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CD28, CTLA-4 and their ligands: who does what and to whom?

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INTRODUCTION

T-cell activation is a critical event in the organization of effective cellular and humoral immune responses. Activated T cells are essential for provision of T-cell help, promoting the development of high-affinity antibody production and the generation of cytotoxic T-cell responses. Accordingly, defects in proteins required for T-cell activation give rise to significant infectious pathology and malignancies. However, the decision to allow T-cell activation also has potentially dangerous consequences for the host and must therefore also be tightly controlled. Defects in proteins involved in regulating activated T-cell behaviour therefore tend to lead to autoimmunity. Thus, the major challenge faced in regulating T-cell responses is how to maintain a sufficiently large immune repertoire capable of recognizing all possible foreign antigens, whilst at the same time maintaining T cells in an unresponsive state towards self-antigens.

In recent years significant progress has been made in our understanding of the mechanisms of self-tolerance. In the thymus it is clear that large numbers of potentially 'self-reactive' T cells are eliminated during negative selection in a process termed central tolerance. However, paradoxically, the process of positive selection that permits the expansion of T cells with low avidity for self–major histocompatibility complex (MHC) interactions must also lead to a degree of self-reactivity which is presumably tolerable in peripheral T cells. The question is how such T cells (albeit weakly self-reactive) can be ensured to remain non-reactive amongst a different array of self-antigens in the periphery. In the last few years a number of proteins have been identified that may serve the function of 'quality controlling' peripheral T-cell activation. This review focuses on two proteins, CD28 and cytotoxic T lymphocyte antigen-4 (CTLA-4), and explores how their interactions with their natural ligands may regulate the outcome of T-cell receptor engagement amongst peripheral T cells.

TAKE YOUR PARTNERS: CD28, CTLA-4 AND THEIR LIGANDS

CD28 and CTLA-4 (CD152) are transmembrane protein members of the immunoglobulin gene superfamily containing a single extracellular 'V-like' domain.[1–3] Both proteins are predominantly expressed by T cells and whilst CD28 is found in substantial amounts on the cell surface of the majority of resting T cells, in contrast CTLA-4 surface expression is much more limited.[4] The levels of CTLA-4 expression in most resting T cells are extremely low (or probably absent), and CTLA-4 predominantly appears following T-cell activation. However, despite maximal expression being reported at 48–72 hr post-activation, remarkably little stable surface CTLA-4 is found, although mRNA is equivalent to that of CD28.[5,6] This lower level of cell-surface expression results from a motif in the cytoplasmic domain of CTLA-4 that facilitates its interaction with the clathrin pit adaptor complex (AP-50) causing its rapid internalization from the cell surface.[7–9] Consequently the majority of CTLA-4 is found in intracellular vesicles that may be then targeted to the cell surface at the site of T-cell receptor (TCR) contact.[10] It has been suggested that phosphorylation of the CTLA-4 cytoplasmic domain results in disengagement from the AP-50 internalization system and therefore stabilizes cell-surface expression.[8]

The complexity of the CD28/CTLA-4 receptor interactions stems from the fact that there are two natural ligands CD80 (B7-1) and CD86 (B7-2) for these receptors.[11–17] Whilst these ligands can both interact with either receptor, they are only approximately 25% identical in sequence and it has therefore been attractive to speculate that they may serve different functions. Predictably, for co-stimulatory ligands, CD80 and CD86 are found on professional antigen-presenting cells such as dendritic cells, monocytes and activated B cells, although they can be induced on other cell types including T cells.[13,17–21] In general CD86 is the more abundant in terms of expression, and is increased more rapidly upon activation. In contrast CD80 is not generally found on resting antigen-presenting cells (APCs) and is induced more slowly upon cellular activation. A large variety of stimuli have been investigated in the control of CD80 and CD86 expression. Most of these, such as CD40, interferon-γ (IFN-γ), interferon-α (IFN-α), granulocyte–macrophage colony-stimulating factor (GM-CSF) and lipopolysaccharide (LPS) appear to result in increased expression[18,22–27] whereas others such as interlekin-10 (IL-10) and interleukin-4 (IL-4) may inhibit expression.[28–30] These expression studies, together with findings in CD80 and CD86 KO mice,[31–33] tend to indicate that CD86 is probably the major initial ligand for CD28 during T-cell activation, based mainly on its more rapid and abundant expression on APCs. However, functional data indicate that CD80 is probably the more potent CD28 ligand in terms of activation,[34,35] which is consistent with

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affinity measurements. Whilst affinity estimates have varied, the interaction of CD80 with both CD28 and CTLA-4 (4 μM and 0·4 μM, respectively) appears substantially better than that of CD86 (approx. 15–40 μM and 4 μM, respectively), although overall these interactions are still relatively weak.[36,37] An additional factor in these studies may relate to the fact that structural data indicate that CD80 is expressed as a dimer.[38] Thus in summary, CD28 can be considered a highly expressed but low-affinity receptor, whereas CTLA-4 is a low abundance but higher-affinity receptor where both receptors interact with CD80 and CD86. A diagram depicting these interactions in general is shown in Fig. 1.

CD28: AN ENHANCER FOR T-CELL ACTIVATION

Current interest in the CD28 molecule stems from the concept that efficient activation of T cells requires signals from both the TCR and an additional co-stimulatory receptor. In the absence of this second signal, T cells either remain unresponsive or become actively tolerant to antigens. This concept was stimulated experimentally in a series of experiments by Jenkins *et al.*, which involved the use of chemically modified peptide-pulsed APCs.[39,40] These APCs were highly impaired in antigen presentation and T cells subsequently became unresponsive (T-cell anergy) to the same antigen. Anergy could also be prevented by provision of a co-stimulatory signal.[41,42] Similar conclusions were reached in studies of transgenic expression of MHC molecules on non-APCs.[43,44] Thus the concept of a 'co-stimulatory signal', which could rescue from anergy if provided at the same time as TCR engagement, began to emerge and was consistent with the functions of the newly identified CD28-ligand, CD80.[12,25] In particular CD28 was shown to be important in enhancement of proliferation and cytokine production by T cells, as well as in the preventing T-cell anergy, thus identifying it as a key second signal for T-cell activation.[45–47]

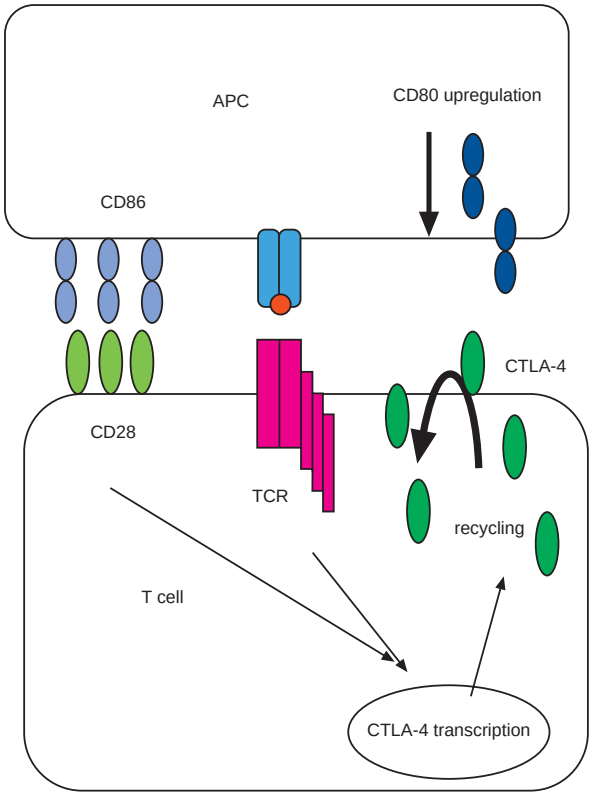

Figure 1. A schematic diagram of CD28 and CTLA-4 interactions with their ligands is depicted. CD86 is generally expressed at higher levels on antigen-presenting cells and is found more widely than CD80. CD80 is induced upon activation with a number of stimuli (see text) and is generally expressed at lower levels with later kinetics. Consequently, CD86 is the more likely primary ligand for CD28. On T cells CD28 expression is constitutive whereas CTLA-4 is not expressed by resting T cells. Both T-cell receptor stimulation and CD28 co-stimulation synergize to up-regulate CTLA-4, although CD28 stimulation is not essential. CTLA-4 expression is thought to be transient at the cell surface and rapidly re-internalized by a clathrin pit mechanism. It should be noted that both ligands can interact with both receptors.

Whilst this two-signal model is likely to be an over-simplification (there are an increasing array of alternative co-stimulatory molecules) these studies provided impetus for the concept that T-cell tolerance in the periphery might be maintained by restricting the provision of co-stimulatory signals. This has resulted in the use of a recombinant molecule (CTLA-4–Ig) which acts as a high-affinity antagonist of both CD80 and CD86 co-stimulatory ligands. Results using this protein have demonstrated considerable potential for blocking CD28/CTLA-4 interaction with their ligands.[48–52] However, the mechanism by which CTLA-4–Ig works is not entirely clear, as both preventing T-cell activation and engineering tolerance are possible. In this regard several studies have indicated that CTLA-4 engagement may actually be necessary for tolerance induction (see below).[53–55] At first sight this appears to be at odds with data from CTLA-4–Ig treatment, which should theoretically remove the ability of CTLA-4 to interact with its only known ligands, CD80 and CD86. One possibility, is that the doses of CTLA-4–Ig used do not entirely blockade CD80/CD86 interactions, but selectively promotes CTLA-4–ligand interactions by restricting the amount of available ligand. Overall, *in vivo* studies have yielded impressive results in transplantation models and early results in human trials look encouraging.[56] Whatever the mechanisms, these studies have provided support for the view that one way to maintain peripheral tolerance is to limit the provision of co-stimulatory signals through CD80 and CD86.

Despite considerable evidence that CD28 is critical in T-cell regulation, it is not entirely clear how its effects are mediated. The signalling pathways emerging from CD28 ligation have been studied in some detail, and have been reviewed elsewhere.[57] However, the absolute requirement for CD28 *in vivo* for T-cell proliferation has been brought into question by CD28KO mice.[58–60] Here, the response of T cells to antigen is not as severely impaired as might have been predicted. Nonetheless, there are substantial defects in the maintenance of responses and particularly in T-cell survival, which along with other studies supports a role for CD28 in maintaining T-cell responses.[61–64] Perhaps the most striking defect *in vivo* in CD28 KO mice is the lack of germinal centres, suggesting a gross defect in the ability of T cells to interact with B cells. This feature may well relate to the ability of T cells to express the chemokine receptor CXCR5 which is strongly influenced by CD28.[65] Thus CD28-deficient T cells may fail to migrate to appropriate sites of interaction with B cells.

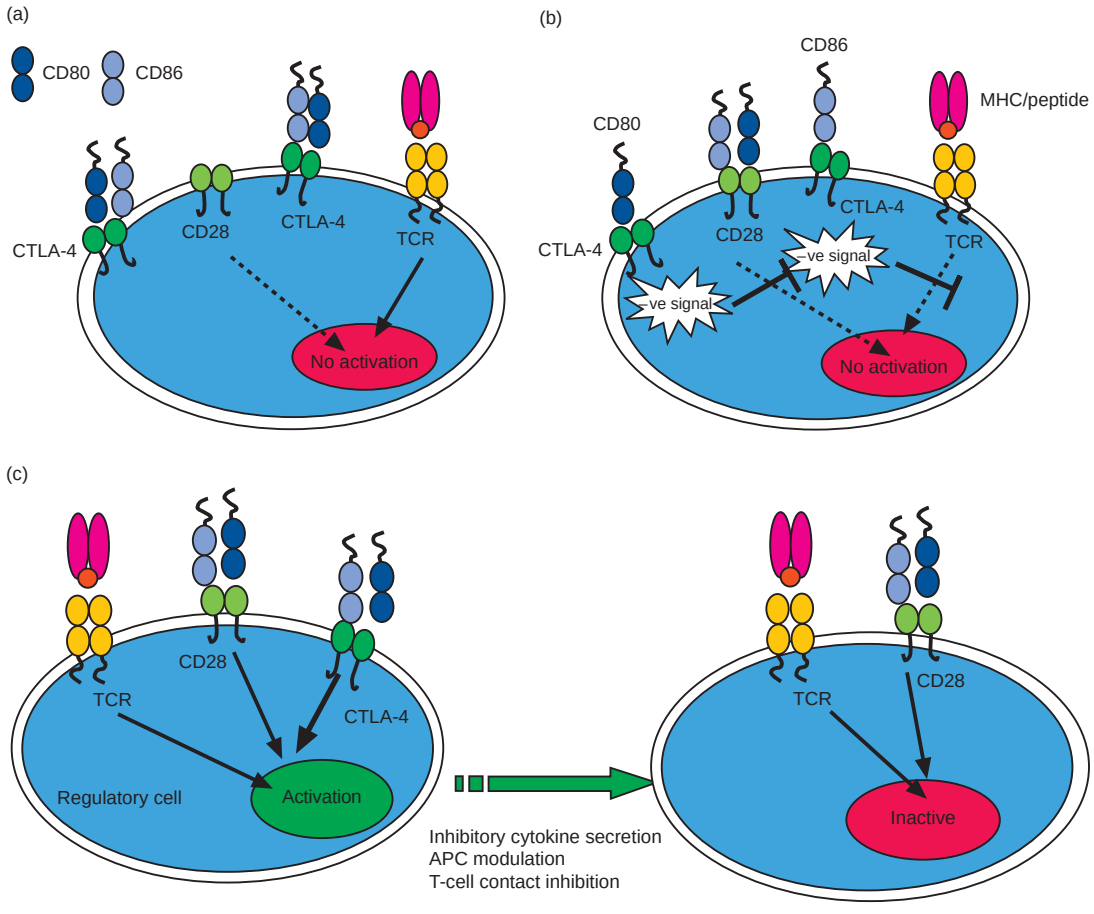

Figure 2. Three models of CTLA-4 function are shown. (a) The ligand competition model is shown. This model requires that CTLA-4 is expressed at sufficient levels to sequester ligands away from CD28 thus preventing co-stimulatory signals from being received. This model does not require a CTLA-4 signalling component and relies on the higher affinity of CTLA-4 for both ligands compared with CD28. In (b) a CTLA-4 signalling model is shown. Here CTLA-4 ligation results in signals that most likely inhibit T-cell receptor signalling resulting in lack of activation signals. In the third (regulatory cell) model (c), a CTLA-4-expressing regulatory cell is stimulated via CTLA-4 to exert suppression over other T cells. This mode of suppression may be either by cell contact or inhibitory cytokines. It should be noted that none of these mechanisms is mutually exclusive of the others.

Mechanistically, there is evidence that CD28 may exert its co-stimulatory effects by lowering the threshold for T-cell activation, consistent with the presence of CD28 in lipid 'rafts' that are rich in signalling proteins.[66,67] In addition, CD28 is also thought to exert effects on the cytoskeleton and promote its reorganization to the TCR contact site.[68,69] One of the more interesting recent observations has been the suggestion that CD28 may be involved in the control of a population of $CD25^+$ regulatory T cells.[70] Here, both CD28KO and CD80–CD86 double knockout (KO) mice crossed on to a non-obese diabetic (NOD) background demonstrated exacerbated diabetes that may be attributed to the lack of regulatory T cells. This study would suggest that CD28 co-stimulation may well be required for the proliferation and survival of this important T-cell subset.

Overall, there is now an overwhelming body of data implicating CD28 as a critical molecule in the T-cell activation process and inhibition of CD28 functions can prevent or substantially decrease T-cell activation. However, some of these strategies are complicated by the fact that the same ligands also control the functions of CTLA-4, which appears to have opposite functions to CD28.

CTLA-4: AN INHIBITOR OF T-CELL ACTIVATION

Whilst studies on CD28 have demonstrated a co-stimulatory role in T-cell activation, the role of CTLA-4 has been more difficult to elucidate. It is now generally accepted that CTLA-4 plays a role in the inhibition of T-cell activation;[71–73] although there are some more controversial suggestions of a stimulatory role.[74] Nonetheless, several laboratories have shown that blocking CTLA-4 enhances T-cell proliferation whereas ligating CTLA-4 with agonistic antibodies suppresses T-cell proliferation, consistent with the function of a negative regulator.[75] However, the most compelling evidence for a regulatory function for CTLA-4 has come from CTLA-4 knockout mice that develop fatal lymphoproliferative disease at 3–4 weeks of age, suggesting a critical role for CTLA-4 in maintaining self-tolerance.[76,77] This phenotype results from polyclonal activation of peripheral T cells that then infiltrate

and cause multiorgan destruction. This disease can be effectively cured by preventing CD28 co-stimulation either using CD80 and CD86 double KO mice, CTLA-4–Ig[78,79] or by crossing on to single TCR transgenic mice.[80–82] In addition the lymphoproliferation has been suggested to be $CD4^+$ dependent.[83] Very recently, CTLA-4 blockade in normal mice has been shown to give rise to spontaneous autoimmunity.[84] Collectively, these studies suggest that one possible function of CTLA-4 may be to 'threshold out' weak TCR engagements that may exist for large numbers of potentially autoreactive circulating T cells. Thus in the absence of CTLA-4, B7 ligands provide unopposed stimulatory signals through CD28 that permit weakly self-reactive T cells to become fully activated.

Whilst the importance of CTLA-4 is unquestioned, the nature of this inhibitory pathway is as yet poorly understood. Data from two laboratories indicate that CTLA-4 blocks T-cell function at a relatively early stage (within 24 hr), preventing up-regulation of activation markers, entry into cell cycle, and the generation of IL-2.[72,73] However, most strikingly these functional effects are seen when surface levels of CTLA-4 are undetectable. Our own analysis of CTLA-4 expression in humans (unpublished data) support the view that resting T cells express little or no CTLA-4 but that CTLA-4 transcription can rapidly up-regulate the protein within 6 hr of activation. However, whether this up-regulation is sufficiently rapid to prevent activation, or whether expression is actually a reflection of activation, is not yet clear. Interestingly, where CTLA-4 protein is detected at later timepoints (24–72 hr) after activation, it is exclusively confined to activated, proliferating T cells which, by definition, are not those inhibited through CTLA-4. This poses the question as to whether CTLA-4 is highly efficient in extremely low amounts or whether there are alternative explanations for this early inhibitory function (see below and Fig. 2). When interpreting data using CTLA-4 monoclonal antibodies it is important to consider that anti-CTLA-4 antibodies do not experience competition with CD28, in contrast with the natural ligands, and this may lead to inhibitory responses at significantly lower levels of CTLA-4 expression.

Whilst it is clear that natural ligands stimulate CTLA-4 function *in vivo*, the circumstances under which CTLA-4 function predominates have yet to be clearly established. For example, does CTLA-4 regulate all types of T-cell stimulation or are its effects confined to certain 'qualities' of TCR stimulation? So far, the majority of T-cell experiments using transfected ligands *in vitro* have indicated that engagement of CD80/CD86 in the presence of anti-CD3 effectively delivers proliferative signals via CD28 with relatively little evidence for CTLA-4-dominated functions under these circumstances.

STOP AND GO: THE BALANCE BETWEEN CTLA-4 AND CD28

The data discussed above provide a working model in which CD28 enhances and CTLA-4 inhibits T-cell responses yet both interact with the same ligands. This raises the obvious question of 'How do T cells choose between using CD28 and CTLA-4?' The answer to this question depends to a large extent on which models of CTLA-4 function are being considered. Several possibilities are outlined below; however, it should be noted that none of the models is mutually exclusive and all of these may operate under defined circumstances.

LIGAND COMPETITION

One of the most frequently cited models of CTLA-4 regulation is the concept that CTLA-4 may act as a competitive inhibitor for the ligands required for CD28 activation (Fig. 2a). Thus, by virtue of its higher affinity, CTLA-4 should be capable of 'out-competing' CD28 for ligand binding. An extension of this hypothesis is that CTLA−4 interactions would be favoured where the levels of ligands are low (for example on resting APCs). However the concept that low levels of ligand are relevant to control of CTLA-4 has yet to be convincingly demonstrated. Our own experiments aimed at testing this hypothesis (C. Ellwood *et al*., submitted) do not generally support this model. Nonetheless, the competition hypothesis is almost certainly correct given the relative affinities of CD28 and CTLA-4 for their ligands. There are, however, some caveats to this hypothesis. Competition is unlikely to have a significant effect in regulating the activation of resting T cells as these express undetectable levels of CTLA-4, making competition highly unlikely at this initial stage. In contrast, activated cells express more CTLA-4 and therefore ligand competition becomes possible during secondary stimulation of T cells with the directed expression of intracellular CTLA-4 at the 'immunological synapse'.[10] This concept is also supported by several studies that indicate more impressive CTLA-4 effects on secondary responses.[80,85] In addition, further support for ligand competition comes from studies of CTLA-4 KO mice that have been made transgenic for a CTLA-4 protein lacking a cytoplasmic domain.[86] Here, some but not all of the features of CTLA-4 KO mice were prevented, suggesting that competition is a distinct mechanism that accounts for only some of the features of CTLA-4 regulation. Whilst the temporal control of CTLA-4 expression is lost in this model, it nonetheless indicates that competition for ligand can be an effective mode of CTLA-4 operation. The most obvious problem with the competition model is the fact that agonistic antibodies to CTLA-4 act as potent inhibitors of T-cell proliferation.[75] Clearly as there are no ligands present in this context, competition alone cannot be the sole mode of CTLA-4 action. This observation provides support for a CTLA-4 signalling mechanism.

CTLA-4 SIGNALLING

As mentioned above, support for a CTLA-4 signalling model (Fig. 2b) comes most clearly from studies with agonistic monoclonal antibodies. Whilst signalling studies have been hampered by the low level of expression of this protein, generally these indicate that CTLA-4 can interfere with TCR-derived signals and block early signalling events.[87–90] The fact that CTLA-4 function can be observed in the absence of CD28 expression also supports the view that CTLA-4 may regulate TCR signals.[91] One possibility is that CTLA-4 recruits a tyrosine phosphatase SHP-2 (SYP, PTP-1D) through a phosphorylated YVKM motif in the cytoplasmic domain. This interaction is then thought to be involved in de-phosphorylating the TCR signalling machinery, thereby blocking early activation signals. However, in contrast to the

models where phosphorylated tyrosine residues in the CTLA-4 cytoplasmic domain are required for recruitment of signalling molecules,[88,92] several recent studies have also shown that tyrosine residues are not essential for CTLA-4 function.[88,93,94] Thus at present the nature of CTLA-4 inhibitory signalling mechanisms are still unclear. Whilst it is not strictly a part of the signalling model, the most common interpretation of the signalling hypothesis is that each individual T cell undergoes a 'fate' decision based on the relative in dominance of CD28 versus CTLA-4 signals (i.e. where CTLA-4 signals are dominant a given T cell will not be activated due to inhibition of TCR and CD28 activation signals). However, this is not the only possible interpretation of CTLA-4 signalling as discussed below.

REGULATORY CELL/CYTOKINE HYPOTHESES

One possible explanation of the biological effects of CTLA-4 observed when CTLA-4 expression levels are very low, is that these effects are actually mediated by a small number of regulatory cells that already express CTLA-4 (Fig. 2c). This possibility is supported by a number of recent findings and overcomes some of the problems discussed above. First, a recent study by Bachmann,[95] demonstrated that CTLA-4 functions were not necessarily T-cell autonomous. Here, CTLA-4 KO bone marrow was transferred into $RAG2^{-/-}$ mice either alone or mixed with wild-type CTLA-4 expressing bone marrow. Whilst CTLA-4-deficient bone marrow caused fatal T-cell infiltration of multiple organs, wild-type bone marrow could suppress disease, clearly demonstrating an ability of CTLA-4-positive cells to regulate CTLA-4 negative cells. Other studies have also suggested a link between CTLA-4 and the cytokine transforming growth factor-β (TGF-β).[96] This is consistent with similarities between TGF-β KO mice and CTLA-4 KO mice, although this link is still somewhat controversial at present. Consistent with this overall concept is the relatively recent re-emergence of regulatory T cells that cause the development of autoimmunity when removed[97,98] (for review see reefs 99,100). Two recent studies, and our own observations, suggest that that a relatively small number of $CD4^+$ $CD25^+$ T cells may also express CTLA-4 (C.Ellwood, unpublished observations).[84,101] Thus, the concept that important CTLA-4 functions may be mediated by a rare subset of regulatory T cell that then influences the function of the majority of other T cells is now a distinct possibility.

CD80 AND CD86: WHAT'S THE DIFFERENCE?

Given that CD28 and CTLA-4 have such contrasting functions it is therefore somewhat perplexing that they should share ligands. The most obvious and attractive hypothesis is that these ligands have clearly separate and discrete functions, yet to date this has been extremely difficult to demonstrate convincingly. Nonetheless there are numerous studies that indicate differences between these ligands. Initial studies using transfectants revealed no obvious differences and were consistent with the inescapable conclusion that both ligands are indeed capable of co-stimulation via CD28.[102] However, in general, CD80 appears to be the more potent stimulatory ligand.[34] Other studies that have attempted to address the differences between ligands have exploited antibody-blocking approaches; however, this has yielded conflicting results. In disease studies, blocking either molecule can exacerbate disease or inhibit disease depending on the model studied.[103–105] These and other studies also provide evidence that CD80 or CD86 can bias towards T helper 1 (Th1) or T helper 2 (Th2) phenotypes.[106] However, whilst there is no overall consistency, there are sufficient reports to suggest that these pathways do play a role in T-cell differentiation.[107,108] One of the major problems in interpreting these studies is that each uses a precise and highly variable set of conditions, e.g. mouse strain, type of antigen, adjuvent use, route of immunization, timing of observation, etc., making any attempts at generalization extremely difficult. It is therefore likely that in many cases, fundamentally different immunological processes are being investigated, which then compounds the problem of defining the specific functions of CD80 and CD86. Given the different models outlined above for CTLA-4 function, then any or all of these mechanisms may be differentially regulated by CD80 or CD86, and it is therefore necessary to know precisely which mechanism is being studied in any given model.

Further attempts to clarify the role of CD80 and CD86 have been made using genetic approaches and in particular KO mice.[31–33] Here, the roles of CD80 and CD86 have been confirmed as co-stimulators and the more dominant initial functions of CD86 have been substantiated. These studies also rule out the concept that either CD80 or CD86 is strictly required for Th1 or Th2 cytokine production. However, a different problem emerges with KO approaches for CD28/CTLA-4 ligands in that by removing a given ligand, both the stimulatory and inhibitory functions of that ligand are eliminated. It is therefore theoretically possible that these two opposing effects may compensate for each other and not truly reveal the functions of a given ligand. For example, based on affinity data, CD80 is both the best ligand for CD28 and CTLA-4; therefore, it is conceivable that CD80 knockouts do not display a CTLA-4 KO-like phenotype because the proliferative drive via CD28–CD80 interactions has also been eliminated. Thus, true differences between CD80 and CD86 are an integrated function of their interactions with both CD28 and CTLA-4, and therefore requires study of both pathways together.

Whilst it is difficult to speculate, clear differences have been observed that could support a model where CD80 might be considered a preferential (but not exclusive) inhibitory ligand for CTLA-4. First, the predominant early expression of CD86 on APCs would generally promote the initial establishment of T-cell responses, whereas the later and generally more restricted expression of an inhibitory CD80 molecule could then potentially regulate responses subsequently. Second, blocking antibodies to CD80 clearly exacerbate diabetes in NOD mice.[103] This model of disease is also known to be controlled by CTLA-4 regulation at an early stage and T-cell infiltration is exacerbated by CTLA-4 blockade.[109] Third, in a transgenic model of diabetes, CD86 expression promoted T-cell infiltration whilst CD80 did not. In this model, if one accepts that CD80 is a more potent co-stimulator, this would argue for an additional role for CD80 in T-cell regulation that is not seen in the CD86 trangenic. Fourth, in transplant models, CD80 has been indicated to be the preferential CTLA-4 ligand controlling graft survival.[51] An interesting feature that links these studies is that CTLA-4 function is being studied in

the context of spontaneous reactivity to auto- or alloantigens and that T-cell responses are not the result of an immunization protocol in the presence of adjuvant as in some models. One possibility is that these studies may all involve a consistent and distinct 'mode' of CTLA-4 function that may contrast with its role in the presence of 'danger signals' or during activation of the innate immune system. Fifth, structural data suggest that CD80 is organized as a dimer, which has not been shown for CD86, a factor that may influence its ability to differentially signal via CTLA-4.[38] Sixth, transgenic expression of CD80 and CD86 on T cells results in hyperproliferation only in the CD86 transgenic, but not in those expressing CD80.[110] Again, this would be consistent with a lack of regulatory ability within CD86. Consistent with this idea, transgenic expression of CD80 has previously been shown to provide a negative regulatory function.[111] Finally, our own recent data (C. Ellwood *et al.*, submitted) directly comparing CD80 and CD86 transfectants, only reveal CTLA-4 inhibition when it is ligated to CD80 and not CD86. Whilst these are clearly highly selected arguments, and numerous alternative interpretations are possible, they serve to illustrate that there is still little data that conclusively argue against one ligand performing preferential functions in relation to CTLA-4. Whilst it is clear that both ligands can and do interact with both receptors, their mechanisms of action are thus not necessarily similar. Given the likelihood that several discrete modes of CTLA-4 function exist, it seems that without models where the mode of T regulation by CD28 or CTLA-4 is very clearly defined, it will continue to be difficult to effect comparisons between CD80 and CD86.

The CD28/CTLA-4 pathway offers important opportunities as targets for immunomodulation and insights into our ability to tolerate self-antigens. Whilst our understanding of these molecules has advanced considerably in the last few years, significant questions still remain regarding why two ligands share receptors that have opposing functions. The still unresolved confusion in this area suggests we are in need of more precise models of the functions of each of the participants in order to resolve this important paradox.

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