PD-L2 are known ligands of PD-1 expressed by T, B, and antigen-presenting cells. The binding of PD-1 to PD-L1 induces resistance to activation by positive signals from the T cell receptor (TCR) and CD28 in conventional T cells.[6] CTLA-4 (CD165) is expressed on activated $CD4^+$ T cells and competitively binds B7.1 and B7.2, acting as a coinhibitory signal to down-regulate early T cell activation and proliferation.[7] Constitutive CTLA-4 expression on regulatory T (Treg) cells enhances their regulatory function through suppression of antigen-presenting cells.[8] BTLA (CD272) is expressed in single-positive thymocytes,

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Received: 21 August 2020 Accepted: 28 October 2020
Published online: 24 November 2020

Table 1. Pathologies and treatments associated with different T cell immunoreceptors in the fields of autoimmunity, cardiovascular disease, and cancer

Immunoreceptor	T cell type	Disease	Treatment	Ref.
PD-1				
Autoimmunity	T cell	Type 1 diabetes		81
	gd T cell	Psoriasis	Nivolumab	210
	Treg	Inflammatory bowel disease	Anti-PD-L1 monoclonal antibodies: MIH2	230
	Treg	RA	TNF blockade	94
	Treg	MS		231
CVD	T cell	Atherosclerosis		123
	CD8 T cell	Atherosclerosis	Anti-PD-1 monoclonal antibody (clone 29E.2A3, BioLegend)	121
	T cell	DC and myocarditis	Anti-PD1 and anti-PD1L antibodies	152
	Treg	ACS		122
Cancer	$CD8^+$ T cells/Treg cells	Melanoma, lung cancer, kidney cancer, BC, head and neck cancer, urothelial carcinoma, hepatocellular carcinoma, gastric cancer, metastatic Merkel cell carcinoma and Hodgkin lymphoma	Anti-PD-1 mAbs: atezolizumab, pembrolizumab and nivolumab	191,210
	CD8 T cell	AML		232
CTLA-4				
Autoimmunity	CD8 T cell	RA	Fusion protein: CTLA4 Ig	97
CVD	T cell	DC and myocarditis	Anti-CTLA4 antibody	152
		Chronic heart failure		233
Cancer	CD8 T cell	Melanoma	Ipilimumab	178
	T cell	Melanoma, NSCLC, breast cancer, prostate cancer, pancreatic cancer, hepatocellular carcinoma, and mesothelioma	Anti-CTLA-4 mAbs: ipilimumab and tremelimumab	187
LAG-3				
Autoimmunity		MS		234
CVD	CD4 T cells	Cardiac dysfunction in chronic HIV	Anti-LAG-3 immunotherapy	235
	CD4 T cells	CAD		128
Cancer	CD8 T cells	Multiple human cancers, breast cancer, ovarian cancer	Anti-LAG-3 immunotherapy	12,206
	CD8 T cells	Melanoma	Anti-LAG3 mAb: relatlimab	206
	CD8 T cells	Metastatic breast cancer	LAG3-Ig fusion protein: EOC202	206
Tim-3				
Autoimmunity	CD4 T cell, CD8 T cell, NKT cell	RA		12,103
	CD8 T cell	Asthma and allergy		12
	Th1 cell	MS		12
	Treg	Autoimmune hepatitis		236
CVD	CD8	Atherosclerosis	Anti-Tim-3 mAb: F38-2E2	121
Cancer	T cells	Hepatocellular carcinoma		237
	CD8 T cell	Prostate cancer		238
	$CD8^+$ T cells/Treg cells	Hepatocellular, ovarian, colon and cervical carcinomas		237
TIGIT				
Autoimmunity	Treg cell	Type 1 diabetes		12
		MS		12
		RA		12
Cancer	CD8 T cell	NSCLC, colon cancer, melanoma, and myelogenous leukemia		12,88
	Treg cell	Melanoma		88

Table 1. continued

Immunoreceptor	T cell type	Disease	Treatment	Ref.
2B4				
Autoimmunity	CD8 T cell	RA		18
	CD8 T cell	SLE		18
Cancer	CD8 T cell	Multiple myeloma		239
	CD8 T cell	Melanoma, hepatocarcinoma, and AML		239
	CD8 T cell	AML		239
VISTA				
Cancer	CD8 T cells, Tregs	Endometrial and ovarian cancers, solid tumors	Anti-VISTA mAb: JNJ 61610588 (clinical trial)	207
	T cell, Treg	Solid tumors and lymphomas, NSCLC	CA-170 (clinical trial)	207
	T cells	Prostate cancer		131
CD69				
Autoimmunity	T cells	RA		4,111
	CD4 T cells	Type 1 diabetes		90
	T cells	Osteoarthritis		4
	Treg cells	SLE		4,44,68,69
	Th17 cells	Wegener's granulomatosis		4
	Th17 cells	MS		4
	T cells	Optic neuromyelitis		4
	T cells/Treg cells	Autoimmune thyroiditis		4,74
CVD	T cells	MI		149
	Th17/Treg cells	Atherosclerosis		4,32,149
	CD4 T cells	Coronary heart disease		240
Cancer	CD4 T cells	Lung adenocarcinoma		241
	Treg cells	Hepatocellular carcinoma		4
	CD4 T cells	NSCLC		242
BTLA				
Autoimmunity	CD4 and CD8 T cells	RA		243
	$CD4^+$ T cell	Ulcerative colitis		243
	$CD4^+$ T cells	SLE		243
	$CD8^+$ T cells	MS		243
	$CD4^-CD8^-$ T cells	Vasculitis		243
	$CD4^+$ T cells	Vasculitis (Behçet's disease)		243
CVD	Treg	Atherosclerosis		243
Cancer	T cells	Epithelial ovarian carcinoma		243
	$CD8^+$ T cell	Melanoma		243
	T cells	Diffuse large B cell lymphoma		243
	$CD4^+$ T	Hepatocellular carcinoma		243
CD5				
Autoimmunity	T cell	CTCL	Anti-CD5 mAb: T101; and radioimmunoconjugate 90Y-T101	244
	T cells	MS		244
Cancer	T cell	Lung carcinoma		244
	T cell	Melanoma		244

CVD cardiovascular diseases, *mAb* monoclonal antibody, *CD* cluster of differentiation, *NKT* natural killer T, *gd T cell* gamma delta T cell, *Treg* regulatory T cell, *TNF* tumor necrosis factor, *Ig* immunoglobulin, *Th* T helper, *PD1* programmed cell death, *PD1L* programmed cell death ligand, *CTLA4* cytotoxic T-lymphocyte-associated protein-4, *LAG3 Lymphocyte-activation gene 3, TIM3* T cell immunoglobulin and mucin domain-containing protein 3, *TIGIT* T cell immunoreceptor with Ig and ITIM domains, *VISTA* V-domain Ig suppressor of T cell activation, *BTLA* B and T lymphocyte associated, *AML* acute myeloid leukemia, *NSCLC* non-small cell lung cancer, *MS* multiple sclerosis, *SLE* systemic lupus erythematosus, *CAD* coronary artery disease, *RA* rheumatoid arthritis, *DC* dilated cardiomyopathy, *MI* myocardial infarction, *ACS* acute coronary syndrome

mature T cells, B cells, macrophages, and dendritic cells. BTLA maintains T cell immune tolerance[9], and the binding of BTLA to its ligand, herpes virus entry mediator, prevents excessive activation of T cells.[10]

LAG-3 is a transmembrane protein homolog to the CD4 coreceptor that binds to major histocompatibility complex (MHC) class II with high affinity.[11] LAG-3 (CD223) is expressed in activated $CD4^+$ T cells, Treg cells, Tr1 cells, activated $CD8^+$ T cells, natural killer cells, dendritic cells, B cells, and exhausted effector T cells.[12] The interaction of LAG-3 with MHC II leads to decreased proliferation and cytokine secretion by antigen-specific $CD4^+$ T cell clones.[11] T cell immunoglobulin and mucin domain 3 is a transmembrane protein belonging to the Ig superfamily that is expressed in CD4 helper 1 (Th1) cells, CD8 T cytotoxic 1 (Tc1) cells, Treg cells, dendritic cells, natural killer cells, monocytes, macrophages, and mast cells. Galectin-9, the first reported Tim-3 ligand, was shown to induce apoptosis in Th1 cells,[13] and carcinoembryonic antigen cell adhesion molecule 1 forms a heterodimer with Tim-3 to mediate T cell inhibition and exhaustion.[14] TIGIT is a coinhibitory transmembrane protein expressed by regulatory and memory $CD4^+$ T cells, $CD8^+$ T cells, and natural killer cells. The poliovirus receptor (CD155) shared with CD226, poliovirus receptor-related 2 (CD112, also known as Nectin-2), and Nectin4 are the ligands for TIGIT and deliver stimulatory signals.[15,16] TIGIT also blocks T cell activation, proliferation, and maturation by targeting downstream TCR signaling pathways.[17] The signaling lymphocyte activation molecule 2B4/CD244 is a CD2-related receptor expressed in antigen-experienced $CD8^+$ αβ T cells, natural killer cells, dendritic cells, and myeloid-derived suppressor cells (MDSCs).[18] The engagement of 2B4 by its receptor, CD48, contributes to $CD8^+$ T cell exhaustion and cell function inhibition.[19] VISTA is expressed in T cells, natural killer cells, and myeloid cells.[20] VISTA is a member of the B7 family, and although the receptor shares homology with members of the CD28 family, the gene is located on a different chromosome. VISTA can act as both a ligand and a receptor in regulating $CD4^+$ and $CD8^+$ T cell proliferation and cytokine production.[21]

The T cell receptor-inhibiting molecule CD5, also known as Leu-1 in humans, is a transmembrane glycoprotein that belongs to the highly conserved SRCR.[22] CD5 is constitutively expressed on lymphocyte precursors, mature T cells, and B1a cells and is associated with both TCR/CD3 and B cell receptor.[23] The known ligands for CD5 are CD5L, CD72, and CD5 itself, but little is known about their physiological functions. CD5 clusters with the TCRζ/CD3-pMHC complex and inhibits signaling through immunological synapses in thymocytes and peripheral T cells.[24,25]

Last but not least, the early leukocyte activation antigen CD69 is a type II C-lectin membrane receptor[26] that belongs to a family of immunomodulatory receptors involved in the immune response, the NK complex.[4] CD69 is expressed early after cell activation in all hematopoietic cells except erythrocytes[27] and has emerged as a new immunoregulatory receptor expressed by T cells in the last decade.[28] Additionally, a number of ligands for CD69 with the ability to modulate T cell responses through different pathways and triggers have been identified recently. Galectin-1 (Gal-1) binds to CD69, inhibiting naive and helper T cell activation and differentiation,[29,30] and interaction with the S100A8/S100A9 complex is required for the differentiation of regulatory T cells.[31] Oxidized low-density lipoprotein (OxLDL) has been shown to bind specifically to $CD69^+$ human T lymphocytes, enhancing Treg cell differentiation and inhibiting Th17 cells.[32] Interestingly, the CD69 cluster with the aromatic amino acid-transporter complex LAT-1-CD98 in γδ T cells regulates L-tryptophan transport and cytokine secretion in these cells.[33] The immunomodulatory T cell receptors, their ligands, and the cellular functions triggered by signaling through each receptor are summarized in Fig. 1.

OPPORTUNITIES RELATED TO IMMUNOMODULATORY T CELL RECEPTORS IN HUMAN AUTOIMMUNE DISEASES

Autoimmune diseases are the consequence of deterioration or loss of self-tolerance resulting from defects in the thymic elimination of potentially self-reactive T cells (central tolerance) or in the control mechanisms of potentially self-reactive T cells in the periphery (peripheral tolerance). The nature of autoimmune diseases is multifactorial since the mechanisms that trigger these conditions can be genetic, epigenetic, molecular, and/or cellular in origin. However, the generation of pathogenic inflammatory responses in peripheral tissues upon activation of autoantigen-specific T cells is a common denominator in all autoimmune diseases.[34] T cells with a potentially autoreactive TCR escape thymic selection, and powerful mechanisms are required to control these autoreactive T cells and maintain peripheral tolerance.[34] Therefore, approaches targeting immunomodulatory T cell receptors have emerged in recent years as therapeutic opportunities to restore tolerance in autoimmune diseases (Table 1).

Systemic lupus erythematosus (SLE)

The immunomodulatory receptors PD-1, CTLA-4, and BTLA play nonredundant roles in the modulation of central tolerance during the thymocyte selection process and in maintaining peripheral tolerance. We analyzed the performance of these receptors in the development of SLE since patients with this autoimmune disease display abnormal levels of these receptors in T cells with a characteristic hyperactive phenotype. PD-1 has a pivotal role in the negative regulation of positive TCR-α/β thymocyte selection by affecting the threshold of pre-TCR/CD3 complex downstream signaling.[35,36] In addition, *PD-1* gene ablation in mice with the additional *lpr/lpr* mutation also results in the development of lupus-like glomerulonephritis and destructive arthritis with age,[37] which indicates the involvement of this immunomodulatory receptor in the maintenance of peripheral self-tolerance. Different animal models demonstrate that PD-1 expression can be tuned finely with anti-PD-1 monoclonal antibodies (mAbs) to preserve the number of $Foxp3^+$ T cells or reduce the number of $CD4^+PD\text{-}1^+$ T cells and alleviate lupus-like nephritis.[38,39] However, a completely opposite effect was observed with anti-PD-1 mAb therapy in experimental autoimmune encephalomyelitis and NOD diabetes animal models.[40,41] The discovery that the PD-1 pathway modulates T follicular helper cell-mediated humoral immunity by negatively regulating T follicular regulatory (Tfr) cells[42] indicates that PD-1 blockade may preferentially influence the Tfr cell function controlling the development of lupus, reconciling previous contradictory results. In patients with SLE, it has been reported that PD-1 expression levels in $CD4^+$ T cells are very low[43] and the frequency of $PD\text{-}1^+$ cells is greatly decreased. It was found that patients with the PD-1.3 A/G allele have significantly lower expression of PD-1 in $CD4^+$ T cells than other patients. Although PD-1 expression is higher in $CD25^+$ Tregs than in effector T cells, PD-1 expression is significantly lower in SLE patients than in healthy subjects, which leads to functional and numerical reductions in Tregs in SLE patients. This was confirmed in another study in which PD-1 was found to be highly induced in $CD4^+CD25^+$ and $CD4^+CD69^+$ T cells from healthy controls but not in those from patients with SLE.[44] A recent study found elevated levels of coinhibitory IgG autoantibodies against PD-1 in the serum of new-onset SLE patients that were associated with SLE Disease Activity Index (SLEDAI) scores, revealing a new pathway of PD-1 modulation.[45] In contrast, a pilot study with a small number of patients showed that PD-1 expression levels in peripheral blood mononuclear cells (PBMCs) were significantly increased in SLE patients and associated with SLEDAI scores, suggesting that PD-1 inhibitors are useful tools in the treatment of SLE.[46] In summary, these data indicate that both the regulation and aberrant expression of PD-1 play key roles in the regulation of

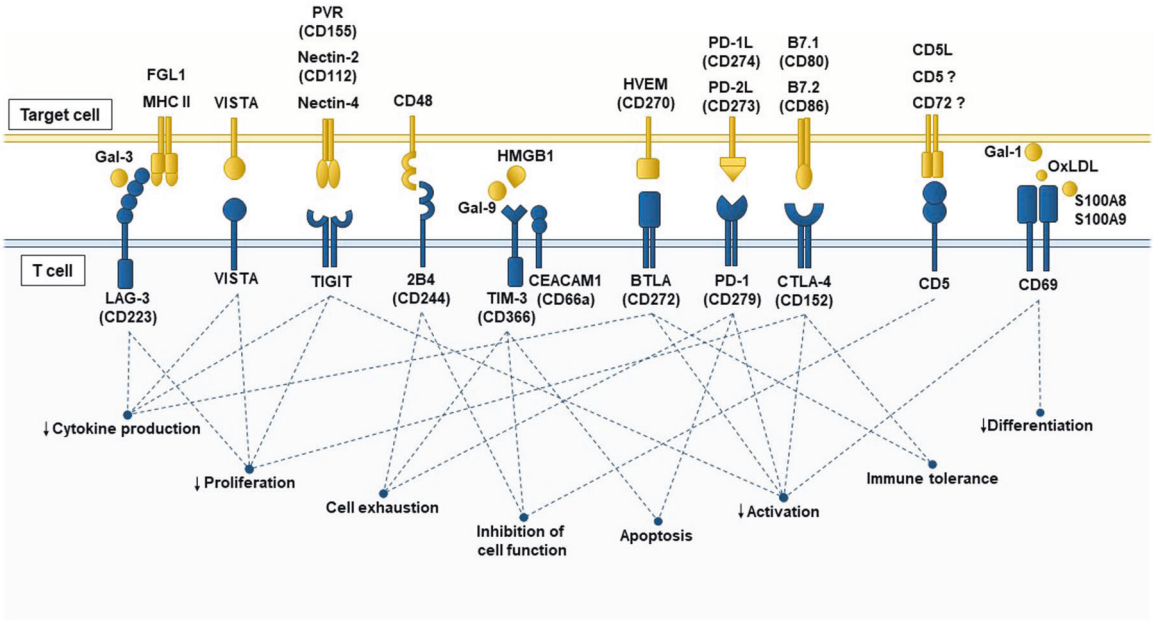

Fig. 1 Membrane receptors with immunomodulatory activity in T cells and their ligands. T cell responses are controlled by multiple inhibitory signals to prevent excessive inflammation that can occasionally cause more damage than repair. T cells express a battery of different receptors with immunomodulatory capacity in the membrane, such as LAG-3, VISTA, TIGIT, 2B4, TIM-3, CEACAM-1, BTLA, PD-1, CTLA-4, CD5, and CD69, which can interact with either soluble ligands or membrane proteins expressed by antigen-presenting cells (APCs), inflammatory cells, tumor cells, or damaged cells. Ligand–receptor interactions induce inhibitory signals in the T cell and sometimes also in the target cell to control inflammation, and the effects include decreasing proliferation, differentiation, activation, cytokine production and cell function and promoting apoptosis, T cell exhaustion and tolerance. LAG-3 *Lymphocyte-activation Gene 3*, MHC II major histocompatibility complex class II, *Gal-3* Galectin-3, *VISTA* V-domain immunoglobulin suppressor of T cell activation, TIGIT T cell immunoreceptor with immunoglobulin and ITIM domains, PVR poliovirus receptor, Tim-3 T cell immunoglobulin and mucin domain 3, CEACAM-1 carcinoembryonic antigen cell adhesion molecule 1, Gal-9 Galectin-9, HMGB1 high-mobility group box 1, BTLA B- and T-lymphocyte attenuator, HVEM herpes virus entry mediator, PD-1 programmed cell death protein, PD-L1/PD-L2 PD-1 ligand 1/PD-1 ligand 2, CTLA-4 cytotoxic T-lymphocyte-associated protein-4, Gal-1 Galectin 1, CD5L CD5 antigen-like, OxLDL oxidized low-density lipoprotein

this autoimmune disease. However, the clinical efficacy of manipulating this pathway requires more basic research and clinical studies. In this regard, an ongoing clinical trial (ClinicalTrials.gov Identifier: NCT03816345) is now testing the efficacy of an anti-PD1 mAb (nivolumab) in SLE and several other autoimmune diseases (Chron's disease, inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis (RA), ulcerative colitis, etc.).

CTLA-4-deficient mice develop lethal lymphoproliferative syndrome and autoimmunity,[47,48] as well as a rapid and severe lupus-like autoimmune syndrome.[49] These mice exhibit global T cell dysregulation promoting systemic humoral autoimmunity that triggers lupus-like autoimmunity.[49] The relevance of the *CTLA* gene in the susceptibility to SLE is supported by evidence from different human populations with different polymorphisms.[50–52] Interestingly, SLE patients display increased expression of CTLA in isolated responder T cells (Foxp3$^-$) compared to healthy controls and patients with other autoimmune rheumatic diseases (RA or psoriatic arthritis).[53] However, these T cells exhibit defective inhibition of T cell activation by CTLA-4 after CD3/CD28 costimulation. CTLA-4 receptors are displaced from membrane microdomains in SLE patients and are unable to regulate the intracellular signaling molecules triggered by T cell activation.[53] Therefore, the receptor CTLA-4 in responding T cells could be a possible target to restore the function of T cells in patients with SLE, and there is in vitro evidence that soluble CTLA-4 from lupus patient PBMCs regulates effector responses.[54] The administration of abatacept, a fusion protein comprising CTLA-4 linked to the Fc portion of IgG1, has been tested clinically in several autoimmune diseases. Abatacept has also been tested in different clinical trials as a therapy for SLE[55] and lupus nephritis[56] patients in randomized studies and, although it showed evidence of biological activity, it did not meet the endpoint criteria of achieving a complete response during treatment.[56]

BTLA expression in effector T cells is associated with SLEDAI scores.[57,58] Lupus patients have a defect in the upregulation of BTLA expression upon activation of CD4$^+$ T cells in comparison with healthy controls.[58] BTLA is recruited to the immunological synapse and acts as an inhibitory receptor that negatively regulates the immune response; this function is defective in SLE patients but can be corrected by restoring intracellular trafficking and lipid metabolism in lupus CD4$^+$ T cells.[58] This gives BTLA characteristics similar to those of CTLA-4 and PD-1, and human anti-BTLA antibodies have been developed;[59,60] however, the potential of BTLA as a possible target in autoimmune diseases has not yet been tested.

CD69-deficient mice are healthy and do not present an autoimmune phenotype,[61] although the expression of this receptor in Treg cells is necessary for the development of natural Treg cells in the thymus[62] and their suppressive activity in inflammatory conditions,[63] indicating that the immunomodulatory receptor CD69 plays a pivotal role in the maintenance of central and peripheral tolerance.[4,26] CD69 expression in Tregs negatively regulates the production of proinflammatory cytokines and is necessary for the suppressive function of these cells in mouse models of autoimmune colitis.[64,65] CD4$^+$CD69$^+$ cell numbers are increased in lupus-prone (NZBxNZW)F_1 mice and pristine-induced SLE mice, and these cells regulate cytokine production by effector T cells.[66,67] SLE patients also present an increased CD69/CD3 ratio in PMBCs compared to healthy controls, which correlates with SLEDAI scores,[68,69] and increased levels of CD4$^+$CD69$^+$TGF-β$^+$IL-10$^+$Foxp3$^-$ Treg cells.[70–72] The CD69$^+$ Treg cell population appears to be increased in a number of

autoimmune and inflammatory disorders.[73–75] However, although increased in number, $CD69^+TGF\text{-}\beta^+$ $Foxp3^-$ Treg cells from SLE patients are not able to inhibit the release of cytokines by autologous lymphocytes, indicating that they do not contribute to the regulation of autoimmunity in these patients.[70] Moreover, SLE patients who do not respond to immunosuppressive therapy have significantly higher levels of P-glycoprotein $(P\text{-gp})^+CD69^+CD4^+$ cells in the blood and renal tissue than responsive patients. P-gp expression in lymphocytes plays a role in the active efflux of intracellular drugs, and the high proportion of $P\text{-gp}^+CD69^+CD4^+$ cells in nonresponsive SLE patients is suggestive of corticosteroid resistance and renal damage.[76] Therefore, CD69 plays a role in regulating the secretion of cytokines and could be a potential target to control SLE in patients refractory to immunosuppressive therapy. Clinical trials need to be conducted to underscore the real value of this molecule as a therapeutic target.

Autoimmune diabetes

The PD-1-PD-L1 interaction has central roles in regulating the initiation and progression of autoimmune diabetes in nonobese diabetic (NOD) mice. PD-1 blockade rapidly precipitates diabetes in prediabetic NOD mice,[41] while overexpression of PD-L1 in pancreatic beta cells prevents diabetes in these animals.[77,78] PD-1 expression is downregulated specifically in $CD8^+$ T cells from type 2 diabetes (T2D) patients,[79] suggesting that immunomodulatory receptors have a role in the development of T2D. Moreover, PD-L1 is expressed in beta cells from type 1 diabetes (T1D) mellitus patients, possibly to attenuate the autoimmune response mediated by type I and II interferons through IRF1.[80,81] Along with animal evidence, these data provide the rationale for developing new therapies to target this costimulatory pathway in this disease without eliminating the protective role of the PD-1–PD-L1 pathway.

In contrast, CTLA blockade only negatively regulates autoimmune diabetes in neonatal NOD mice,[40] and CTLA-4 Ig or anti-B7-2 mAb treatment was shown to reduce the incidence of diabetes in young mice, whereas treatment with any of these reagents had no effect on disease progression in older mice.[82]

Targeting the BTLA pathway in NOD mice with an mAb that selectively depletes pathogenic helper $CD4^+$ T cells increases the proportion of Treg cells and protects against spontaneous disease onset in NOD mice.[83] Adoptive transfer of OVA-specific $CD8^+$ T cells isolated from BTLA-deficient mice into RIP-mOVA-recipient mice (expressing membrane-bound OVA in pancreatic beta cells) induces diabetes.[9] Moreover, BTLA limits γδ T cell numbers and sustains normal γδ T cell subset frequencies by restricting interleukin-7 (IL-7) responsiveness and $CD27^-ROR\gamma t^+$ population expansion.[84] γδ T cells have been implicated in the development of diabetes; however, the putative role of BTLA targeting in clinical autoimmune diabetes treatment has not been addressed. LAG-3-deficient NOD mice exhibit accelerated autoimmune diabetes development mediated by expansion of pathogenic T cell clones in the islets, which are normally restrained by LAG-3.[85] However, the onset of diabetes is accelerated even more in animals doubly deficient in LAG-3 and PD-1, which also develop other autoimmune diseases, such as myocarditis.[86] More recently, LAG-3 has been shown to act by limiting the function and proliferation of Tregs due to enhanced IL-2–Stat5 signaling pathway and Eos expression.[87] TIGIT is a repressor of the CD226-activating pathway that functions by binding the common ligand CD155. This costimulatory axis has also been postulated to be a therapeutic target for the treatment of T1D, as targeting TIGIT would control the suppressive function of Tregs.[88]

Finally, the expression of CD69 has been associated with the development of T2D complicated by coronary artery disease in patients.[89] Although whether there is a causal relationship is unknown, CD69 could exert its function through the regulation of the hypoxia-inducible factor short isoform I.1.[90]

Rheumatoid arthritis

T cells play a central role in the pathogenesis of RA; however, the first attempts to target T cells for therapeutic purposes produced a high risk of infection. Thus, the immunomodulatory receptors on T cells have been explored as better targets for the treatment of this often progressive and destructive chronic joint disease. CTLA-4 deficiency affects both central tolerance and peripheral tolerance as well as Treg-mediated suppression. Mice deficient in CTLA-4 develop severe collagen-induced arthritis,[91] and patients with CTLA-4 mutations show expansion of Treg cells, which leads to subsequent inflammation and autoimmunity probably through the production of organ-specific autoantibodies.[92,93] The therapeutic agent *abatacept* (CTLA-4-Ig) has shown efficacy in a broad spectrum of RA patients from early-stage disease to refractory disease resistant to tumor necrosis factor-α (TNF-α) blockers.[94] *Abatacept* treatment results in significant improvement in the signs and symptoms of RA[95] and in patients with juvenile idiopathic arthritis who did not respond to traditional TNF blockers.[96] Treatment with *belatacept*, an alternative human CTLA-4 Ig, in a phase I/II clinical trial evaluating multiple doses revealed the preliminary efficacy of this drug in the treatment of RA.[97]

BTLA polymorphism is associated with susceptibility to RA,[98] and the expression of BTLA is decreased in T cells from patients with RA.[99] $LAG\text{-}3^+$ Treg cells have been associated with the development of RA, and their frequency is lower in patients with RA than in healthy individuals.[100] Moreover, $LAG\text{-}3^+Foxp3^-$ Tregs are highly effective in relieving joint severity and local and systemic inflammation.[101] TIM-3 is associated with RA disease activity[102] and involved in the immune dysregulation mediated by TNF-α, IL-17, and interferon-γ (IFN-γ) in this disease;[103] thus, TIM-3 may play an important role in the pathogenesis of RA. TIGIT overexpression in vivo improves the severity of RA by decreasing the production of IFN-γ and IL-17, increasing IL-10 cytokine levels and removing anti-collagen II antibodies.[104] $CD4^+CD28^-$ T cells from RA patients overexpress 2B4 together with CD226 and CRACC, suggesting that these receptors could modulate this disease.[105] The roles of VISTA and CD5 in RA have been studied, and the relevant expression of these molecules in the pathology of RA relies on macrophages[106] and B cells,[107] respectively. Signaling by these receptors begins in the innate phase of inflammation, and their roles in T cells during RA need to be explored further. The above data suggest that these immunomodulatory receptors could have therapeutic roles in the development of RA, although at the moment, there are no data on their clinical effectiveness.

CD69-deficient mice show no overt signs of autoimmunity,[97] although they are more susceptible to asthma,[63] contact dermatitis,[108] myocarditis,[109] colitis,[64] and RA[110] than wild-type mice due to exacerbated Th1 and Th17 responses. The role of CD69 was first described in the synovial fluid T cells of RA patients;[111] these T cells fail to express CD25 or produce IL-2, and consequently, they are not able to proliferate properly. Moreover, CD69 expression by synovial T cells in RA patients correlates with disease activity.[112] CD69-deficient mice show a higher incidence and severity of collagen-induced arthritis (CIA), with exacerbated T and B cell responses, than wild-type mice.[110] These mice also show reductions in the levels of tumor growth factor-β1 (TGF-β1) and TGF-β2, which are protective cytokines in CIA, in inflammatory foci and parallel increases in the levels of proinflammatory cytokines, such as RANTES and IL-1β, leading to increased joint inflammation and cartilage and bone erosion. The immunomodulatory receptor CD69 expressed by T cells regulates the immune response through control of TGF-β production[26] and regulates the differentiation and activation of Treg and Th17 cells through control of the Stat5/miR-155/SOCS1 (ref. [62]) and Jak3–Stat5 pathways,[28] respectively. CD69 is also a hallmark of tissue-resident memory T cells, together with CD103 and CD49a, with

direct implications on this pathology and autoimmune diseases in general.[113] However, the potential of this receptor as a possible therapeutic tool in RA has not been explored in the clinic.[4] The potential roles of T cell immunoreceptors and their involvement in autoimmune pathologies are outlined in Fig. 2.

The efficacy of these immunomodulatory receptors in modulating autoimmune diseases is experimentally associated with certain changes in immune markers, which can be exploited as biomarkers. Some of the receptors mentioned above may indicate the immune process that is taking place due to different treatments. In the next few years, more research will be needed to decipher whether these receptors should be exploited not only to predict the efficiency of treatments and identify autoimmune patients that could possibly benefit from the treatments but also to predict possible adverse effects of treatments.

IMMUNOMODULATORY T CELL RECEPTORS IN CARDIOVASCULAR DISEASE

Increasing numbers of clinical trials have been conducted in patients with acute myocardial infarction (MI) and heart failure, such as cardiomyopathy and myocarditis, using a variety of cell types, including bone marrow stem cells, mesenchymal stem cells, and cardiac resident stem cells. However, none of these preclinical trials or studies have yielded clear results in terms of regeneration of cardiac tissue. In this scenario, immunomodulation has gained interest for its use not only as an adjuvant in cellular therapies but also as cardioprotective therapy in different cardiovascular diseases. T cell responses are important in the onset, progression, and resolution of acute myocardial events and chronic heart failure.[114] Here, we review the roles of immunomodulatory T cell receptors in different cardiovascular diseases, including atherosclerosis as the trigger of MI.

Immunomodulation in atherosclerosis treatment

T lymphocytes are present during all stages of development in human atherosclerotic lesions,[115] although their role in the disease has been controversial for decades. Increasing evidence demonstrates the modulatory role of the immune system in experimental and clinical arteriosclerosis. The most recent and definitive evidence comes from the CANTOS (Canakinumab Antiinflammatory Thrombosis Outcome Study) clinical trial in which anti-inflammatory treatment was found to be associated with reduced cardiovascular risk.[116] These works provide strong evidence that immunomodulatory therapies have therapeutic potential in the development and management of clinical atherosclerosis and other chronic inflammatory conditions. Even statins have been seen to regulate certain immune molecules, such as modulating MHC II through arterial cells and restraining T cell responses[117] through IFN-gamma.[118] T cell-mediated immunity plays a significant role in atherosclerosis development.[119] The role of $CD4^+$ T cells is quite complex; Th1 cells have a proatherogenic role, while Treg cells protect against disease development. However, the roles of Th2, Th17, and follicular helper T cells are still controversial. $CD8^+$ T cells have pleiotropic effects on the development of atherosclerosis; they can induce an inflammatory response via inflammatory cytokines, and cytotoxic activity towards endothelial cells increases the progression of atheroma plaques, whereas the same cytotoxic activity towards macrophages and regulatory $CD8^+$ T cell subsets can inhibit inflammatory responses and atherosclerosis progression.[120] Interestingly, $CD8^+$ T cell function is regulated by the PD-1 and Tim-3 signaling pathways in atherosclerosis.[121] In humans, the $CD8^+PD\text{-}1^+Tim3^+$ T cell subset, which is enriched in central memory T cells, is abundant in patients with atherosclerosis presenting with increased antiatherogenic cytokine production and decreased proatherogenic cytokine production. In patients with acute coronary syndrome, the expression of PD-1 and PD-L1 on circulating T cells is very low.[122,123] Blockade of $CD8^+PD\text{-}1^+Tim3^+$ T cells increases TNFα and IFNγ production. Ab-mediated inhibition of TIM-3 was shown to aggravate atherosclerosis by limiting efferocytosis and T cell responses in mice.[124] In parallel, pretreatment of apolipoprotein-deficient (apoE−/−) mice with CTLA-4-IgG can reverse disease acceleration, blocking the signaling pathway in T cells and T cell activation,[125] whereas CTLA-4-blocking antibodies strongly increase atherosclerotic lesion numbers,[126] and the overexpression of transgenic CTLA-4 in apoE−/− mice enhances Treg-mediated suppression and prevents atherosclerosis development.[127] Together, these studies demonstrate that CTLA-4 limits plaque development by inducing anti-inflammatory T cell responses, positioning CTLA-4 as a promising target in atherosclerosis.

The lipoprotein scavenger receptor BI (SCARB1) rs10846744 noncoding variant has been associated with atherosclerosis, independent of traditional cardiovascular risk factors, and with LAG3. A novel study proposed plasma LAG3 as an independent predictor of HDL-C levels and coronary heart disease risk.[128]

BTLA was recently described to be mostly expressed on B cells in patients with cardiovascular disease and in follicular B2 cells in Ldlr−/− mice fed a high-fat diet. However, the use of an agonistic anti-BTLA antibody was shown to inhibit atherosclerosis development in an animal model with significant increases in regulatory B and T cell numbers,[129] indicating an immunomodulatory effect mediated through T cells in atherosclerosis.[129] These findings suggest BTLA as a new promising target for the treatment of atherosclerosis.

Treatment with agonistic anti-TIGIT antibodies inhibits $CD4^+$ T cell responses, although this treatment does not affect atherosclerotic lesions in LDLr−/− mice fed a high-fat diet.[130] These data suggest that myeloid cells can increase their activity by crosstalk with T cells, negatively influencing disease. Similarly, VISTA is a receptor and a ligand with immunosuppressive effects on IFNγ and TNFα in both T cells and macrophages,[131] which adopt an anti-inflammatory M2 phenotype that can also play a role in atherosclerosis development.[132]

Although CD5 is expressed in T cells from atherosclerotic lesions of apoE−/− and ldlr−/− mice,[133] the role of CD5 expression in T cells during disease development is unknown. However, the role of CD69 in atherosclerosis was described recently.[32] An interaction between CD69 and OxLDL was identified to be responsible for the anti-inflammatory phenotype of T cells needed to restrain plaque development. The binding of OxLDL to CD69 in mouse and human T cells induces the expression of NR4A1 and NR4A2, members of the NR4A subfamily of human nuclear receptors involved in regulatory T cell differentiation.[134] CD69 depletion in the lymphoid compartment of ldlr−/− mice results in reduced expression of NR4A1 and NR4A3 in T cells in mice fed a high-fat diet. The inhibition of these signaling pathways favors the development of Th17 cells, promoting a proinflammatory environment and accelerating the development of atheroma plaques.[32,135] In addition, the expression of the CD69 and NR4A1 genes in peripheral blood cells was studied in a cohort of subjects with exhaustive characterization of subclinical atherosclerosis belonging to the *Progression of Early Subclinical Atherosclerosis* (PESA) study,[136] including in healthy and asymptomatic individuals. The data showed that CD69 and NR4A1 mRNA levels decreased with the progression of the atheroma plaque, validating the data from the in vivo model. Analysis of the CD69 receptor in the PBLs of this cohort showed that the loss of CD69 expression was very significantly associated with the development of atheroma plaques, even when this association was corrected for other cardiovascular risk parameters, such as the levels of OxLDL. Further studies on the new regulatory OxLDL/CD69 pair in

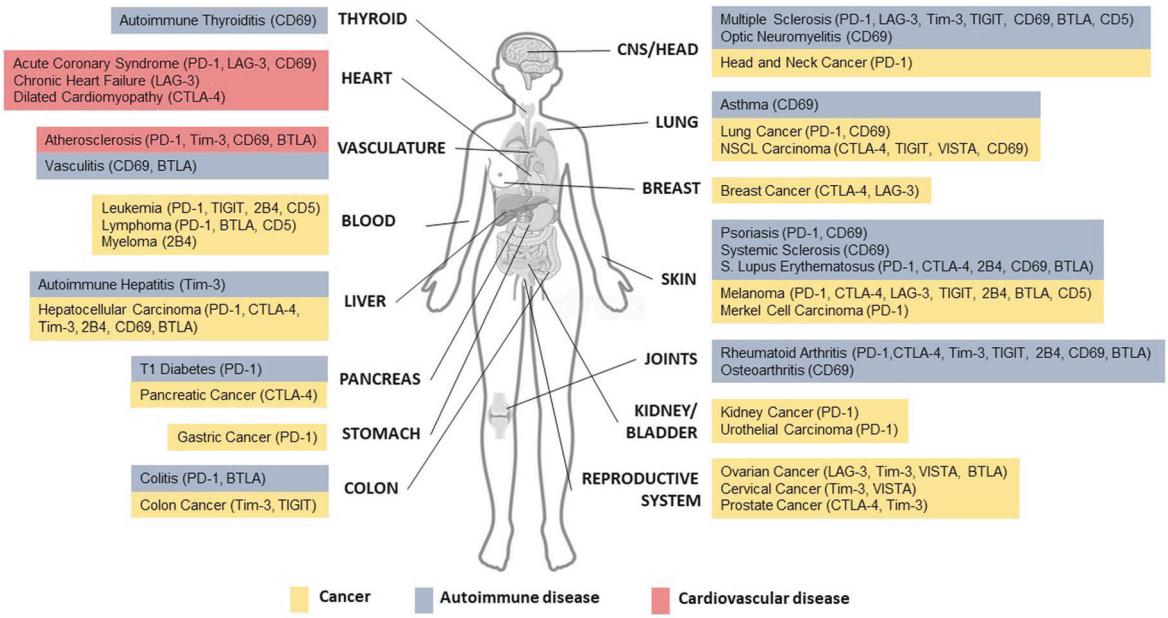

Fig. 2 Contributions of inhibitory receptors on T cells to human autoimmune diseases (blue), cardiovascular diseases (red), and cancer (yellow) in different organs. Signaling through immunomodulatory membrane receptors is pivotal to promoting immune tolerance and avoiding excess deleterious T cell activation and reactivity. Activation or inhibition of immunomodulatory receptors on T cells may be beneficial or detrimental, depending on the disease context. In different cancer types, immunotherapy based on the blockade of these inhibitory receptors leads to increased and relatively long-lasting T cell responses against tumor cells, resulting in efficient tumor clearance. However, autoimmunity due to self-reactivity, which is related to excessive T cell activation, is the main side effect of cancer immunotherapy. Additionally, dysfunction of inhibitory receptors on T cells is linked to different autoimmune disorders, such as thyroiditis, vasculitis, hepatitis, type 1 diabetes, colitis, multiple and systemic sclerosis, optic neuromyelitis, asthma, psoriasis, systemic lupus erythematosus, rheumatoid arthritis, and osteoarthritis. Favoring signaling through these receptors has emerged as a strategy to restore tolerance in autoimmune patients. The T cell response is also an important mediator of atherosclerosis and acute and chronic myocardial disease development and progression. Thus, different immunomodulatory receptors on T cells are therapeutic candidates in cardiovascular diseases. This schematic summarizes the immunomodulatory T cell receptors (in parentheses) that have been implicated in different human diseases

human lymphoid cells during atherosclerotic disease progression will provide novel insights into targeting these pathways for the prognostic evaluation/treatment of cardiovascular diseases.

Acute myocardial infarction

MI results from occlusion of the coronary arteries, mainly due to a thrombus caused by rupture of a lipid atherosclerotic plaque, which causes ischemia in a region of the heart. Ischemia causes subsequent cardiomyocyte death and triggers inflammatory and repair mechanisms. In recent years, multiple studies have highlighted the roles of T cells not only in the development of atherosclerosis but also in the progression after MI, contributing to cardiac function and remodeling.[137] Since immunomodulatory T cell receptors are also checkpoints in the inflammation underlying atherosclerosis, ICI therapy may create an increased risk for atherosclerotic cardiovascular events, such as MI, in cancer patients.[138] Acute cardiovascular manifestations derived from atherosclerosis appear progressively after several years of subclinical disease. Although ICI therapy has been implemented in the clinic for the past decade, it may be too early to have a clear picture of the effect of ICIs on the incidence of acute MI. However, in recent cumulative studies, MI has been reported to be a complication in patients treated with ICIs, with an incidence ranging from 1 to 3%.[139,140] Some studies indicate that MI occurs less than a year after ICI treatment, suggesting that immunomodulatory therapy can accelerate the progression and instability of pre-existing atherosclerotic lesions rather than cause de novo plaque formation.[141] An analysis of coronary plaques suggested that the T cell proportion was higher in ICI-treated patients than in untreated patients,[142] consistent with an exacerbated T cell response after ICI therapy in the coronary arteries, a possible trigger of acute events.[142]

Beyond their roles in atherosclerotic plaque instability and rupture, some immunomodulatory receptors have been proposed to be involved in regulatory mechanisms limiting the degree of inflammation after myocardial ischemic events. Variants of the PD-1-[143] and CTLA-4-encoding genes[144] are associated with an altered risk of MI,[145] and proteomic profiling analysis identified CD5 antigen-like and others as marker proteins associated with new-onset atherosclerotic cardiovascular disease risk.[145] PD-1 expression is upregulated in peripheral leukocytes when they increase in number during the first hours after MI and then decrease after reperfusion. In contrast, lower levels of PD-1 expression in T cells are associated with larger infarction lesions.[146]

Furthermore, analyses of the peripheral blood phenotype of MI patients revealed that circulating $CD4^+$ T cells overexpress CD69 (ref. [147]) and that regulatory T cells overexpress CTLA-4 early after MI.[148] T cells show evident CD69 expression not only in the peripheral blood but also in the culprit coronary artery plaque.[149] This evidence suggests that anti-inflammatory molecules are rapidly stimulated after infarction to prevent excessive inflammation and damage, although little is known about the particular mechanisms underlying these processes.

Myocarditis and dilated cardiomyopathy

Inflammation of the myocardium, or myocarditis, can be caused by infectious or noninfectious triggers. Self-reactive $CD4^+$ T cells are a common feature of different types of myocarditis. These cells recognize cardiac antigens and increase the inflammatory response. If inflammation persists, cardiomyocyte death results

in the loss of myocardial tissue and fibrosis, leading to the development of dilated chronic cardiomyopathy.[150] Thymic epithelial cells present self-antigens to immature T lymphocytes to deplete the lymphocytes with self-reactive potential. Interestingly, some cardiac antigens, such as alpha myosin heavy chain (αMyHC), are poorly represented in the thymic epithelial cells (TEC) repertoire of autoantigens. As a result, heart-specific autoreactive T cells escape thymic negative selection.[151] These naive T cells can recognize cardiac autoantigens presented by dendritic cells in the lymph nodes that drain the heart, but their activation may be dampened by peripheral tolerance mechanisms. Peripheral tolerance includes suppression by Treg cells and immunomodulatory receptors or immune checkpoints. Dysfunction or inhibition of immune checkpoints can cause activation and expansion of these self-reactive T cell clones. In addition, a storm of costimulatory signals occurring in the context of heart infection or tissue damage can contribute to overcoming peripheral tolerance and triggering autoimmune responses to the heart.[152] The potential roles of T cell immunoreceptors and their involvement in cardiovascular diseases are outlined in Fig. 2.

CARDIOVASCULAR IMMUNE-RELATED ADVERSE EVENTS (IRAES) OF IMMUNOMODULATORY RECEPTOR THERAPY

Myocarditis is one of the irAEs of ICI therapy for cancer. Patients with ICI-related myocarditis have T cell infiltration of the myocardium usually accompanied by a severe clinical presentation, such as a decreased ejection fraction, cardiogenic shock, severe arrhythmia, and advanced atrioventricular block.[153–155] The lack of myocarditis-specific biomarkers makes diagnosis difficult, so only severe cases are usually reported, which could explain the observed low incidence and high mortality. Approximately 0.1–1.1% of patients treated with ICI therapy develop myocarditis, although myocarditis is fulminant in up to 50% of these patients. According to different studies, the time to onset of symptoms of ICI-induced myocarditis varies between 1 and 2.5 months after the start of treatment with an ICI.[154,156,157]

ICI-induced myocarditis has been described in patients administered an anti-PD-1 antibody, an anti-CTLA-4 antibody or a combination of both,[154] although additional studies with larger sample sizes should be performed to attribute specific myocardial adverse effects to each ICI.

Preclinical data indicate critical roles for the PD-1/PD-L1 and CTLA-4 pathways in regulating autoimmune responses to the heart. Depletion of functional PD-1 in BALB/c mice but not in BALB/c Rag2−/− mice leads to the spontaneous development of fatal autoimmune dilated cardiomyopathy,[158] suggesting a critical role for PD-1 in the regulation of cardiac autoimmunity mediated by lymphoid cells. Subsequent studies have described the increased susceptibility of PD-1-deficient mice to experimental autoimmune myocarditis, which is established by immunization with an αMyHC peptide.[159] Mice lacking CTLA-4 die within the first month from severe lymphoproliferative disease with involvement of the heart and development of fulminant myocarditis.[160] In addition, the administration of anti-PD-1 plus anti-CTLA-4 combination therapy to nonhuman primates results in the development of multiple organ toxicities, including myocarditis, with cardiac infiltration of mononuclear cells consisting primarily of T cells and with a composition similar to that observed in patients with ICI-induced myocarditis.[161] PD-L1 was first described as highly expressed in the mouse heart.[162] In addition, different studies have identified PD-L1 expression in the myocardium of patients with ICI-induced myocarditis.[153] Other studies have shown that the cardiac endothelium positively regulates PD-L1 in response to IFNγ as a mechanism of tissue-based tolerance and T cell depletion[163] in a negative feedback loop in the heart to maintain resistance to local effector T cell-mediated responses.

The exact mechanism of ICI-induced myocarditis development remains unclear. A suggested explanation is that T cells may recognize homologous antigens in the heart and tumors by a molecular mimicry mechanism.[153] Specific muscle antigens, such as troponin and desmin, were found in tumor biopsies, supporting the idea that the same T cell clones can detect antigens present in both the myocardium and tumors. Consistently, myocarditis secondary to treatment with ICIs is more common when there is also autoimmune involvement of the skeletal muscle or myositis.[164] As an alternative explanation, T cell clones that recognize different antigens can undergo aberrant activation. As discussed above, some T cell clones that recognize cardiac epitopes escape negative selection in the thymus, so when peripheral tolerance mechanisms are blocked by immunotherapy, the expansion of a heart-specific T cell response can be triggered.[151]

Although CD69 involvement in human disease remains unexplored, preclinical studies indicate that a lack of other immunomodulatory T cell receptors increases susceptibility to myocarditis and dilated cardiomyopathy. CD69 deficiency leads to exacerbated Th17-mediated autoimmune myocarditis and subsequent cardiomyopathy after immunization with the αMyHC peptide.[109] Although LAG-3 deficiency alone does not induce cardiac autoimmunity, depletion of LAG-3 in PD-1-deficient mice increases the susceptibility to spontaneous T cell-mediated fulminant myocarditis.[86] Finally, administration of an anti-Tim-3 antibody exacerbates male myocardial inflammation in a mouse model of viral myocarditis.[165,166] Taken together, this evidence suggests that multiple inhibitory signals in T cells prevent cardiac autoimmunity.

ICIS IN CANCER

In recent years, there has been a large increase in the development and implementation of cancer immunotherapies. FDA approval of the use of blocking antibodies specific for CTLA-4 (ipilimumab) or PD-1 (nivolumab) in humans and biologics such as CTLA-4-Ig (abatacept) has been key to producing significant improvements in the treatment of various types of cancer, especially melanoma. Unlike radiotherapy and chemotherapy, which are intended to directly interfere with the growth and survival of tumor cells, immunotherapies target tumors indirectly, favoring an increase in antitumor immune responses that arise spontaneously in many patients. ICI therapy could therefore be used to modulate the immune response to improve the treatment of many types of cancer (Fig. 2).

Cancer mechanisms to bypass the immune system

To understand the modes of action of ICIs, it is important to recognize the dynamic interaction between cancer and the immune system that occurs during the course of the disease. Cancer cells are genetically unstable, which contributes to their uncontrolled proliferation and expression of antigens that the immune system can recognize. These antigens include normal proteins overexpressed by cancer cells and new proteins that are generated by mutation and genetic rearrangement.[167] Cytotoxic $CD8^+$ T cells are particularly effective in mediating antitumor immune responses by recognizing tumor-specific antigens presented by MHC I. $CD8^+$ T cells become licensed effector cells after appropriate stimulation by antigen-presenting cells, which collect antigens at the tumor site. In addition to displaying antigenic peptides presented on MHC molecules, antigen-presenting cells must provide costimulatory signals via surface receptors, such as CD28, and cytokines, such as IL-12, for effective stimulation of T cells.[168]

Tumor cells adopt a variety of mechanisms to prevent immune recognition and immune-mediated destruction. Established

tumors arise through the selection of clones that can evade the immune system, a process known as immunoediting.[169] Tumor cells can evade immune recognition directly by inhibiting molecules that make them vulnerable, such as tumor antigens or MHC class I.[170] Alternatively, tumors can evade immune responses by taking advantage of mechanisms the body has developed to prevent immunopathologies. The mediators involved in these mechanisms include inhibitory cytokines, such as IL-10 and TGF-β; inhibitory cell types, such as Tregs, regulatory B cells, and MDSCs; metabolic modulators, such as indoleamine 2,3-dioxygenase; and immunomodulatory receptors, such as PD-1 and CTLA-4.[171,172]

Immune exhaustion also contributes to immune dysfunction in cancer. Originally described in the context of chronic viral infection, where the host cannot eliminate the pathogen, it is now evident that exhausted T cells can also promote cancer development.[173,174] Under these conditions, the persistent high antigenic load leads T cells to regulate immunomodulatory receptors, whose signaling subsequently leads to a progressive loss of proliferative potential and effector functions and, in some cases, exhausted T cell elimination.[175] Therefore, exhaustion is a physiological mechanism designed to limit immunopathology during persistent infection and a major obstacle to antitumor immune responses.[176]

Anti-CTLA4 treatment

CTLA-4 blockade has been shown to promote T cell activation and intratumoral Treg cell depletion.[177] Ipilimumab (IgG1) and tremelimumab (IgG2) are the two human anti-CTLA-4 antibodies that have undergone clinical evaluation. In a 2010 phase III clinical trial, in previously treated patients, treatment with ipilimumab at a dose of 3 mg/kg with or without administration of a gp100 peptide vaccine was evaluated and compared to peptide vaccine administration alone, finding an improvement in overall survival (OS) in both groups of patients who received ipilimumab, which led to the approval of ipilimumab for patients with metastatic melanoma.[178] A similar test was performed with a combination of dacarbazine with or without ipilimumab at a dose of 10 mg/kg, which improved survival but increased liver toxicity.[179] In addition, a pharmacokinetic analysis showed that ipilimumab had linear pharmacokinetics in the dose range of 3–10 mg/kg.[180] The efficacy of ipilimumab was validated in a randomized, double-blind, phase III trial after complete resection of high-risk stage III melanoma.[181] Studies in other types of cancer have shown the efficacy of ipilimumab in some renal cell carcinoma patients, including patients who did not respond to other immunotherapies.[182] In patients with B cell lymphoma, CTLA-4 blockade with ipilimumab had antitumor activity and was well tolerated at doses of 1 and 3 mg/kg;[183] however, a phase III trial did not show a significant difference in terms of survival between the ipilimumab group and the placebo group in patients with castration-resistant metastatic prostate cancer.[184] Tremelimumab, an alternative antibody that blocks CTLA-4, continues to be investigated in clinical trials and has also demonstrated long-lasting responses and acceptable tolerability in patients with melanoma,[185] refractory metastatic colorectal cancer,[186] hepatocellular carcinoma,[187] or malignant mesothelioma.[188]

Anti-PD1 treatment

PD-1 regulates the activation of T cells in peripheral tissues and is expressed in activated T cells, Treg cells, activated B cells, and NK cells. The endogenous PD1 ligands, PD-L1 and PD-L2, are expressed in activated immune cells and nonhematopoietic cells, including tumor cells; tumor cell expression is the mechanism by which these cells circumvent the immune system[189]. Inhibition of these interactions with therapeutic antibodies improves the T cell response and stimulates antitumor activity.[162]

The first anti-PD-1 inhibitor evaluated was nivolumab (BMS-936558), a human mAb (IgG4) that blocks the immunomodulatory receptor PD1.[190]

A phase I trial in which different doses were tested found that nivolumab is safe and produces positive responses in 16–31% of pretreated patients across all types of solid tumors tested, with a duration of at least 1 year.[191] For ipilimumab, both adverse effects and efficacy depend on the dose, but for nivolumab, these correlation are not observed, which can be explained by the high correlation between the receptor and antibody even at low doses.[192]

Pembrolizumab (MK-3475) is an alternative humanized high-affinity IgG4 antibody recognizing PD1. It has been evaluated in several phase I clinical trials, and three different doses have been tested in patients with metastatic melanoma who were never previously treated and in a cohort patients including patients previously treated with *ipilimumab* and those who were not previously treated with *ipilimumab*. Most of the adverse effects observed were seen with the highest dose, and no significant differences among the three treatment subgroups were, but patients with a smaller tumor burden had a greater capacity to respond to pembrolizumab.[193]

Pidilizumab (formerly CT-011) is a humanized anti-PD-1 IgG1 antibody. It was one of the first anti-PD-1 agents used in cancer patients. Several clinical trials at different stages, which treated patients with hematological malignancies or metastatic melanoma, have shown that treatment is generally well tolerated, with no treatment-related deaths, but in some patients, previous treatment with ipilimumab did not produce an advantage in treatment response. Pidilizumab appears to be associated with lower response rates in melanoma than nivolumab or pembrolizumab, but the 1-year OS rate is similar to that reported in studies of nivolumab.[193,194]

Anti-PD-L1 treatment

MPDL-3280A is an anti-human IgG1 antibody engineered to block PD-L1, with the Fc domain of IgG1 mutated to completely nullify the effects of antibody-dependent cellular cytotoxicity and complement-dependent toxicity. Furthermore, PD-L1 is particularly expressed in tumors infiltrated by immune cells, which implies that this treatment mainly benefits patients with more inflamed tumors.[193] This antibody exerts its mechanism of action by blocking PD-1/PD-L1 signaling, and unlike antibodies that block PD-1, those that block PD-L1 avoid the side effects that result from blocking the interaction of PD1 with PD-L2 and that of PD-L1 with CD80. *MPDL3280A* has been evaluated in multiple tumor types, with safety and preliminary efficacy identified in melanoma, renal cell carcinoma, non-small cell lung carcinoma, colorectal cancer, gastric cancer, and squamous cell carcinoma of the head/neck. Other PD-L1-inhibiting antibodies are *BMS-936559*, which has been shown to be safe and clinically active in various types of tumors in phase I clinical trials, and *MEDI-4736*, which is currently in clinical development.[191]

Combination therapies

Blocking immune checkpoint molecules with multiple inhibitory antibodies can improve treatment efficacy. For example, for ipilimumab, the percentage of patients with a reduction in tumor size or the ORR (objective response ratio) achieved with monotherapy was 6%, and the OS time was 10 months.[178] In phase I work, nivolumab had an ORR of 31%, and the OS time was 17 months. However, in a phase I trial combining ipilimumab with nivolumab, the ORR was 53% at the maximum tolerated dose, and all subjects who responded to treatment showed a ≥80% decrease in their tumor burden after 12 weeks of treatment.[195] Patients treated with this combination therapy showed a more frequent rate (53%) of adverse effects. This combination, and other

combinations of mAbs, should be confirmed in other phase III clinical trials, which are currently underway.[191] Combination therapy with other noninhibitory immune checkpoint agents is also being evaluated. The feasibility of other combination therapy strategies is being explored in different trials, each with compelling preclinical justification. The most direct combination therapy approach would be a combination of immunotherapy and chemotherapy, as there is a multitude of preliminary data from patients who have been included in clinical trials evaluating immunoregulators after an insufficient response to conventional chemotherapy; in particular, there are treatments that combine ipilimumab with cycles of chemotherapy with carboplatin, etoposide or paclitaxel in patients with lung cancer[196–198] and advanced melanoma.[199]

The combination of ICIs with alternative treatments that favor systemic stimulation of the immune system, such as vaccines, cytokine therapy, and adoptive cell therapy,[200–202] would be an interesting approach. The combination of immunomodulatory agents with agents producing a local effect that causes tumor regression, such as radiotherapy, is based on the *abscopal* effect, which describes the phenomenon of tumor regression outside the irradiated field caused by the release of cytokines and antigens induced by irradiation that enhances the systemic immune response against the tumor. The combination of radiotherapy with ipilimumab has been evaluated in phase I/II trials in prostate cancer, which showed an improvement in treatment response with tolerable adverse effects.[203] Similar to the combination with radiotherapy, other combinations are being investigated with different alternatives strategies for local control, such as cryoablation and electrochemotherapy. The *abscopal* effect is also observed after treatment with cytotoxic therapies, such as B-Raf protein or VEGF inhibitors, capable of stimulating the immune system by favoring the production of antigens.[202,204] Some of these studies, such as one combining ipilimumab with GM-CSF, showed an improved survival rate with few side effects for the patients treated with the combination therapy compared to those treated only with ipilimumab. The combination of ipilimumab with bevacizumab, a VEGF-blocking antibody, improves the immune responses against tumors with tolerable toxicity. However, some combined therapies, such as treatment with vemurafenib and ipilimumab, produce high liver toxicity.[205] In summary, the use of combined therapies seems to be a step forward in the search for a cure for cancer, but more studies are needed to adjust the doses very precisely and reduce adverse effects.

The dual blockade of LAG-3/PD-1 was recently identified to be clinically relevant and is being tested in clinical trials. A recombinant fusion protein with four extracellular domains of LAG-3 has been tested safely in combination with pembrolizumab or nivolumab in patients with melanoma with progressive disease after immunotherapy.[206] Similarly, the combination of anti-VISTA molecules with anti-PD-1 therapy is under investigation. Combination of CA-170, an oral inhibitor of VISTA, with PD-1 has shown positive results in different clinical trials.[207]

ICI-based therapies have been able to increase the average life expectancy of cancer patients, yet mortality remains high among patients with advanced-stage disease, highlighting the need for further innovation in the field. These therapies appear to be most effective in patients with pre-existing antitumor immunity, suggesting that, in patients without such immunity, these drugs cannot mediate de novo antitumor immune responses. It would therefore be interesting to implement de novo generation of antitumor immunity with these treatments. To achieve this objective, it is essential to improve the understanding of the mechanisms of action of these treatments to identify ways to improve their use not only through specific guidance in certain types of patients who are more likely to respond but also through a correct combination with other therapies that will increase the responses of patients in whom these treatments are less efficient. Furthermore, as the understanding of the mechanisms of action of these therapies improves, it will also be easier to prevent possible adverse effects caused by these therapies.

ICIS AND IRAES

Although there is no doubt that treatments targeting these immunomodulatory T cell receptors have created a new era in cancer treatment and increased patient survival, different adverse events related to the immune system have been observed. These adverse events are called irAEs and are directly related to the mechanisms of action of PD-1 and CTLA-4.[139] Under physiological conditions, these molecules prevent autoimmunity and limit activation of the immune system, but inhibition of these two receptors as a cancer therapy causes a wide range of side effects that resemble autoimmune reactions. Analysis of sera and biopsies from patients with irAEs has revealed that these reactions are mediated by activated $CD4^+$ and $CD8^+$ T cells infiltrating tissues, as well as by increases in the levels of proinflammatory cytokines.[208] IrAEs can impact multiple organs and systems, including the skin, gastrointestinal tract, liver, endocrine system, eyes, kidneys, nervous system, pancreas, and heart. Almost all patients treated with ICIs experience mild side effects, such as diarrhea, fatigue, itching, rash, nausea, and decreased appetite. Apart from myocarditis mentioned earlier in this review, serious adverse reactions include severe diarrhea, colitis, increased alanine aminotransferase levels, inflammatory pneumonitis, and interstitial nephritis.[209] Patients who experience exacerbation of pre-existing autoimmune conditions, such as psoriasis,[210] or who develop new autoimmune conditions, such as T1D mellitus,[211] have also been reported. Particularly serious side effects may require cessation of treatment, although these patients may still respond thereafter.

Anti-CTLA-4 antibody-induced IrAEs are common but generally low grade, although severe and life-threatening cases have also been reported. The skin and gastrointestinal tract are more frequently affected, while hepatic, endocrine, and neurological events are less common.[212] Rash is generally the first irAE to manifest after the first or second dose of ipilimumab.[213] Colitis and diarrhea are the most common gastrointestinal IrAEs after the first few months of ipilimumab treatment, which can be very serious, particularly in rare cases where perforation of the gastrointestinal tract occurs. A phase III trial in patients with advanced melanoma demonstrated that ipilimumab adverse events were mild, with sporadic life-threatening cases.[214] Anti-CTLA-4 antibodies can induce a severe and extensive form of inflammatory bowel disease, suggesting the need to avoid nonsteroidal anti-inflammatory drugs in patients treated with anti-CTLA-4 therapy.[215] Hepatic irAEs are rare and may manifest as an acute hepatitis or biliary pattern, which can be reversed with corticosteroids.[216] In addition, autoimmune hypophysitis has been reported in up to 17% of patients with melanoma or renal cell carcinoma treated with anti-CTLA-4 therapy, which may be related to pituitary gland enlargement and hormonal deficiencies.[217] The risk of irAEs in the case of ipilimumab treatment depends on the dose, and the mean time to onset of adverse effects is 10 weeks.[218]

In anti-PD-1/PD-L1 treatment, 30–40% of patients experience skin toxicities.[219] The most common skin toxicities are lichenoid reactions, vitiligo, pruritus, and eczema. Vitiligo is observed only in patients with metastatic melanoma. Antibodies targeting the PD-1/PD-L1 axis have shown a favorable toxicity profile in preliminary trials with rates of relatively severe adverse events of 13% in patients receiving *MK-3475*, 9% in patients receiving *BMS-936559*, and 14% in patients receiving *nivolumab*. A unique and life-threatening toxicity for these agents is pneumonitis, which was the cause of death in three patients (1%) in a phase I clinical trial of nivolumab despite corticosteroid treatment.[219] Cardiac irAEs

due to PD-1 axis blockade have attracted attention due to the high mortality rates but have an incidence of less than 1%. Endocrinopathies derived from PD1 axis blockade include hypophysitis, thyroiditis, hypothyroidism, hyperthyroidism, and T1DM. In addition to the previously described pathologies, there are some rare ICI-mediated toxicities that have neurological, ocular, or hematological manifestations.

The incidence of adverse effects is higher in patients treated with combination therapies, who are more susceptible to acute kidney injury than those treated with ipilimumab, nivolumab, or pembrolizumab monotherapy. Up to 7% of people treated with anti-PD-1 monotherapy and up to 33% of patients treated with combination anti-PD-1 and anti-CTLA-4 therapy develop hepatitis.[220] Combined treatment with these ICIs causes a decrease and blockade in Treg cell function, which also produces other pathologies that are included within the irAEs, such as increases in the incidence of proatherogenic lesions or pneumonitis. Another event considered an irAE is the generation of autoantibodies, as occurs in thyroiditis, since treatment with PD1 inhibitors can promote the mobilization of pre-existing autoantibodies against the thyroid.[220] The study of the pathophysiology of these adverse effects and toxicities should produce a more precise understanding of the functions of ICIs in the tumor and tissue contexts and thus favor the improvement of therapies, with avoidance of possible adverse effects.

Autoimmune diabetes mellitus is related to blockade of CTLA-4, PD-1, its ligand (PD-L1), and combination ICI therapy.

Recently, it has been demonstrated that a constitutive reduction in CTLA-4 signaling does not accelerate SLE in a susceptible NZM2328 animal model.[221] Although a patient with de novo SLE nephritis after ipilimumab treatment was reported,[222] SLE patients treated with ICIs do not seem to exhibit disease worsening.[223] De novo cases of SLE have yet to be reported in patients treated with other ICIs.

In general, the knowledge of possible biomarkers in the field of immunotherapy is very limited in aspects related to predicting the efficiency and suitability of different treatments. The field of biomarkers for predicting possible adverse effects induced by immunotherapy is even less investigated. Therefore, the development of the field of biomarkers to cover the negative aspects of immunotherapy and potentially predictand the suitability of treatments will be very important.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

T cells are key in the development and control of inflammatory diseases of autoimmune origin or cancer; very recently, it has been shown that they also play key roles in pathologies such as acute MI, atherosclerosis, and myocarditis, highlighting the relevance of T cells in cardiovascular disease.[224] Different molecular tools blocking immunomodulatory receptors on T cells have emerged as some of the most promising cancer therapies in the last decade. However, the potential of targeting immunomodulatory T cell receptors in other diseases, such as autoimmunity or cardiovascular disease, which is the leading cause of death worldwide,[225] has been little explored. In addition to the most well-known receptors in clinical practice, PD-1 and CTLA-4, there is a battery of immunomodulatory receptors whose immunoregulatory properties have been described more recently, but their therapeutic potential in clinical practice remains unexplored. In contrast, other T cell receptors, such as CD69, have roles in the development of autoimmunity and in cardiovascular disease in mice and humans, and blockade of this receptor has antitumor properties;[226] however, its role in human cancer has never been addressed.[227] In this review article, we have summarized the mechanisms of action of these immunomodulatory receptors expressed by T cells, paying special attention to the less explored receptors, including their potential development in the clinic for treatment of cancer, cardiovascular disease, or autoimmune diseases, such as diabetes, lupus, or RA.

The use of inhibitory drugs and mAbs against these immunomodulatory receptors can be equally effective in cancer and autoimmune diseases. An example is *abatacept* (CTLA-4-Ig), which is used mainly in melanoma, but recent clinical trials show that it has potential in the treatment of autoimmune diseases, such as RA, in patients refractory to treatment with TNF-α blockers, indicating the potential of immunomodulatory receptor-based therapies in several pathologies that have not yet been explored. In this regard, the integration of technologies to deliver and control drug or mAb release has become one of the more important challenges in the field of immunotherapy. The strategies to deliver these drugs to the target organ are almost as important as the mechanism of action. For this reason, research in nanotechnology, one of the known strategies to improve the nanodelivery of chemotherapeutic agents, is becoming of interest in the field of immunotherapy.[228] In addition, the development of cellular therapies (e.g., CAR T cells) as targeted vehicles for use with regulatory molecules and/or combination with immunotherapy is a trend that we will see developed in the coming years and that, hopefully, will further improve the therapeutic potential not only in cancer but also in autoimmune and cardiovascular diseases.[229] In parallel, we need to advance the knowledge on the molecular and cellular mechanisms triggered by these receptors in each tissue, especially to prevent the triggering of adverse immune effects associated with these therapies in the most vulnerable tissues.

The field of biomedicine faces a challenge in the coming years, in which the study of the immunomodulatory receptors of T cells in the context of numerous pathologies can represent a great step forward in the treatment of fatal or chronic diseases that are highly prevalent in the world and lack cures. The enormous success of targeting the immune checkpoints PD-1 and CTLA4 in cancer should be enough to encourage the scientific community to study the other immunomodulatory T cell receptors in depth in the coming years and bring therapies related to these receptors closer to clinical practice in autoimmune and cardiovascular diseases.

ACKNOWLEDGEMENTS

This study was supported by competitive grants from the Ministerio de Ciencia, Innovación y Universidades, through the Carlos III Institute of Health-Fondo de Investigación Sanitaria (PI19/00545) to P.M.; CIBER Cardiovascular (Fondo de Investigación Sanitaria del Instituto de Salud Carlos III and cofunding by Fondo Europeo de Desarrollo Regional FEDER) to P.M.; and CAM (S2017/BMD-3671-INFLAMUNE-CM) from the Comunidad de Madrid to P.M. The CNIC is supported by the Instituto de Salud Carlos III (ISCIII), the Ministerio de Ciencia e Innovación and the Pro CNIC Foundation and is a Severo Ochoa Center of Excellence (SEV-2015-0505). R. B.-D. is supported by the Formación de Profesorado Universitario (FPU16/02780) program of the Spanish Ministry of Education, Culture and Sports.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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