Dendritic cells (DCs) are central in maintaining the intricate balance between immunity and tolerance by orchestrating adaptive immune responses. Being the most potent antigen presenting cells, DCs are capable of educating naïve T cells into a wide variety of effector cells ranging from immunogenic CD4+ T helper cells and cytotoxic CD8+ T cells to tolerogenic regulatory T cells. This education is based on three fundamental signals. Signal I, which is mediated by antigen/major histocompatibility complexes binding to antigen-specific T cell receptors, guarantees antigen specificity. The co-stimulatory signal II, mediated by B7 family molecules, is crucial for the expansion of the antigen-specific T cells. The final step is T cell polarization by signal III, which is conveyed by DC-derived cytokines and determines the effector functions of the emerging T cell. Although co-stimulation is widely recognized to result from the engagement of T cell-derived CD28 with DC-expressed B7 molecules (CD80/CD86), other co-stimulatory pathways have been identified. These pathways can be divided into two groups based on their impact on primed T cells. Whereas pathways delivering activatory signals to T cells are termed co-stimulatory pathways, pathways delivering tolerogenic signals to T cells are termed co-inhibitory pathways. In this review, we discuss how the nature of DC-derived signal II determines the quality of ensuing T cell responses and eventually promoting either immunity or tolerance. A thorough understanding of this process is instrumental in determining the underlying mechanism of disorders demonstrating distorted immunity/tolerance balance, and would help innovating new therapeutic approaches for such disorders.

Keywords: activation, tolerance, co-stimulation, co-inhibition, dendritic cells, T cell priming
by soluble DC-derived cytokines, there are indications that signal II may also contribute to T cell polarization. A final putative DC-derived signal is suggested to provide polarized T cells with homing directions to the site of infection or injury (Sigmundsdottir and Butcher, 2008). Thus, DCs control the delicate balance between immunity and tolerance through the signals they convey to T cells.

Although the combined effect of all DC-derived signals is important for full blown T cell responses, signal II is key for allowing these responses and licensing them to become either immunogenic or tolerogenic. Here, we shed light on the multifaceted signal II by reviewing current knowledge of to date identified co-stimulatory and co-inhibitory pathways (Figure 1), their mode of action, relation to disease, and any possible clinical applications based on utilizing these pathways.

**CO-STIMULATORY MOLECULES**

**CD80/CD86/CD28 PATHWAY**

Following the discovery of the CD80/CD28 interaction, B7-2 (CD86) was identified as a second ligand for CD28 (Azuma et al., 1993). The CD80/CD86/CD28 pathway was suggested to deliver the strongest co-stimulatory pathway as CD28-deficient cells failed to proliferate in the presence of APCs (Green et al., 1994). The consequences of CD28 engagement by its ligands comprise stimulation of T cell proliferation, dramatic upregulation of IL-2 (Linsley et al., 1991a), promotion of T cell survival by enhancing Bcl-XL expression (Boise et al., 1995), and enhanced glycolytic flux to meet energetic requirements associated with a sustained response (Frauwirth et al., 2002). Those effects were shown to be dependent on activating the signaling cascades of phosphoinositide-3 kinase (PI3K), protein kinase B (PKB, also known as Akt), and nuclear factor kappaB (NF-κB; Song et al., 2008).

Several reports pointed out a possible role for CD28 signaling in T cell polarization. Murine T cells were shown to produce enhanced levels of IL-4 and IL-5, characteristic for Th2, upon strong CD28 stimulation (Baliffson et al., 1997). Strong CD28 signaling was also demonstrated to inhibit Th17 responses (Perus et al., 2010). Although it is generally accepted that memory T cells, unlike naive, are less dependent on co-stimulation via CD28, it was shown that this co-stimulatory pathway is important in controlling T cell recall responses (Ndejemb et al., 2006).

In addition to its key role in initiating and sustaining efficient T cell responses, the CD28 pathway is also involved in controlling immune tolerance. Co-stimulation of developing thymocytes by CD28 was shown to induce the expression of Foxp3 and promote the differentiation of regulatory T cells (Tregs; Tai et al., 2005). Furthermore, T cell activation in the absence of CD28 co-stimulation leads to a state of anergy characterized by dramatically reduced production of IL-2 and other effector cytokines upon subsequent TCR triggering (Schwartz, 1997). There is ample evidence that DCs utilize this mechanism to maintain tolerance to self. At steady state conditions, immature DCs present self-derived antigens accompanied by low levels of CD80/CD86 and therefore fail to supply specific T cells with adequate signal II, leading eventually to the deletion, anergy, or regulation of auto-reactive T cells that escaped thymic selection (Steinman and Nussenzweig, 2002). Thus, the CD80/CD86/CD28 pathway is as involved in promoting tolerance as in mediating immunity.
Since many immunogenic tumors lack expression of CD80 and CD86, it was postulated that tumor-infiltrating T cells would receive chronic TCR stimulation without co-stimulation leading to T cell anergy. This hypothesis was tested by inducing the expression of CD80/CD86 molecules on tumor cells prior to injection into mice. Forced expression of CD80/CD86 in tumor cells resulted in CD8+ T cell-dependent tumor rejection (Towensend and Allison, 1993). However, this method had barely any effect on pre-established tumors (Fallarino et al., 1997), implying that other pathways promoting immune tolerance toward established tumors are involved.

CD40/CD40L PATHWAY

CD40 was the first co-stimulatory molecules to be identified from the tumor necrosis factor (TNF) receptor (TNFR) family. First discovered as B cell receptor, CD40 is also expressed by DCs, macrophages, epithelial cells, and even activated T cells. Its ligand (CD40L or CD154), a member of the TNF family, is expressed not only by activated T cells, but also by natural killer (NK) cells and plasmacytoid DCs (pDCs, Querzada et al., 2004). In addition to promoting humoral immunity by activating B cells, the CD40/CD40L pair is pivotal for cellular immunity as it mediates a dialog between T cells and DCs. Indeed, CD40 engagement on DCs was shown to activate NF-κB pathway (Querzada et al., 2004) and consequently inducing DC maturation (Caux et al., 1994) and enhancing DC longevity (Miga et al., 2001). Initially, CD40-induced maturation of DCs was suggested to be sufficient in licensing CD8+ T cells (Schroenberger et al., 1998). However, further investigation in the CD40 pathway revealed that additional signals are necessary for optimal DC activation. CD40 cross-linking alone is not enough to induce IL-12 production, necessary for cytotoxic and Th1 responses, but DC pre-activation by microbial products followed by CD40 ligation dramatically increased IL-12 production (Scholmerich et al., 2000). This finding indicates that combined triggering of CD40 and PRRs, like Toll-like receptors (TLRs), is critical for DC licensing. The CD40-induced IL-12 also implies a central role for CD40/CD40L pathway in T cell differentiation, by favoring Th1 polarization. Blocking CD40/CD40L interactions lead to abrogated Th1 responses with reciprocal upregulation of Th2 cytokines (Hancock et al., 1998).

The adjuvant effect of CD40 ligation, reflected by DC activation, prompted the application of agonistic anti-CD40 antibodies for cancer therapy. Injecting agonistic anti-CD40 antibodies evoked cytotoxic T cell responses and eradicated the tumor in a mouse model of lymphoma (French et al., 1999). Furthermore, application of fully humanized anti-CD40 agonistic antibody resulted in objective partial responses in 14% of advanced solid tumor patients (Vonderheide et al., 2007). A similar approach was based on the administration of soluble CD40L, which was less efficient as it led to partial responses in 6% of treated tumor patients (Vonderheide et al., 2001). More clinical trials applying CD40 ligation, singularly or in conjunction with other therapeutic modalities, were carried out and showed promising results (Zheng et al., 2012).

Due to its activatory nature, the CD40/CD40L is decisive in regulating tolerance. It was shown that DCs derived from CD40-deficient mice conferred tolerance by priming IL-10 secreting Tregs (Martin et al., 2003). This effect on tolerance prompted investigating the possibility of exploiting CD40 blocking to enhance allograft survival. Although applying anti-CD40L antibodies as a monotherapy was able to block many effector mechanisms, it failed to induce sufficient allograft tolerance (Jones et al., 2008). However, combinations with other immunosuppressive therapies such as cytokine T lymphocyte (CTL)-associated antigen-4-immunoglobulin (CTLA-4-Ig, Larsen et al., 1996) and rapamycin (Li et al., 1998) were shown to result in long-term graft survival. Collectively, CD40/CD40L pathway, in conjunction with other pathways, is vital for initiating active immunity and regulating tolerance.

ICOSL/ICOS PATHWAY

The inducible T cell co-stimulator (ICOS) was identified as the third member of the CD28/CTLA-4 family of co-stimulatory molecules (Hutloff et al., 1999). ICOS expression by T cells requires prior TCR activation and CD28 co-stimulation (McAdam et al., 2000). The ligand (ICOSL) is expressed by DCs (Wang et al., 2000), B cells, and a variety of non-hematopoietic tissues (Ling et al., 2000). ICOSL/ICOS pathway exerts its co-stimulatory effects on already activated T cells by supporting proliferation and cytokine production (Hutloff et al., 1999). Additionally, ICOS is proposed to play an important role in T cell polarization. Initially, ICOSL/ICOS was suggested to support Th2 responses. Blocking ICOSL/ICOS interactions was shown to block Th2 lead airway responses without influencing Th1-mediated inflammation (Coyle et al., 2000). Similarly, another study showed that the majority of T cells expressing ICOS in vivo co-produced Th2-type cytokines (Loehning et al., 2003). In contrast, disrupting ICOSL/ICOS pathway was found to inhibit Th1-mediated disorders like allograft rejection (Guo et al., 2002) and experimental allergic encephalomyelitis (Rotman et al., 2001). ICOS was shown to be involved driving Th17 responses (Park et al., 2005), further complicating the role of ICOSL/ICOS in T cell polarization. An attempt to resolve this controversy was by showing that engaging ICOS on activated T cells amplified the effector responses of these cells regardless of their polarized state (Wassink et al., 2004).

Benefiting of the activatory effect of ICOSL/ICOS pathway in the context of cancer therapy was evaluated. Induced ICOSL expression on tumor cells was demonstrated to promote tumor regression by inducing CD8 cytotoxicity (Liu et al., 2001). Nevertheless, this strategy was ineffective in case of weakly immunogenic tumors (Arc et al., 2013). Surprisingly, it was recently revealed that tumor cell-expressed ICOSL augments Treg activation and expansion within the tumor local environment (Martin-Orozco et al., 2010). This suggests that targeting ICOSL/ICOS pathway may not be the most optimal option for cancer treatment. On the contrary, blocking its ICOSL/ICOS-mediated suppression may be beneficial in cancer therapy. The tolerogenic effect of ICOSL/ICOS pathway is not restricted to tumors, as there are indications of its involvement in maintaining immune tolerance. ICOS-deficient mice displayed reduced numbers of natural Tregs (nTregs), which may be owed to a decrease in survival and/or proliferation of these cells (Burmester et al., 2008). Another indication of ICOS involvement in tolerance...
is the finding that ICOS triggering on T cells dramatically increased the production of the anti-inflammatory cytokine IL-10 (Hurdliff et al., 1999). Consistently, high ICOS expression by T cells was selectively associated with the anti-inflammatory IL-10 (Lohning et al., 2003). These findings argue for targeting ICOSSL/ICOS pathway to induce tolerance for therapeutic purposes. However, it is very important to clearly dissect the conditions under which this pathway induces activation or tolerance.

**CD70/CD27 PATHWAY**

CD70 is another member of the TNF family of co-stimulatory molecules. Its ligand CD27 was identified first as a novel T cell differentiation antigen (van Lier et al., 1987). The contribution of CD27 to immunity was later recognized to be dependent on its binding partner CD70, which is expressed under the control of antigen receptors and TLRs in lymphocytes and DCs, respectively (Tesselaar et al., 2003). Similar to CD40, engaging CD27 induced the activation of NF-κB pathway (Alisb et al., 1998). The first indication of the co-stimulatory properties of the CD70/CD27 pathway was provided by triggering CD27, which augmented CD3-induced T cell proliferation (van Lier et al., 1987). This effect was later explained by promoting survival of newly stimulated T cells, in contrast to CD28 that prompts cell cycle entry and induces proliferation (Hendriks et al., 2003). This survival effect relies completely on IL-2 receptor signaling and the autocrine production of IL-2 (Peperazk et al., 2010).

The contribution of CD70/CD27 pathway to T cell polarization is debatable. CD8⁺ T cells from CD27 knockout mice maintained the capacity of differentiation into CTLs and interferon-gamma (IFN-γ) production, implying that CD27 is not involved in the development of cytotoxic CD8⁺ responses (Hendriks et al., 2003). On the other hand, transgenic expression of CD70 on steady state immature DCs was found to break CD8⁺ tolerance and permit the differentiation of effector CD4⁺ and CD8⁺ cells from naive precursors (Keller et al., 2008). Moreover, the murine CD8α⁺ DC subset was revealed to favor the differentiation of Th1 cells in a CD70-dependent and IL-12-independent mechanism (Sories et al., 2007). This is further supported by showing that human Langerhans cells (LCs), an epidermal subset of DCs, are capable of inducing CD8⁺ anti-viral responses in a CD70-dependent manner (van der Aar et al., 2011). A recent study also demonstrated that the CD70/CD27 pathway impedes the differentiation of Th17 effector cells and attenuates accompanying autoimmunity in a mouse model of multiple sclerosis (Coquet et al., 2012). These findings argue for targeting CD70 signaling (Coquet et al., 2012) to induce tolerance and permit the initial priming of naïve T cells into effector T cells, bares functional specialization. Whereas CD28 is essential for the initial priming of naïve T cells into effector T cells, CD40 is crucial for the expansion (later proliferation) and survival of these effector cells.

Several studies have pointed out a central role for CD40 in regulating the balance between Th1 and Th2 responses. Co-stimulating T cells through CD40 was shown to induce IL-4 expression and inhibited IFN-γ production (Flynn et al., 1998). Furthermore, DC treatment with thymic stromal lymphopoietin (TSLP), known for its Th2 skewing properties, leads to the expression of the anti-apoptotic molecules Bcl-2 and Bcl-xL (Rogers et al., 2005). OX-40-favored Th2 response was proposed to be mediated by an initial induction of nuclear factor of activated T cells (NFAT) c1 in an IL-4 receptor-independent manner, followed by an IL-4 receptor-dependent effect on GATA3 (So et al., 2006). However, it was shown later that DC-derived OX-40L maintained both Th2 and Th1 responses, owed to OX-40L-enhanced survival of effector T cells regardless of their polarization (Jenkins et al., 2007). Thus, it seems that the role of OX-40/OX-40L in the differentiation of Th2 cells is restricted to promoting the survival of already established Th2 cells that differentiated under the effect of other DC-derived factors. OX-40/OX-40L is also involved in controlling immune tolerance. The first evidence of this role is the expression of significant amounts of OX-40 on naturally occurring FoxP3⁺ Tregs. OX40 signaling appears to be dispensable for the development of FoxP3⁺ Tregs.
since this population exists in OX-40-deficient mice. However, OX-40 signaling is important for the survival of nTregs as OX-40-deficient mice displayed lower counts of this population of Tregs (Takeda et al., 2004). The effect of OX-40 triggering on the functions of nTregs remains controversial. Whereas one study showed that OX-40 signaling in CD4+ T cells render them resistant to suppression by nTregs (Takeda et al., 2004), another study reported abrogated suppression following OX-40 triggering on nTregs (Valanzina et al., 2003). Another mechanism by which OX-40/OX-40 is assumed to contribute to tolerance regulation is by influencing the development of induced Tregs (Tregs). Under conditions promoting Treg differentiation, OX-40 engagement on T cells was shown to inhibit Foxp3 expression by these T cells (Bo and Ciofani, 2007). Nevertheless, the surrounding environment during Treg differentiation seems to determine the outcome of OX-40 signaling, which was reported to promote the expansion of Tregs if IL-4 and IFN-γ were absent from the milieu (Ruby et al., 2009). In conclusion, OX-40/OX-40 appears to be central in maintaining the survival of T cells in general, but its influence on T cell functions requires further elucidation.

4-1BB/L/4-1BB PATHWAY

4-1BB (CD137) is yet another member of the TNFR family. Its expression is induced on T cells following TCR activation (Polllik et al., 1993). The ligand, 4-1BBL of the TNF family, is expressed on activated APCs (Vinay and Kwon, 1998). Engagement of T cell 4-1BB was reported to induce IL-2 production independently of CD28, when accompanied by strong TCR signaling (Sassuli et al., 1998). Furthermore, 4-1BB interaction with its ligand was demonstrated to provide a co-stimulatory signal particularly to CD8+ T cells, enhancing proliferation, cytokinetics (Shiford et al., 1997), and survival (Lee et al., 2002). Similar to other TNFR family members, 4-1BB enhanced survival is dependent on NF-κB activation, which in turn induces the two pro-survival molecules: Bcl-xL and Bcl-2 (Kim et al., 1998). 4-1BB interaction with its ligand was demonstrated to promote the expansion of Tregs if IL-4 and IFN-γ were absent from the milieu (Ruby et al., 2009). In conclusion, OX-40/OX-40 appears to be central in maintaining the survival of T cells in general, but its influence on T cell functions requires further elucidation.

4-1BB/L/4-1BB PATHWAY

Glucocorticoid-induced TNFR related gene (GITR) was first discovered as a dexamethasone-induced molecule in murine T cell hybridomas (Nectini et al., 1997). The expression of the human ortholog was subsequently identified in human lymphocytes and shown to be independent of glucocorticoid treatment. Similar to the TNFR family members OX-40 and 4-1BB, GITR is only expressed on recently activated T cells, implying a role in promoting effecter functions rather than involvement in initial priming of naive T cells (Gurney et al., 1999). The GITR ligand (GITRL) is expressed by APCs and is upregulated upon activation (Tone et al., 2003). GITRL/GITR pathway provides co-stimulation to naïve T lymphocytes demonstrated by enhanced proliferation and effecter functions in the setting of suboptimal TCR stimulation (Ronchetti et al., 2004). Additionally, GITR triggering promoted naïve T cell survival through the activation of NF-κB and mitogen-activated protein kinase (MAPK) pathways, though it was not sufficient to inhibit activation-induced cell death initiated by TCR signaling (Esparza and Arch, 2005). GITRL/GITR pathway does not seem to have an impact on T cell polarization. Although applying an agonist antibody against GITR initially enhanced Th2 responses in a mouse model of helminth infection, this effect was short lived and GITR-independent (van der Werf et al., 2011).

A role for GITR/GITL pathway in immune tolerance was initially demonstrated by the constitutive expression of GITR on Tregs (Shimizu et al., 2002). Factually, Tregs isolated based on the expression of GITR could prevent the development of colitis induced in an adoptive transfer model (Iraishikara et al., 2003). However, engaging Treg-expressed GITR, by agonist antibodies, was shown to abrogate their suppressive capacity (Shimizu et al., 2002). In the beginning, this effect was interpreted by mere activation of Tregs upon GITR stimulation, but this explanation was underscored by the fact that Treg preconditioning with anti-GITR did not cause the subsequent loss of suppression (Shimizu et al., 2002). Eventually, it was revealed that triggering GITR on effector T cells rendered them resistant to suppression by Treg (Stephens et al., 2004), providing a plausible explanation for the anti-tolerogenic effects of GITR stimulation. This postulates a model where APC-expressed GITRL would bind GITR on recently stimulated T cells allowing them to resist suppression. Simultaneously, GITR ligation on Tregs would allow their expansion and their subsequent domination at later stages of the immune response (Stephens et al., 2004).

Based on the activatory nature of GITR/GITL pathway and its characteristic inhibition of tolerance, employing this pathway in cancer therapy was evaluated. The administration of an agonistic antibody against GITR has been shown to augment CD8+ anti-tumor immunity (Cohen et al., 2006). In addition to mobilizing anti-tumor responses, triggering GITR was also shown to attenuate Treg-mediated suppression within the tumor (Ko et al., 2005), making GITRL/GITR a promising target for cancer therapy.
LIGHT/HVEM PATHWAY

The TNFR family member herpes virus entry mediator (HVEM) was initially discovered as a receptor for herpes simplex virus (Montgomery et al., 1996). It is expressed on resting T cells, monocytes, and immature DCs. HVEM has multiple binding partners: LIGHT and lymphotyphoceptor-α (LT-α) from the TNF superfamily; and CD160 and B and T lymphocyte attenuator (BTLA) from the Ig superfamily. HVEM interaction with these ligands creates a complex network of pathways, which collectively regulates adaptive immune responses (Ward and Sedy, 2011). In this section we will only focus on the co-stimulatory pathway resulting from LIGHT/HVEM interactions. LIGHT is expressed by immature DCs (Tamada et al., 2000a) and is induced upon activation on T cells, in contrast to HVEM (Morel et al., 2000). LIGHT/HVEM interaction was revealed to be required for DC-mediated allogenic T cell responses. Indeed, activating T cell HVEM enhanced T cell proliferation at suboptimal TCR stimulation conditions (Tamada et al., 2000a). Disrupted LIGHT/HVEM interaction was shown to result in inhibited T cell proliferation, further supporting the importance of this pathway in co-stimulation (La et al., 2002). Similar to other TNFR family members, HVEM mediates its effects by activating NF-κB pathway (Harrop et al., 1998). Interestingly, LIGHT/HVEM pathway can also contribute to T cell activation indirectly by inducing DC maturation, reminiscent of the role of CD40 in inducing DC maturation (Morel et al., 2001). LIGHT/HVEM pathway is also suggested to contribute to T cell polarization. T cells co-stimulated through HVEM displayed enhanced production of Th1 cytokines (Tamada et al., 2000b). Accordingly, LIGHT-deficient mice showed reduced IFN-γ levels, prolonging allolograft survival in these mice (Ye et al., 2002). Due to the complexity of the signaling network of HVEM and LIGHT, reported findings should be interpreted as these observations may involve other pathways.

TIM FAMILY

In addition to the CD28/B7 and TNFR/TNF co-stimulatory family, the recently identified TIM (T cell Ig domain and mucin domain) family is a new contributor to signal II. This family of genes was initially identified while searching for Th1-specific markers (Monney et al., 2002). In humans, three TIM family members, TIM1, TIM3, and TIM4 have been identified thus far. Mice possess an additional member: TIM2 (Kuchroo et al., 2008). In this section we will only focus on TIM3 and TIM4, which were reported to be expressed by DCs. TIM3 was first discovered as a specific marker for Th1 cells (Monney et al., 2002), and was shown to induce the death of these cells by binding to its ligand galectin-9 (Zhu et al., 2005). TIM3 expression was also detected on DCs, and its ligation by galectin-9 induced the production of the inflammatory cytokine TNF-α. The absence of TIM3 signaling was shown to result in impaired TLR responsiveness, implying a synergistic relation between TIM3 and TLR signaling pathways (Anderson et al., 2007). Although TIM3 triggering on T cells and DCs leads to ERK (extracellular signal-regulated kinases) phosphorylation and IκB degradation, different tyrosine phosphorylation patterns in T cells and DCs were detected, providing a plausible explanation for the differential effects of TIM3 between different cell types (Anderson et al., 2007). Thus far, interactions between DC-expressed TIM3 and T cell-expressed galectin-9 have not been investigated. However, previous findings prompt a model where DC-expressed TIM3 promotes inflammation and the differentiation of TIM3-expressing Th1 cells. IFN-γ-induced galectin-9 would interact with TIM3 from other T cells, inducing cell death and thereby self-limiting the immune response. Additionally, TIM3 is suggested to contribute to tolerance. A crucial role for TIM3 in clearing apoptotic cells by phagocytosis was recently revealed. Blocking this function resulted in inhibited cross-presentation of self-antigens and the development of auto-antibodies (Nakayama et al., 2009). In a completely different mechanism, TIM3 expressed by tumor-infiltrating DCs was shown to interact with the alarmin HMGB1, disturbing the recruitment of tumor cell-derived nucleic acids into DC endosomes, attenuating immune responses to these tumors (Chiba et al., 2012).

In contrast to the other members of the TIM family, TIM4 is exclusively expressed by APCs and not by T cells (Meyers et al., 2005). Through binding to TIM1 on T cells, TIM4 was shown to provide T cells with a co-stimulatory signal promoting T cell expansion, cytokine production, and survival. These effects were mediated by induced phosphorylation of the signaling molecules LAT (linker of activated T cells), Akt, and ERK1/2 in stimulated T cells (Rodríguez-Manzanet et al., 2008). Notably, the strength of TIM4 signal is decisive in determining the stimulatory effect, as weak TIM4 signaling inhibits T cell proliferation instead of potentiating it (Meyers et al., 2005). Similarly, TIM4 was shown to inhibit the proliferation of naive T cells, which lack the expression of TIM1 (Mizui et al., 2008). These data imply that TIM4 has at least two binding partners: an activating ligand (TIM1) and an inhibitory one to be identified. Through these ligands, TIM4 exerts bimodal regulation of immune responses. Analogous to TIM3, the role of TIM4 in regulating immunity is also evident through mediating the engulfment of apoptotic cells. In vivo blocking of TIM4 resulted in the development of auto-antibodies (Miyanishi et al., 2007).

ADHESION MOLECULES PROVIDING CO-STIMULATORY SIGNALS

Leukocyte adhesion and detachment from other cells is tightly regulated by adhesion molecules. A specific set of these molecules is involved in regulating DC/T cell interactions. This set includes the following molecules: intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3), expressed by DCs, and their respective ligands LFA-1 and CD2, expressed by T cells. The seminal discovery of the involvement of LFA-1 in mediating T cell functions prompted a hypothesis that LFA-1 would act by enhancing adhesion and thereby increasing the range of avidities that can promote antigen recognition (Springer et al., 1982). Subsequently, ICAM-1 was identified as the ligand of LFA-1 (Rothlein et al., 1986). LFA-1 ligation by ICAM-1 was shown to induce proliferation of TCR-stimulated T cells in an IL-2-dependent mechanism, proposing that ICAM-1/LFA-1 interaction as a co-stimulatory pathway (Van Seventer et al., 1999). In addition to co-stimulation, ICAM-1/LFA-1 interaction stabilizes the immunological junction (Bleul et al., 2001) and the ICAM-1/LFA-1 pathway appears to contribute to T cell activation. However, previous findings suggest a model where DC-expressed TIM3 promotes inflammation and the differentiation of TIM3-expressing Th1 cells. IFN-γ-induced galectin-9 would interact with TIM3 from other T cells, inducing cell death and thereby self-limiting the immune response. Additionally, TIM3 is suggested to contribute to tolerance. A crucial role for TIM3 in clearing apoptotic cells by phagocytosis was recently revealed. Blocking this function resulted in inhibited cross-presentation of self-antigens and the development of auto-antibodies (Nakayama et al., 2009). In a completely different mechanism, TIM3 expressed by tumor-infiltrating DCs was shown to interact with the alarmin HMGB1, disturbing the recruitment of tumor cell-derived nucleic acids into DC endosomes, attenuating immune responses to these tumors (Chiba et al., 2012).

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cell differentiation as repeated T cell stimulation with ICAM-1 promoted IFN-γ production by these cells (Semenani et al., 1994).

Moreover, blocking ICAM-1/LFA-1 interactions during T cell stimulation drastically increased Th2 cytokines (Salomon and Bluestone, 1998). More recently, ICAM-1/LFA-1 interaction during CD8+ T cell priming was demonstrated to be essential for the establishment of effective T cell memory (Schoeler et al., 2008). The effects of the ICAM-1/LFA-1 pathway are believed to result from influencing multiple cellular signaling cascades. LFA-1 was found to interact with the transcriptional co-activator JAR1, implying an influence on c-Jun-driven transcription (Bianchi et al., 2000).

In parallel, T cell CD2 interaction with its ligand LFA-3 was recognized for contributing to T cell activation by strengthening the adhesion between T cells and APCs and thereby enforcing TCR contact with its ligands (Davis and van der Merwe, 1996). Moreover, CD2 signaling was also shown to restore responsiveness in anergic human T cells (Boussiotis et al., 1994). CD2 blocking in vivo was revealed to induce T cell unresponsiveness, further supporting the notion that LFA-3/CD2 pathway contributes to immune activation (Xu et al., 2004). Conversely, specific mobilization of LFA-3/CD2 interactions was demonstrated to induce, single handedly, non-proliferating Tregs secreting high amounts of IL-10 (Wakkach et al., 2001). In light of these contradictions, further characterization of the role of LFA-3/CD2 co-stimulatory pathway is required.

CO-INHIBITORY MOLECULES

CD80/CD86:CTLA-4 PATHWAY

Cytotoxic T lymphocyte-associated antigen-4 (CD152) is a CD28 homolog that was discovered in 1987 (Brunet et al., 1987). The closely related structures of these two molecules suggest overlapping functional qualities. Indeed, CTLA-4 binds to CD80 and CD86, though at greater affinities. However, CTLA-4 was the first described co-stimulatory molecule with inhibitory effects in a stark contrast to the activatory properties of CD28 (Linsley et al., 1991b). The effects of CTLA-4 include inhibition of proliferation, cell cycle progression, and IL-2 synthesis (Watanabe et al., 1996). Additionally, CTLA-4 seems to have an influence on T cell polarization. T cells lacking CTLA-4 expression were shown to adopt a Th2 phenotype (Bour-Jordan et al., 2003). Furthermore, neutralizing CTLA-4 signaling in T cells was recently shown to enhance IL-17 production and promote the differentiation of Th17 cells (Ying et al., 2010).

The prominent role of CTLA-4 in tolerance is clearly demonstrated by CTLA-4-deficient mice, which succumb at 3–4 weeks of age to massive lymphoproliferative disease (Tivol et al., 1995). Furthermore, the suppressive functions of naturally occurring Tregs, which constitutively express CTLA-4, were dependent on CTLA-4 signaling (Rudd et al., 2009). First, CTLA-4 antagonizes the CD28 stimulatory signaling by competing with CD28 on binding to CD80/CD86. Interestingly, CTLA-4 expression on cells is induced in a CD28-dependent mechanism (Angre et al., 1996), implying that CTLA-4 serves as an internal checkpoint that prohibits excessive stimulation by CD28. Extrinsic inhibitory effects of CTLA-4 are suggested to be exerted through different mechanisms. CTLA-4 molecules expressed by Tregs were shown to engage CD80/CD86, expressed by DCs, promoting the activity of IDO. The modified catabolic properties of DCs lead to localized deprivation of tryptophan and thereby reduced T stimulatory capacity of these DCs (Fallarino et al., 2003). Another suggested mechanism for the extrinsic effects of CTLA-4 was demonstrated by the capacity of CTLA-4 to capture CD86, expressed by APCs, internalize it for ensuing degradation in a process called trans-endocytosis (Qureshi et al., 2011). Tregs were also observed to suppress T cells by establishing a direct interaction through CTLA-4, which binds to CD80 and CD86 expressed by those T cells (Taylor et al., 2004). Finally, unstimulated T cells were revealed to produce a soluble form of CTLA-4, which may possibly convey the inhibitory effects to other cells (Maggiorelli et al., 1999). Collectively, CTLA-4 is unequivocally vital for tolerance.

Due to its role in maintaining tolerance, blocking CTLA-4 interaction with CD80 and CD86 was postulated to promote anti-tumor immunity. Indeed, in vivo administration of blocking antibodies against CTLA-4 resulted into effective anti-tumor immunity and tumor rejection (Leach et al., 1998). Nevertheless, CTLA-4 blockade efficacy in tumor therapy was correlated with the stage and immunogenicity of the tumor. At early stages small tumors were sensitive to the effects of CTLA-4 blockade (Shrikant et al., 1999), whereas advanced tumors were resistant due to the strongly tumor-induced T cell tolerance (Sotomayor et al., 1999). In an attempt to circumvent this hurdle, anti-CTLA-4 blocking antibodies were tested in combination with other therapeutic modalities. Combined anti-CTLA-4 application and Treg depletion resulted in maximal tumor rejection, which was dependent on the expansion of tumor-specific CD8+ T cells (Sutmüller et al., 2001). Those promising experimental observations lead to the development of two fully human anti-CTLA-4 antibodies: ipilimumab (Bristol-Myers Squibb, New York, NY, USA) and tremelimumab (Pfizer, New York, NY, USA). Early clinical trials in metastatic melanoma and ovarian carcinoma patients demonstrated that blocking CTLA-4 resulted in extensive tumor necrosis with lymphocyte and granulocyte infiltrates in a large number of patients (Hodi et al., 2003). Further large scale clinical trials have shown irreifiable evidence of the efficacy of anti-CTLA-4 antibodies, leading eventually to FDA approval of these antibodies (Kirkwood et al., 2012). Despite its novelty, this therapeutic strategy is challenged by autoimmune complications resulting from the administration of anti-CTLA-4 antibodies (Sanderson et al., 2005).

The tolerogenic effects arising from CTLA-4 engagement with CD80/CD86 can also be utilized for inducing tolerance toward transplanted tissues. This notion has been supported by observations in animal experimental models. Administration of recombinant CTLA-4-Ig fusion protein after renal or cardiac transplantation enhanced allograft acceptance and reduced inflammatory responses (Arama et al., 1998). This led to the development of humanized CTLA-4-Ig (Belatacept). Kidney transplantation patients receiving Belatacept showed reduced allograft rejection and maintained better renal functions, compared to patients receiving cyclosporine. These findings resulted in gaining FDA approval for using Belatacept for the prevention of acute rejection post-renal transplant (Vincenti et al., 2011).
PD-L1/PD-L2/PD-1 PATHWAY

Programmed cell death-1 (PD-1) is another member of the CD28 family that is expressed by activated T and B cells (Agata et al., 1996). Two ligands were identified to interact with PD-1: PD-L1 (Dong et al., 1999) and PD-L2 (Latchman et al., 2001). Those ligands are characterized by differential expression patterns. PD-L1 is constitutively expressed and further enhanced on activated lymphocytes, including Tregs and DCs. It is also expressed by a wide variety of non-hematopoietic cell types including the vascular endothelial cells, neurons and pancreatic islet cells. In contrast, PD-L2 expression is restricted to DCs and macrophages under certain conditions (Greenwald et al., 2003). Interestingly, PD-L2 displays three times higher binding affinity to PD-1 in comparison to PD-L1, which on the other hand was also identified to bind to CD80 (Butte et al., 2007). The varying binding and expression properties of PD-L1 and PD-L2 suggest distinct functions in regulating T cell responses. Along with its ligands PD-1, is recognized for its vital role in regulating adaptive immune responses (Sharpe et al., 2007). Indeed, triggering of PD-1 by one of its ligands during TCR signaling can block T cell proliferation, cytokine production and cytolytic activity, and impair T cell survival (Riley, 2009). The intracellular domain of PD-1 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) as well as an immunoreceptor tyrosine-based switch motif (ITSM), which are phosphorylated upon ligand engagement. Subsequently protein phosphatases, such as Src homology phosphatase-1 (SHP-1) and SHP-2, are recruited to TSM where they are activated and inhibit proximal TCR signaling events by dephosphorylating key intermediates in the TCR signaling cascade (Chemnitz et al., 2004). Similar to CTLA-4, triggering PD-1 limits glucose metabolism and Akt activation, albeit through different mechanisms (Chemnitz et al., 2004). Consistently, a recent study also demonstrated that PD-1 exerted its inhibitory effects by affecting Akt and Ras pathways and thereby inhibiting cell cycle progression and T cell proliferation (Patsouli et al., 2012).

The first indication of the importance of PD-1 in immune tolerance came from PD-1-deficient mice, which developed strain-specific autoimmunity. The absence of PD-1 caused the development of cardiomyopathy secondary to the production of auto-antibodies against cardiac troponin in BALB/c mice (Nishimura et al., 1999), while CSFRII−/− developed a lupus-like autoimmune disease (Nishimura et al., 2001). In humans, polymorphisms in the PD-1 gene were also associated with susceptibility to several autoimmune diseases including systemic lupus erythematosus (Proksauna et al., 2003), type 1 diabetes (Nielsen et al., 2003), and multiple sclerosis (Kroter et al., 2005). These observations were supported by functional studies demonstrating the contribution of the PD-L1/PD-L2/PD-1 pathway to central tolerance. In the thymus, interactions between PD-1, expressed by CD4+CD8− thymocytes, and PD-L1 broadly expressed in the thymic cortex, were deemed crucial in regulating positive selection (Nishimura et al., 2000). PD-1 was also shown to participate in thymic negative selection (Blank et al., 2003). Gene expression profiling studies of central tolerance in non-obese diabetic (NOD) mice also implicated PD-1 and PD-L1 in central tolerance (Zucchelli et al., 2005). PD-L1/PD-L2/PD-1 pathway also contributes to peripheral tolerance through multiple mechanisms.
PD-1, prolonged the survival of cardiac allografts in mice (Ozkaynak et al., 2002). Furthermore, PD-L1 expression on murine liver allografts is central for spontaneous tolerance (Morita et al., 2010).

**B7-H3 PATHWAY**

B7-H3 belongs to the B7 family of co-stimulatory molecules. Similar to other Ig superfamily members, B7-H3 is a transmembrane molecule. It possesses a short cytoplasmic tail with no known signaling domain. B7-H3 is expressed on a wide variety of tissues and tumor cell lines. However, its expression on leukocytes is only detectable following stimulation. B7-H3 expression can be induced on DCs and monocytes by inflammatory cytokines, whereas a combination of phorbol myristate acetate and ionomycin can induce it on T cells. B7-H3 was shown to bind a receptor expressed by activated T cells. This receptor is distinct from CD28, CTLA-4, ICOS, and PD-1 and yet to be identified. Triggering receptor expressed on myeloid cells (TREM)-like transcript 2 (TLT-2), constitutively expressed by CD8+ T cells and activation-induced on CD4+ T cells, was proposed to be the binding partner of B7-H3 (Hashiguchi et al., 2008). However, this was strongly refuted by another study providing evidence of non-existing interaction between B7-H3 and TLT-2 (Lettinier et al., 2009). Initially, B7-H3 was suggested to be a positive co-stimulatory molecule that induces T cell proliferation, IFN-γ production and CTL generation in humans (Chapoval et al., 2001). Nevertheless, this was contradicted by another study demonstrating that B7-H3 is a potently inhibited T cell stimulation under different conditions and regardless of the stimulation status of the T cells in question (Lettinier et al., 2009). This is corroborated by data from murine studies where applying an agonistic fusion protein, B7-H3-Ig, was shown to inhibit proliferation, IL-2 and IFN-γ production of TCR-stimulated T cells. This inhibitory effect was demonstrated by exacerbated airway inflammation in B7-H3-deficient mice compared to wild type counterparts (Suh et al., 2003). Moreover, blocking B7-H3 caused enhanced T cell proliferation in vitro and worsened EAE in vivo. This effect may be explained by the inhibitory influence of B7-H3 signaling over NF-κB, NFA T, and AP-1 that are involved in regulating T cell activation (Prasad et al., 2004). Notably, the effects of B7-H3 were overridden by CD28 co-stimulation, implying that B7-H3 functions optimally in the absence of co-stimulation (Suh et al., 2003). Of interest, tumors are suggested to hijack the B7-H3 to evade anti-tumor immune responses. This is demonstrated by increased disease severity when cancer cells upregulated B7-H3 expression (Holmeyer et al., 2008). Collectively, further characterization of the B7-H3 pathway is required to resolve functional discrepancies, which may be explained by the existence of two receptors for B7-H3 with opposite functions, yet to be identified.

**B7-H4 PATHWAY**

B7-H4 is the last among the B7 family members that was identified. Unlike other B7 family members, which are type I membrane molecules, B7-H4 is characterized by a glycosylphosphatidylinositol (GPI) domain that links to the cell membrane (Prasad et al., 2003). In humans, B7-H4 mRNA was detected in a variety of tissues. However, immunohistochemical analysis did not reveal any B7-H4 protein expression by these tissues. Likewise, no B7-H4 expression could be detected on freshly isolated T cells, B cells, monocytes, and DCs, but it was induced after activating these cells in vitro. The ligand of B7-H4 has not been identified yet, but it is suggested to be expressed by stimulated T cells and to be distinct from other CD28 family members (Sica et al., 2003). B7-H4 is widely regarded as a co-inhibitory molecule. Indeed, treatment of TCR-stimulated T cells by a fusion B7-H4-Ig protein resulted in inhibited T cell proliferation and cytokine production, an effect that required B7-H4 cross-linking (Sica et al., 2003). The inhibitory effects of B7-H4 are proposed to arise from arrested cell cycle progression in T cells (Sica et al., 2003), and impaired induction of Jun, known for its role in inducing IL-2 production in activated T cells (Prasad et al., 2003). A recent study also showed that B7-H4 signaling inhibits phosphorylation of MAP kinases, ERK, p38, Jun N-terminal kinase (JNK), and Akt, usually elicited upon TCR triggering of T cells (Wang et al., 2012b).

In line with in vitro findings, mice suffering from graft versus host disease demonstrated prolonged survival upon the in vivo application of B7-H4-Ig (Sica et al., 2003). Expectedly, in vivo administration of an antagonizing antibody against B7-H4 blocked the inhibitory effect of B7-H4 pathway and led to accelerated disease development in a mouse model of EAE (Prasad et al., 2003). Furthermore, B7-H4-deficient mice showed better control of Leishmania major infection as Th1 responses were augmented in these mice (Suh et al., 2006). B7-H4 deficiency also enhanced neutrophil-mediated immunity, implying that B7-H4 may have a role in regulating innate immunity too (Zhu et al., 2009). In addition to its role as a co-inhibitory molecule, B7-H4 seems to mediate the effect of Tregs. It was shown that Tregs, but not conventional T cells, induce high levels of IL-10 production by APCs and consequently trigger B7-H4 expression that renders these APCs immunosuppressive (Kryczek et al., 2006a). The overall tolerogenic effect of B7-H4 can be exploited by tumors to evade immune responses. B7-H4 expression was reported for several tumors including lung cancer, ovarian cancer (Choi et al., 2003), gastric cancer (Jang et al., 2010), and tumor-associated macrophages (Kryczek et al., 2006b). Blockade of B7-H4 on these macrophages was actually effective in reversing their suppressive effect and restored anti-tumor T cell immunity (Kryczek et al., 2006b). Additionally, manipulating B7-H4 pathway has potential in the field of transplantation. A recent study showed that B7-H4 expression was shown to prolong islet allograft survival in mice (Wang et al., 2012b). Thus, the B7-H4 pathway serves as an interesting therapeutic target in different diseases, though several aspects of this pathway remain elusive.

**HVEM/BTLA/CD160 PATHWAY**

As mentioned earlier, the molecules HVEM, BTLA, CD160, and LIGHT interact directly with each other forming a complex pathway network regulating adaptive immune responses. HVEM, expressed by immature DCs, can provide negative co-stimulatory signals through binding to its ligands BTLA and CD160 on T cells (Ware and Sedy, 2011). BTLA belongs to the Ig superfamily and is a structural homolog of CTLA-4 and PD-1. It is also a transmembrane glycoprotein that can be phosphorylated on tyrosines located in conserved cytoplasmic ITIM motif (Watanabe et al., 2006).
and B7-H4, the inhibitory effects of BTLA can be exploited by rheumatoid arthritis, but not with systemic lupus erythematosus. The study also revealed an association between another BTLA SNP and susceptibility to rheumatoid arthritis (Lin et al., 2006). Another ITIM region of BTLA was reported to associate with increased 2008). Moreover, a single-nucleotide polymorphism (SNP) in the leading to a hepatitis-like syndrome with advancing age (Oya et al., 2006) and to cause the development of auto-antibodies pong et al., 2006) and to cause the development of auto-antibodies expressing by T regs. This was supported by showing that T regs from also shown to mediate Treg suppression by interacting with HVEM wild type controls (Watanabe et al., 2003). BTLA deficiency was also reported to exacerbate allergic airway inflammation (Deppong et al., 2006) and to cause the development of auto-antibodies leading to a hepatitis-like syndrome with advancing age (Oya et al., 2008). Moreover, a single-nucleotide polymorphism (SNP) in the ITIM region of BTLA was reported to associate with increased susceptibility to rheumatoid arthritis (Lin et al., 2006). Another study also revealed an association between another BTLA SNP and rheumatoid arthritis, but not with systemic lupus erythematous or Sjogren’s syndrome (Oki et al., 2011). Similar to B7-HD and B7-H4, the inhibitory effects of BTLA can be exploited by tumors to evade immunity. Melanoma-specific CD8+ T cells were shown to persistently express BTLA. Interrupted BTLA signaling, achieved by applying CpG oligonucleotide vaccine formulations, lead to functional recovery of melanoma-specific CD8+ T cells (Derre et al., 2010). Herpes virus entry mediator can also interact with CD160, a GPI anchored membrane molecule that is mainly expressed by CD8+ T cells and activated CD4+ T cells. Cross-linking CD160 with a specific antibody on stimulated T cells was shown to strongly inhibit T cell proliferation and cytokine production. Similarly, the inhibitory effect of CD160 was also elicited by binding to its ligand HVEM (Cai et al., 2008). Although both BTLA and CD160 bind to the cysteine-rich domain-1 (CRD-1) of HVEM with comparable affinity, CD160 dissociates from HVEM at a slower rate compared to BTLA. Moreover, mutagenesis study of HVEM revealed that CD160 has a distinct binding site on HVEM, albeit overlapping with BTLA (Kojima et al., 2011). These differences between CD160 and BTLA, though subtle, suggest that these molecules do not have redundant functions. Further delineation of the elusive HVEM/CD160 pathway and its functional implications are required to unravel its specific role in regulating immune responses.

IL3T AND IL3T/HLA-G PATHWAYS

The inhibitory receptor Ig-like transcript-3 (IL3T, Cella et al., 1997) and IL4T (Colonna et al., 1998), both expressed by monocytes, macrophages, and DCs, belong to a family of Ig-like inhibitory receptors that are closely related to the killer cell inhibitory receptors. Both IL3T and IL4T were shown to transmit signal through a long cytoplasmic tail containing ITIM motifs, which inhibit cell activation by recruiting the protein phosphatase SHP-1 (Cella et al., 1997; Colonna et al., 1998). In the case of IL3T, the extracellular region consists of two Ig-like domains, which are speculated to contain the putative binding site of the yet to be identified IL3T ligand (Cella et al., 1997). On the other hand, the binding partner of IL4T was shown to be the MHC class I molecule human leukocyte antigen G (HLA-G, Colonna et al., 1998). In addition to triggering an inhibitory signal, IL3T cross-linking was shown to lead to its internalization and delivery into an antigen presenting compartment, suggesting a role in antigen processing (Cella et al., 1997). DC expression of IL3T and IL4T was shown to be induced under the effect of CD8+ CD28- alloantigen-specific T suppressor cells (Chang et al., 2002). Immature monocyte-derived DCs (MoDCs) also upregulated IL3T and IL4T expression upon treatment with either IL-10 or/and IFN-α (Manavalan et al., 2003). ViTt treatment only induced IL4T expression in MoDCs (Manavalan et al., 2003) and primary human blood BDCA1+ DCs (Chu et al., 2012). Interestingly, IL3T expression, by both MoDCs and pDCs, was downregulated following activation (Ju et al., 2004).

Tolerogenic DCs over-expressing IL3T or IL4T demonstrated impaired NF-κB activation and consequently reduced transcription capacity of NF-κB-dependent co-stimulatory molecules (Chang et al., 2002). Those DCs were shown to be capable of transforming alloreactive effector T cells into antigen-specific Tregs (Manavalan et al., 2003). Similarly, triggering IL4T by HLA-G tetramers was shown to impair maturation and T cell stimulatory capacity of human DCs (Liang and Horuzsko, 2003). Interestingly, IL3T was shown to maintain its T cell inhibitory effect when it was expressed as soluble IL3T-Fc that lacks IL3T’s cytoplasmic tail, indicating that IL3T delivers its inhibitory signal by binding to its partner on activated T cells (Kim-Schulze et al., 2006). Recently it was shown that IL3T capacity to convert T cells into suppressive cells is dependent on BCL6 signaling in these T cells (Chang et al., 2010). IL3T is also proposed to be important for controlling inflammation, as silencing IL3T expression in DCs enhances TLR responsiveness, which is reflected by enhanced secretion of inflammatory cytokines such as IL-1α, IL-1β, IL-6, and IFN-α. IL3T-silenced DCs could also attract more lymphocytes by secreting high levels of the chemokines CXCL10 and CXCL11 in response to TLR ligation. Eventually, impaired IL3T expression in DCs rendered them more stimulatory for T cells, which also secreted higher levels of cytokines like IFN-γ and IL-17 (Chang et al., 2009). Another suggested mechanism by which both IL3T and IL4T contribute to tolerance is by possibly mediating the effects of IDO. DCs cultured in tryptophan-deprived local environment upregulated the expression of IL3T and IL4T, favoring the development of Foxp3+ Tregs (Brenk et al., 2008). Finally, IL4T was shown to be central for the development of type 1 Tregs, induced by IL-10-treated DCs (Gregori et al., 2010).
The effects of ILT3 and ILT4/HLA-G pathways are also evidenced in vivo. Immune modulation exerted by ILT4/HLA-G interactions is believed to mediate maternal tolerance toward the semi-allogenic fetus (Hunt et al., 2005). Moreover, in vivo treatment with VitD was shown to upregulate the expression of ILT3 on DCs in healing psoriatic lesions. Nevertheless, ILT3 was revealed to be dispensable for the induction of Tregs and completely overridden by the inhibitory effects of VitD (Penna et al., 2005). Consistently, maternal VitD intake during pregnancy was found to enhance ILT3 and ILT4 gene expression levels in cord blood, pointing out a plausible mechanism for early induction of immune tolerance (Rochat et al., 2010). Enhanced ILT3 and ILT4 levels were also observed at an early stage of venom-specific immunotherapy, implying a possible role in inducing tolerance toward allergic reactions (Bussmann et al., 2010). Owing to its inhibitory effects, ILT3 is suggested to be employed by tumors as a mean of evading anti-tumor immunity. Indeed, soluble ILT3 protein was found at high levels in the serum of patients with melanoma, and carcinomas of the colon, rectum, and pancreas produce. This soluble ILT3 was active in inducing suppressor CD8+ T cells that block anti-tumor immunity, which was restored upon blocking or depleting ILT3 (Suzuki-Foca et al., 2007). A similar mechanism is also utilized by viruses, as demonstrated by a point mutation in one of HIV Gag epitopes that increased binding to ILT4 and consequently programed myelomonocytic cells to become tolerogenic (Lichterfeld et al., 2007). The inhibitory effects of ILT3 can also be harnessed for allograft acceptance. Indeed, soluble recombinant ILT3-Fc was shown to suppress T-cell-mediated rejection of allogeneic islet transplants in mice (Vlad et al., 2008). In correlation to its inhibitory effect, blood monocytes during multiple sclerosis relapses demonstrated lower ILT3 expression, which was restored upon treatment with IFN-β, unraveling a plausible therapeutic target in the treatment of multiple sclerosis (Jensen et al., 2010). Similarly, a SNP in the ILT3 extracellular region was correlated with low surface expression and increased serum cytokine levels in lupus patients (Jensen et al., 2012).

CONCLUDING REMARKS AND FUTURE PROSPECTS

Since the identification of the CD80/CD86/CD28 classical co-stimulatory pathway, the concept of DC-derived signal II was dramatically expanded to accommodate the ever increasing number of newly discovered co-stimulatory and co-inhibitory pathways. An increasing body of reports reflects the complexity of these pathways and implies possible interactions to form a sophisticated network controlling adaptive immune responses. The existence of multiple co-stimulatory and co-inhibitory pathways postulates for overlapping functions. Nevertheless, this notion of redundancy should be considered carefully. The components of these pathways have distinct expression patterns and kinetics, which means that these pathways are not simultaneously operative. In addition, mobilizing these pathways can trigger distinct signaling cascades and thereby leading to variable outcomes.

Dendritic cell expression of co-stimulatory and co-inhibitory molecules is dictated by several factors. The specific type of DC is a major determinant of this expression. In humans, DCs are classified into groups based on origin, specific expression of certain surface markers, and functional properties. For example, human blood DCs are divided into two major subsets: pDCs and myeloid DCs (mDCs). The latter can be further divided into three subsets: BDCA1+ DCs, BDCA3+ DCs, and CD16+ DCs. In parallel, skin DCs are also classified into epidermal LCs, dermal CD1a+ DCs, and dermal CD14+ DCs. Similar classification can be expected in other tissue-resident DCs. Most of the findings concerning co-stimulatory and co-inhibitory molecules in humans were based on experiments performed on the in vitro generated MoDCs, which serve as a great tool for delineating immunological functions and mechanisms. However, there are strong indications of differential expression of co-stimulatory and co-inhibitory molecules among different DC subsets. These variations can be partially related to the intrinsic qualities of every DC subset. For instance, pDCs and LCs lack the expression of TLR4, and consequently they are not able to upregulate CD80 and CD86, observed in other subsets in response to lipopolysaccharide (LPS).

Another central determinant of co-stimulatory and co-inhibitory molecules expression by DCs is the type of stimulus, to which DCs are exposed. As mentioned earlier, DCs respond to pathogen stimulation by upregulating CD80 and CD86. However, there are indications that certain co-stimulatory molecules are strictly expressed upon activation with a specific class of pathogens. A clear example is CD70 expression by LCs upon TLR3 triggering by double-stranded RNA derived from viruses, granting LCs advantage in eliciting strong anti-viral CD8+ T cell responses. Although dermal DCs and MoDCs express TLR3, they do not upregulate CD70 in response to double-stranded RNA, implying a combined effect of the type of stimulus and the type of DC in inducing CD70 expression. Similarly, pDC stimulation with CpG B, a TLR9 ligand, induced the expression of CD70, which was not observed using another type of stimulation or in other DC subsets (Shaw et al., 2010). Another example demonstrating the effect of pathogenic stimulation is the upregulation of OX40L only upon exposure to the soluble egg antigen from the parasite Schistosoma mansoni. Furthermore, DC treatment with certain immune modulating agents can influence the expression of co-stimulatory and co-inhibitory molecules. VitD-treated DCs displayed induced expression of PD-L1 and ILT3, concurrent with inhibited expression of CD80 and CD86. On the other hand, DCs under the influence of IL-10 had normal expression levels of CD80 and CD86 but over-expressed ILT3 and ILT4. It is also evident that DCs are strongly influenced by cues derived from the local environment. The well-documented effect of VitD, the major component of local skin milieu, is a clear example. The influence of other known tissue-related environmental factors on co-stimulation requires further elucidation. Thus, optimal understanding of the role of DC-derived signal II requires determining the total repertoire of co-stimulatory and co-inhibitory molecules expressed by different DC subsets and under different conditions.

In addition to the differential DC expression of co-stimulatory and co-inhibitory molecules, the respective ligands of these molecules are also described to be expressed by T cells following different kinetics. Some of these ligands are constitutively expressed, like CD86, whereas others are restricted to recently TCR-activated T cells such as 4-1BB and GITR. Furthermore, some of these ligands were shown to be exclusively expressed by...
certain types of effector T cells, like the TH1-specific expression of TIM3. Taken together, the different expression modalities of the co-stimulatory and co-inhibitory pathway components imply that these pathways are mobilized at certain stages of T cell priming and under specific conditions.

Despite the stimulatory or inhibitory nature of signal II, there are some indications pointing out that signal II does not play a role in T cell polarization, typically undertaken by cytokine-based signal III. For instance, OX-40/OX-40L and 4-1BB/4-1BBL pathways are proposed to promote the differentiation of TH2 and TH1 effector cells, respectively. Nevertheless, the observed polarizing effect was in many occasions revealed to be the mere outcome of promoted T cell survival rather than active polarization signaling mediated by these co-stimulatory or co-inhibitory molecules. Therefore, reported contributions of signal II to T cell differentiation should be interpreted carefully and further investigated.

The vast immunological consequences of signal II have transformed its pathways, both stimulatory and inhibitory, into therapeutic targets for the treatment of a wide variety of diseases. Mobilizing co-stimulatory pathways and blocking co-inhibitory interactions showed promising results in promoting anti-tumor immunity and it is proposed to be beneficial for the treatment of chronic viral responses. Assuming that mature DCs provide optimal positive co-stimulatory signals while priming anti-tumor T cells, blocking co-inhibitory pathways may augment the efficacy of these T cells. In that respect, concurrent targeting of multiple co-inhibitory pathways might be necessary. Neutralizing the key inhibitory check point CTLA-4 permits extensive primary T cell activation, but by itself is not sufficient for driving an anti-tumor immune response, especially in the case of advanced tumors. However, the additional circumvention of yet another co-inhibitory check point, which is dictated by the tumor itself, may solve this problem. Selecting the second inhibitory target would highly depend on the type of existing co-inhibitory pathways that are expressed. In some occasions, a combinatorial blocking strategy may not only mount efficient anti-tumor T cell responses, but also allow the persistence of such responses within the local tumor environment.

On the other hand, promoting tolerance by blocking activation and mobilizing co-inhibitory pathways is a promising strategy for raising allograft tolerance. Similarly, immune suppressant agents were also revealed to manipulate these pathways in a comparable manner to induce tolerance. Nevertheless, these therapeutic modalities should be applied with great care to avoid any possible adverse effects like inducing susceptibility to infection or autoimmune reactions. Targeting these therapies to a specific pathway or a specific cellular compartment, like a certain DC subset, may be an option to bypass any possible complications.

ACKNOWLEDGMENTS

This work was supported by a KWO grant (KWF2009-4402), NWO grants (Vidi-917.76.365, 95013002, 95101006), and EU grant (Pharmachild-260353) and a RUNMC PhD grant.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.