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The risks of targeting co-inhibitory pathways to modulate pathogen-directed T cell responses

Helge Frebel and Annette Oxenius

Institute of Microbiology, ETH Zurich, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

The identification of T cell co-inhibition as a central mechanism in the regulation of adaptive immunity during infectious diseases provides new opportunities for immunotherapeutic interventions. However, the fact that T cell activity is frequently downregulated during pathogen-directed responses suggests a pivotal physiological role of co-inhibitory pathways during infectious disease. Reports of exacerbated immunopathology in conditions of impaired co-inhibition foster the view that downregulation of T cell activity is an essential negative feedback mechanism that protects from excessive pathogen-directed immunity. Thus, targeting co-inhibitory pathways can bear detrimental potential through the deregulation of physiological processes. Here, we summarize recent preclinical and clinical interventions that report immune-related adverse events after targeting co-inhibitory pathways.

T cell co-inhibitory pathways as therapeutic targets

Co-signaling is an essential component in T cell activation and differentiation [1,2]. The integration of co-stimulatory and -inhibitory signals during T cell receptor engagement determines the functional capacity of T cells during immunity and tolerance. Several co-inhibitory receptors of the immunoglobulin superfamily such as programmed death (PD)-1 or cytotoxic T lymphocyte-associated antigen (CTLA)-4 were initially identified as crucial for the establishment and maintenance of peripheral tolerance due to their suppression of self-reactive adaptive immune responses [3,4]. Co-inhibitory receptors were also assigned increasing relevance in the regulation of immune responses against altered self and foreign antigen. In cancer, various tumors exhibit high expression levels of co-inhibitory ligands which correlate with poor prognosis [5,6]. Tumor-infiltrating T cells express multiple co-inhibitory receptors that compromise tumor-specific T cell responses [7,8]. In infection, co-inhibitory pathways modulate T cell immunity against all major classes of pathogens, that is, viruses, bacteria, protozoa, fungi, and nematode parasites [9–13]. However, the immunomodulatory function of co-inhibitory pathways has been most extensively investigated in the context of persistent virus infections where they contribute to the downregulation of antiviral effector functions [14–16].

In view of accumulating studies that suggest a profound role of T cell co-inhibition in tumor establishment and pathogen persistence, the interference with co-inhibitory pathways represents a promising approach to enhance protective immunity. Therefore, the benefit of treatment with blocking antibodies that target co-inhibitory receptors or ligands has been investigated in numerous animal models and clinical studies [6,17]. Despite the documented therapeutic potential of co-inhibitory pathway interference during various infectious diseases [17], distinct studies have reported adverse events or overall detrimental consequences when interfering with T cell co-inhibition (Table 1). In line with these reports, our own findings suggest a vital role of T cell co-inhibition in the protection from immune-mediated tissue damage during persistent virus infection [18]. Our results and those of others highlight the release of negative immunoregulation to bear considerable detrimental potential in the form of severe immunopathology.

Here, we discuss the potential risk factors and considerations for the therapeutic targeting of co-inhibitory pathways during infectious diseases. We suggest that therapeutic approaches should take into account the biology of the pathogen and the elicited disease, the biology of the targeted co-inhibitory pathway, as well as specific characteristics of the individual being treated (Figure 1).

Pathogen and disease biology

Despite promising preclinical results from studies using diverse types of pathogens, several aspects of pathogen biology might contraindicate interference with co-inhibitory pathways. For example, programmed death ligand (PD-L)1 blockade prior to *Listeria monocytogenes* infection negatively affects antibacterial CD8 T cell responses [19]. The suppression is suggested to result from increased nitric oxide (NO) production by macrophages after PD-L1 blockade [20]. As macrophage-derived NO is a hallmark of infections with intracellular bacteria, PD-L1 blockade might also compromise T cell responses against other intracellular bacteria. Accordingly, deficient PD-1–PD-L1 signaling precipitates increased mortality after infection with the intracellular bacterium *Salmonella typhimurium* or *Mycobacterium tuberculosis* [21,22]. Increased mortality is attributed to impaired T helper (Th)1 development in the former but to immunopathology due to excessive Th1 responses in the latter infection model. Despite this divergent impact of impaired co-inhibition, the immune responses elicited by

Corresponding author: Oxenius, A. (oxenius@micro.biol.ethz.ch).

  http://dx.doi.org/10.1016/j.it.2012.12.002

Table 1. Adverse events reported for interfering with co-inhibitory pathways during infectious diseases.

		Interference with co-inhibitory			
	Pathogen	**receptor**	**ligand**	**Adverse event**	**Refs**
Viruses	LCMV (persistent)	PD-1	PD-L1	Fatal vascular pathology	[9,18,29]
			PD-L1	Increased destruction of splenic stroma	[28]
			PD-L1 (+high-dose anti-4-1BB)	Increased apoptosis of virus-specific CD8 T cells	[49]
	SIV	CTLA-4 (+vaccination)		Abolished effect of prophylactic vaccination	[47]
		CTLA-4 (+ART + IDO)		Fatal pancreatitis	[50]
	HSV-1		PD-L1	Aggravated herpetic stromal keratitis	[31,78]
			gal-9	Aggravated ocular lesions	[79]
	Adenovirus	PD-1		Aggravated liver pathology	[26]
	Coronavirus (gliatropic)		PD-L1	Aggravated axonal bystander damage	[27]
	Theiler's murine encephalomyelitis virus		PD-L1	Aggravated CNS inflammation and demyelination	[80]
	MHV-3	PD-1		Increased mortality	[38]
	Coxsackievirus B3	TIM-3		Aggravated acute myocarditis	[39]
	Hepatitis C virus		PD-L1 (+CD137L)	Partial inhibition of virus-specific CD8 T cells	[48]
	Influenza virus		PD-L1	Impaired virus-specific CD8 T cell response	[52]
Bacteria	*L. monocytogenes*		PD-L1	Reduced expansion of antibacterial CD8 T cells	[19]
	S. typhimurium		PD-L1	Increased mortality	[21]
	M. tuberculosis	PD-1		Fatal lung pathology	[10,22]
	Citrobacter rodentium	HVEM	CD160	Increased mortality	[70]
	Str. pneumoniae	HVEM		Increased mortality	[70]
	Chlamydia muridarum		PD-L1 (+anti-TIM-3)	Aggravated upper genital tract pathology	[81]
Protozoa	*P. yoelii (strain Py-lethal)*	CTLA-4		Increased mortality	[24]
	Plasmodium berghei	CTLA-4		Increased fatal cerebral malaria	[32]
	P. berghei (in BALB/c mice)	CTLA-4		Increased fatal cerebral malaria	[30]
			PD-L1	Increased fatal cerebral malaria	[30]
	L. mexicana		PD-L2	Aggravated cutaneous lesions	[41]
	L. donovani	HVEM	LIGHT	Decreased hepatic parasite control	[45]
Nematodes	*T. cruzi*		PD-L2	Increased parasitemia	[42]

these intracellular bacteria shared the protective function conferred by PD-1–PD-L1 signaling.

Viral infection renders host cells targets for T cell-mediated killing. Hence, viral tropism can be relevant when interfering with co-inhibitory pathways, because the blockade of T cell co-inhibition can enhance cellular cytotoxicity and might affect vital organ functions due to enhanced killing of infected yet indispensable cell types [18,23]. Of note, this consideration is only relevant for infections with noncytopathic viruses that leave infected host cells viable. Furthermore, strain-dependent variations in pathogen virulence also seem to impact the benefit of targeting co-inhibitory pathways [24]. Although CTLA-4 blockade during infections with strain Py17NL of *Plasmodium yoelii* results in improved parasite clearance devoid of overt immunopathology, treating mice infected with the more virulent strain Py-lethal leads to exacerbated inflammation and increased mortality.

In addition to pathogen biology, several infection-related parameters should be considered when interfering with co-inhibitory pathways. The progression of persistent lymphocytic choriomeningitis virus (LCMV) infection is decisive for the therapeutic impact of PD-L1 blockade: treatment during early infection elicits fatal immunopathology, whereas treatment during established persistent infection enhances antiviral T cell responses without the development of adverse events [9,18]. Moreover, CTLA-4 blockade is without effect if conducted from day 3 of *P. yoelii* (strain Py-lethal) infection, but starting treatment on day 0 post-infection significantly increases mortality [24]. Analogously, PD-L1 blockade from day 0 of *L. monocytogenes* infection compromises antibacterial T cell immunity, whereas a slight delay of treatment enhances antibacterial T cell function [19]. The above studies highlight disease progression as an important factor for co-inhibitory interference, because even minor changes in injection schedules render treatments ineffective or detrimental.

The LCMV model system also suggests that the extent of pathogen dissemination is crucial for the success of co-inhibitory pathway blockade. Mice infected with a rapidly controlled and therefore locally defined low inoculum dose of LCMV docile remain asymptomatic after early PD-L1 blockade. By contrast, high-dose infected mice in which the virus disseminates systemically succumb to lethal immunopathology after early PD-L1 blockade [18]. Studies of *M. tuberculosis* infection also highlight the importance of evaluating co-stimulatory pathway interference *in vivo*, because enhanced effector functions observed *in vitro* with

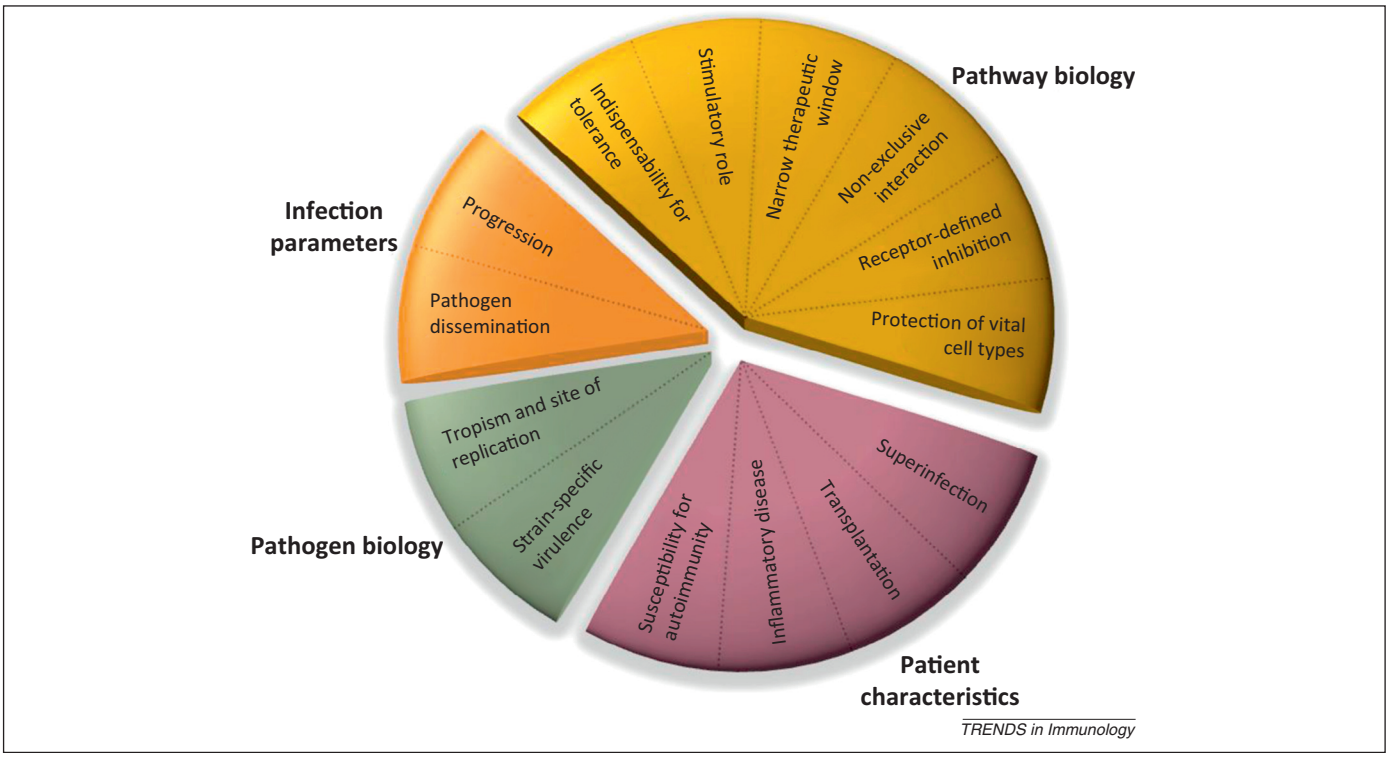

Figure 1. Based on studies evaluating the therapeutic potential of co-inhibitory pathway-targeting treatments during infectious diseases, 14 factors were highlighted that can influence the benefit of this immunotherapeutic intervention. The factors were assigned to four nonredundant categories: infection parameters, pathogen biology, pathway biology, and patient characteristics.

human T cells after PD-1 blockade translate into fatal immunopathology in *M. tuberculosis*-infected PD-1 deficient mice [22,25].

Taken together, pathogen-specific factors such as cell tropism and strain-specific virulence, as well as infection-related parameters such as disease progression and pathogen dissemination, can determine the therapeutic benefit of co-inhibitory pathway interference.

Co-inhibitory pathway biology

Ligand and receptor expression patterns define the physiological role of co-inhibitory pathways during pathogen-directed immune responses. Specific cell types protect themselves or nearby cells from T cell effector functions by upregulating co-inhibitory ligands. PD-L1 expression on hepatic endothelial and Kupffer cells has been reported to inhibit locally T cell effector functions, which protect from aggravated liver pathology during adenovirus infection [26]. PD-L1 expression on oligodendroglia in the central nervous system (CNS) of demyelinating coronavirus-infected mice suppresses T cell effector functions and might offer protection from fatal axonal bystander damage [27]. Moreover, PD-L1 upregulation on fibroblast reticular cells dampens the destruction of the splenic architecture during persistent LCMV infection [28]. In the same infection model, PD-L1-deficiency on cells of nonhematopoietic origin elicits fatal immunopathology 6–7 days after infection [29].

Although ligand expression patterns define the type and localization of protected target cells, co-inhibitory receptor expression determines the cell types and effector functions subject to negative regulation. Hence, interrupting signaling through a specific receptor can manifest in enhanced effector functions in specific cell types, which might be considered for the design of therapeutic approaches. The immunopathological processes observed in the LCMV and coronavirus system have been attributed to excessive pathogen-directed CD8 T cell responses [18,27,29]. Likewise, increased mortality after PD-L1 or CTLA-4 blockade during virulent malaria infection results from increased interferon (IFN)-γ production by CD8 T cells [30]. During herpes simplex virus (HSV)-1 and *M. tuberculosis* infection, impaired PD-1–PD-L1 signaling leads to pathogenic proinflammatory cytokine secretion of CD4 T cells [22,31]. Also, CTLA-4 blockade increases CD4 T cell expansion associated with significantly enhanced mortality during experimental blood-stage malaria [32]. Although most of the above studies have focused on the capacity of T cells to degranulate and produce IFN-γ, elevated levels of the proinflammatory mediators tumor necrosis factor (TNF) and interleukin (IL)-6 are frequently observed systemically in treated mice [14,22,27,30]. Conflicting results have been published on the role of co-inhibitory receptors in the development and function of follicular helper T (Tfh) cells. PD-1-deficiency compromises the capacity of Tfh cells to promote germinal center formation after immunization with nitrophenol-chicken gamma globulin (NP-CGG), and naïve PD-1 knockout mice exhibit reduced fecal IgA levels due to a skewed Tfh cell cytokine profile in the gut [33,34]. However, PD-L1-deficient mice mount enhanced Tfh cell and humoral responses during *Schistosoma mansoni* infection, and the simultaneous blockade of PD-L1 and lymphocyte-activation gene (LAG)-3 boosted pathogen-specific Tfh cell and IgG production during *P. yoelii* infection [35,36]. Although all of these studies shared a positive

effect of impaired PD-1–PD-L1 signaling on the total numbers of Tfh cells, they significantly differed with respect to its impact on antibody production.

The blockade of co-inhibitory pathways might also impact innate immunity. PD-1 is expressed on activated natural killer (NK) cells, and to a lesser degree, on monocytes and myeloid dendritic cells [37]. Thus, impaired PD-1–PD-L1 signaling might also enhance the effector functions of innate immune cells. Exacerbated pathology in PD-1-deficient mice after murine hepatitis virus (MHV)-3 infection could be driven by increased IFN-γ secretion of NK cells [38]. Blocking T cell immunoglobulin mucin 3 (TIM-3) during early coxsackievirus B3 infection decreases the expression level of co-stimulatory molecules on macrophages and mast cells, as well as CTLA-4 expression on regulatory T cells, which is associated with aggravated infection-induced inflammatory heart disease [39].

When interfering with receptor–ligand interactions, the possibility of multiple interaction partners must be taken into account. One receptor can interact with multiple ligands and vice versa, and thus blocking strategies could elicit several concerted maybe even opposing effects. PD-1 is known to interact with PD-L1 as well as PD-L2 and, in turn, PD-L1 with PD-1 and CD80 [40]. Given that PD-1–PD-L1 and PD-1–PD-L2 interaction can exert opposing effects on pathogen-directed immune responses, as seen during *Leishmania mexicana* and *Trypanosoma cruzi* infection [41,42], blocking of a specific ligand might be preferred to targeting the receptor in order to achieve the desired effect. Likewise, the TNF receptor family member herpesvirus entry mediator (HVEM) is known to interact with LIGHT (homologous to lymphotoxins, inducible expression, competes with HSV glycoprotein D for HVEM, a receptor expressed on T lymphocytes), lymphotoxin (LT)α, CD160 and B and T lymphocyte attenuator (BTLA). Interactions with the two former molecules are described to be co-stimulatory, whereas the two latter interactions are co-inhibitory [43,44]. The interaction of HVEM with LIGHT is pivotal for the clearance of hepatic *Leishmania donovani* infection [45]. In the same infection model, the binding of LIGHT to a different receptor, LTβR, inhibits parasite-directed T cell responses during early infection, suggesting opposing roles of LIGHT within one model system due to interactions with distinct molecules.

Interfering with specific co-stimulatory pathways can have a narrow therapeutic window in which the magnitude of enhanced immune responses improves pathogen control without exceeding a threshold of pathogenicity. This concept is supported by the finding that immunopathology is less severe in *M. tuberculosis*-infected PD-L1-deficient mice than in PD-1-deficient mice [22]. The absence of PD-L1 expression is probably compensated by other ligands and therefore leads to an only modest increase in T cell responses, whereas PD-1-deficiency may lead to a stronger release of T cell inhibition and thus more severe pathology.

The view that immune-related adverse events result from an overeffective release of co-inhibition also questions the usefulness of simultaneous targeting of multiple co-inhibitory pathways or their inhibition combined with immunostimulatory treatments. Although promising results have been obtained in murine model systems of persistent viral infection [46] and prophylactic vaccination [47], adverse events have been reported. Treatment of human HCV-specific T cells with PD-L1 blocking antibody and stimulating CD137L *ex vivo* partially inhibit T cell responses due to overstimulation [48]. In line with this, the simultaneous application of PD-L1 blocking and high-dose agonistic anti-4-1BB antibody during persistent LCMV infection leads to only transiently enhanced virus-directed CD8 T cell responses, after which T cells are overstimulated and driven into apoptosis [49]. Furthermore, in simian immunodeficiency virus (SIV)-infected macaques, combining antiretroviral therapy with simultaneous CTLA-4 blockade and indoleamine 2,3-dioxygenase (IDO) inhibition elicits lethal pancreatitis [50].

Contrary to the classical function of co-inhibitory pathways, their signaling can stimulate or sustain pathogen-directed immune responses in distinct conditions and contraindicate its interruption. The upregulation of PD-L1 on T cells prolongs cell survival and enhances protective immunity after ovalbumin (OVA) immunization [51]. Blocking PD-L1 on T cells during *in vitro* priming enhances their production of IFN-γ, which in turn impairs T cell expansion due to increased IFN-γ-driven NO secretion by co-stimulating macrophages [20]. Moreover, PD-L1 deficiency impairs Th1 responses against *S. typhimurium* [21]. PD-L1 expression on T cells is required for efficient dendritic cell maturation during influenza virus infection [52]. PD-L1 blockade on dendritic cells is also reported to compromise their co-stimulatory capacity during acute HSV-1 infection [53], whereas PD-L2 blockade on macrophages decreases NO generation and thus aggravates parasitemia during *T. cruzi* infection [42].

T cell co-inhibition is required for the establishment and maintenance of peripheral tolerance [54]. It therefore is possible that interfering with co-inhibitory pathways has an impact on peripheral tolerance. Although this remains to be investigated in detail, several studies, mostly from the field of human cancer immunotherapy, provide some insight. Clinical evaluations of CTLA-4 blockade in patients with advanced-stage melanoma have shown several immune-related adverse events (irAEs), including enterocolitis, as a result of dysregulated intestinal immunity [55]. Additional irAEs documented during CTLA-4 targeting therapies include endocrinopathy, nephritis, hepatitis, and dermatitis [56]. A single dose of PD-1 blocking antibody elicited inflammatory colitis or hypothyroidism in two out of 39 advanced solid tumor patients [57]. Recent data from a trial evaluating the safety and activity of PD-1 blockade in a larger cohort of advanced solid tumor patients have documented adverse events of potential immune-related origin including hypothyroidism, hepatitis, and diabetes mellitus in 39% of treated individuals [58]. A concurrently performed study assessing analogous aspects of PD-L1 blockade in solid tumor patients reproduced some of the above irAEs, while also observing colitis, pneumonitis, hypophysitis, and thyroiditis [59]. Although most of the reported adverse events were categorized as non-severe, they illustrate the potential of temporary pathway blockades to break immunological tolerance.

In summary, co-inhibitory ligands confer pivotal protection to vital cell types, the blockade of specific receptors releases the inhibition of distinct effector functions, and multiple interaction partners within the co-inhibitory/-stimulatory network, and thus inhibition, might counteract the desired effect or lead to adverse effects. Treatment approaches also require taking into account narrow therapeutic windows, potential immune-stimulating roles of co-inhibitory pathways and their indispensability for maintaining peripheral tolerance.

Patient characteristics

Considering the pivotal role of co-inhibitory pathways in protecting from excessive immunity, patient history, genetic predisposition for specific autoimmune diseases, and the current state of health might be important when interfering with co-inhibition. Deficient co-inhibitory signaling can contribute to the spontaneous or induced development of autoimmune diseases [3,4], and thus certain preconditions would contraindicate pathway blockades. Individuals prone to develop or suffering from diabetes could experience early onset or aggravated disease as reported for anti-PD-L1 treated prediabetic NOD mice [60]. Likewise, patients displaying acute or chronic inflammatory diseases, for example, in the intestine or joints, could be regarded as high-risk patients in view of co-inhibitory pathways contributing to the control of excessive inflammation at these sites [61,62]. Individuals with atherosclerosis or an increased potential to develop inflammatory disorders of the vascular system could suffer from early onset or exacerbated disease, similar to how PD-1–PD-L1 signaling-deficient mice are predisposed to such pathologies [63,64]. Defective PD-1 signaling is also associated with progressive multiple sclerosis in humans and early onset or aggravated experimental autoimmune encephalomyelitis (EAE) in mice, and thus individuals with preconditions for inflammatory CNS pathology may be at additional risk [65,66].

Data from rodent models suggest that co-inhibitory pathways play a role in transplant tolerance and therefore contraindicate co-inhibitory pathway blockade in transplant recipients [67,68]. Pathway interference could also have an impact during superinfections. Although this has not been investigated in detail, considering the high prevalence of *M. tuberculosis* superinfection among HIV-positive individuals and the detrimental impact of PD-1-deficiency on disease severity in the *M. tuberculosis* mouse model, targeting the PD-1–PD-L1 pathway to reinvigorate T cell responses during HIV infection might induce adverse immunopathological events [22]. PD-L1 pathway blockade might also be discontinued in case of influenza virus superinfection, because PD-L1-deficiency can impair influenza virus-specific T cell responses [52]. Recent studies have documented the therapeutic effect of HVEM–CD160 signaling blockade during persistent virus infection, but its requirement for protecting against excessive immunity during pathogenic *Escherichia coli* and *Streptococcus pneumoniae* infection also renders a superinfection with latter pathogens during pathway blockades tenuous [69,70]. Finally, there only are very limited insights into the modulatory role of co-inhibitory pathways during recall responses as studied for secondary LCMV infection [71].

In summary, individuals prone to develop autoimmunity, suffering from acute or chronic inflammatory diseases or having received transplants can be at risk when treated with co-inhibition-targeting therapies. The acquisition of super- or secondary infections during pathway blockades might bear the potential to elicit detrimental adverse events.

Therapeutic indications

Clinical settings of infectious diseases where the blockade of co-inhibitory pathways does not provoke significant adverse events should be considered for this therapeutic approach. In this context, treating local infections should be preferred to systemic diseases, because possible immune-related side effects would be locally confined and easier to compensate or antagonize [72]. Reinvigorating downregulated adaptive immunity during established chronic infections likely bears a lower risk of provoking pathological levels of proinflammatory cytokines or the excessive killing of infected cells than treating during acute infections at the peak of pathogen-directed immune responses due to reduced numbers of antiviral T cells and multiple mechanisms of T cell downregulation, which are present in established chronic viral infections [17,73–75]. The release of co-inhibition could safely be exploited for boosting antigen-specific T cell responses in the context of prophylactic or therapeutic vaccination [76,77]. Furthermore, enhancing pathogen-directed humoral responses might have a lower propensity to elicit immunopathology [36], because most antibody-mediated effector mechanisms do not involve the killing of autologous cells. In cancer, the therapeutic targeting of co-inhibition for boosting antitumor immunity bears a low risk of provoking excessive pathological responses because of relatively small systemic frequencies of tumor-reactive T cells and a confined response to the direct tumor environment. However, precaution should be taken for patients suffering from chronic inflammatory diseases or acute viral infections, and the risk of long-term treatments to release self-reactive T cells from regulation has to be considered.

Concluding remarks

The concept of co-inhibition-mediated protection from overshooting pathogen-directed immune responses is in line with its already recognized physiological role in the suppression of undesired autoreactive T cell responses. It is therefore likely that co-inhibition of adaptive immunity during infectious diseases has evolved to support host survival. However, excessive negative regulation can also facilitate the systemic spread and persistence of pathogens. To this end, the ultimate goal will be to determine where co-inhibition overprotects the host and to design therapies that only release the disease-promoting overprotection without eliciting immunopathology. This review provides a framework of insights into the protective roles of co-inhibitory pathways during infectious diseases and highlights considerations and contraindications for designing therapeutic approaches that target co-inhibition devoid of adverse events.

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