

CTLA4IG AND THE THERAPEUTIC POTENTIAL  
OF T CELL CO-STIMULATION BLOCKADE

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**Abstract**

It is now generally accepted that CD4<sup>+</sup>T cells are critical players in the initiation of adaptive immune responses by contributing to the terminal differentiation of effector B cells or CD8<sup>+</sup> T cells. It is, therefore, not surprising that CD4<sup>+</sup> T cell activation is tightly controlled through the concerted action of a large number of molecular interactions. Activation requires not only the recognition of the appropriate antigen within a MHC molecule by the T cell receptor (TCR), but also the delivery of co-stimulatory signals by the antigen presenting cell (APC). As a consequence, therapeutic modulation of co-stimulatory molecules, for instance with CTLA4Ig, can lead to interference with T cell activation and consequently abrogation of pro-inflammatory manifestations mediated by cell types influenced by CD4<sup>+</sup> T cells, such as B cells, CD8<sup>+</sup> T cells, or macrophages. This type of observations provided the rationale for the use of co-stimulatory blockade in autoimmunity, and other immunopathology characterized by inappropriate immune activation, such as rheumatoid arthritis (RA). Several studies have also suggested that besides the non-specific anti-inflammatory effects, co-stimulation blockade may, in certain conditions, promote the induction of long term immune tolerance.

**Keywords:** Co-stimulation; Abatacept – CTLA4Ig; Rheumatoid Arthritis; Autoimmunity; Immune Tolerance.

**Resumo**

O papel fundamental dos linfócitos T CD4<sup>+</sup> na iniciação de respostas imunitárias adquiridas é actualmente consensual. Estas células contribuem

para a diferenciação final de linfócitos B e para a activação de células CD8<sup>+</sup>. Não é assim surpreendente que a activação dos linfócitos CD4<sup>+</sup> seja alvo de um grande controlo por parte de muitas moléculas diferentes. A activação destas células depende do reconhecimento de antígeno por um receptor de células T (TCR), mas também de sinais de co-estimulação provenientes da célula apresentadora de antígeno (APC). Assim, a modulação terapêutica da co-estimulação, por exemplo com CTLA4Ig (abatacept), pode interferir na activação das células T, contrariando os efeitos pró-inflamatórios decorrentes de células que dependem de linfócitos CD4<sup>+</sup> (como células CD8<sup>+</sup>, linfócitos B, ou macrófagos). Este tipo de observações tem fornecido os argumentos para o desenvolvimento de drogas que bloqueiam a co-estimulação para o tratamento de autoimunidade e outra patologia imunitária, como é exemplo a artrite reumatóide. Vários estudos têm sugerido que para além de efeitos anti-inflamatórios não específicos, o bloqueio da co-estimulação pode, em algumas situações, conduzir a tolerância imunitária prolongada.

**Palavras-chave:** Co-estimulação; Abatacept – CTLA4Ig; Artrite Reumatóide; Autoimunidade; Tolerância Imunitária.

**Introduction**

The initiation of adaptive immune responses requires the CD4<sup>+</sup> T cell activation by a dendritic cell (DC) presenting the appropriate antigen. As a consequence of such T cell – DC interaction, the CD4<sup>+</sup> T cell acquires functional characteristics that influence the subsequent activation and recruitment of other cell types to the ongoing immune response. A consequence of the initial cross-talk between the CD4<sup>+</sup> T cell and the DC is the «licensing» of the DC for efficient priming of CD8<sup>+</sup> T cells.<sup>1-3</sup> In the absence of this DC «licensing» naive CD8<sup>+</sup> T cells cannot be efficiently activated by DCs, even when the DC bears the

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appropriate antigen for CD8<sup>+</sup> T cell recognition.

The differentiation of naive B cells into antibody-producing plasma cells, as well as the selection of the immunoglobulin isotype (for example the decision between producing IgE, IgA, or IgG molecules), is determined by CD4<sup>+</sup> T cells that directly interact with B cells.<sup>4</sup> In addition, phagocytes such as macrophages, eosinophils, and even endothelial cells or fibroblasts can be influenced by the cytokines produced by CD4<sup>+</sup> T cells that consequently can contribute to pathology mediated by those different cell types.

The critical role of CD4<sup>+</sup> T cells in the orchestration of adaptive immune responses is eloquently illustrated by the manifestations of immunodeficiencies characterized by CD4<sup>+</sup> T cell defects, including genetic deficiencies in molecules important for T cell – B cell interaction, such as CD40L.<sup>5</sup> This last situation characterizes X-linked hyper-IgM hypogammaglobulinemia, a disease where the antibody production is compromised due to a defect in a single molecule critical for T cell – B cell crosstalk, leading the absence of germinal centers and incapacity to mount humoral immune responses.<sup>6</sup>

Therefore, T cell activation by DC, as well as the interaction between different types of T cells – including regulatory T cells – and DCs is likely to play an important part in RA pathogenesis.<sup>7,8</sup>

### The signal 1 and signal 2 for T cell activation – the immune synapse

The efficient activation of a T cell by a DC bearing the appropriate antigen, requires three different type of signals: signal 1, consisting on the molecules involved directly in antigen recognition; signal 2, the co-stimulatory molecules; and signal 3, comprising the soluble cytokines in the immediate vicinity of the T cell. In recent years, due to advances in live imaging techniques, the molecular events of T cell activation have become known in great detail.

CD4<sup>+</sup> T cell activation requires the recognition of the appropriate antigen in the context of a MHC class II molecule by the TCR, in a process where the co-receptor CD4 also participates by interacting with the class II molecule.<sup>9</sup> The intracellular signaling triggered by these molecular interactions requires the participation of CD3 – a group of molecular chains containing several intracellular domains critical for the amplification of stimulatory signals.<sup>10</sup> In the case of CD8<sup>+</sup> T cells, the molecular interactions are similar, but the antigen is presen-

ted in the context of MHC class I and the co-receptor CD8 replaces the role of CD4 in T cell activation. These molecular interactions correspond to what is generally designated as signal 1 (Figure 1A).

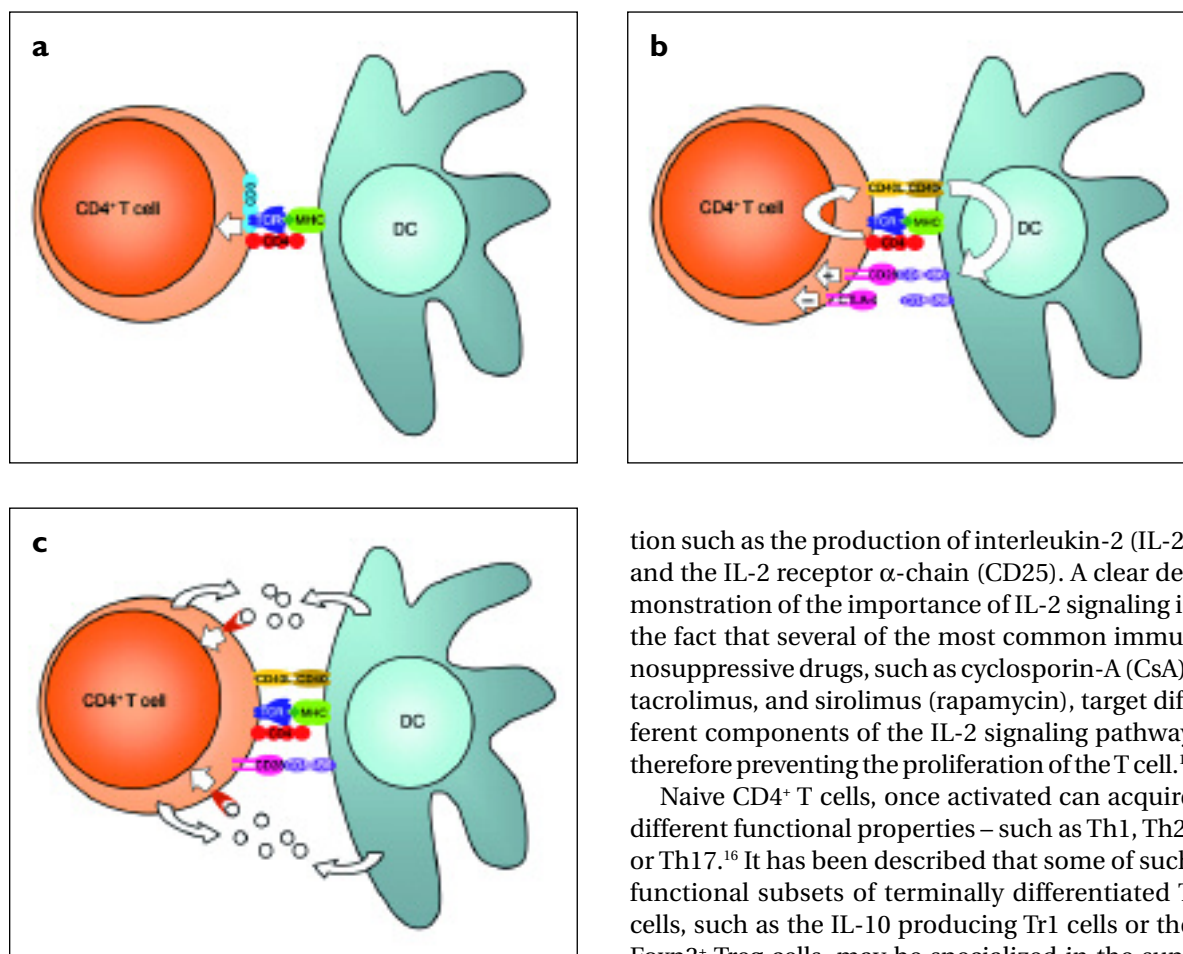
Efficient T cell activation also requires co-stimulation.<sup>11</sup> Although a large number of co-stimulatory molecules have been described, some of the most important for T cell activation are CD80 and CD86 (also known as B7-1 and B7-2), that when present on the APC engage CD28 on the T cell, thus providing strong activation signals. However, the expression of CD80 and CD86 is influenced by a prior interaction between CD40 (on the DC) with CD40L (also known as CD154, on the T cell). Furthermore, CD40L is only present on T cells following the events described as signal 1, comprising antigen recognition. As a consequence, the availability of co-stimulation by the DC is tightly regulated, as the DC will only become “licensed” to provide co-stimulation following the presentation of appropriate peptide to T cell (signal 1), driving the expression of CD40L by the T cell that then signals the DC to express its most potent co-stimulatory molecules – CD80 and CD86. The interaction of CD80/CD86 with CD28 provides strong activation of the T cell. Co-stimulation is usually considered as signal 2 for T cell activation (Figure 1B).

Live microscopy studies have shown that that the molecular interactions leading to T cell activation take place in organized macro-molecular structures named the immune synapse.<sup>12</sup> The immune synapse consists of a central region where molecules involved in signal 1 interact with the molecules involved in co-stimulation sharing the same distribution. This central region is surrounded by an outer ring of molecules involved in cell adhesion. Furthermore, TCR recognition of the antigen induces changes in the adhesion molecules present on the T cell, leading to stronger and more stable interactions with the DC thus facilitating full T cell activation.<sup>13</sup>

It should be noted that co-stimulation in the absence of signal 1 does not support T cell activation and proliferation. But signal 1 in the absence of co-stimulation, as discussed below, is also insufficient for the full activation of pro-inflammatory T cells.<sup>14</sup>

### The signal 3 for T cell activation – the cytokine environment

A consequence of the signaling pathways induced



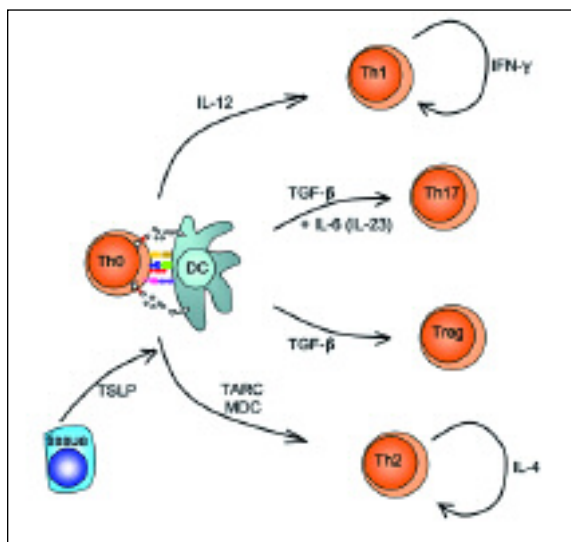
**Figure 1.** The three signals model for T cell activation. **(A)** Signal 1. The events leading to T cell activation start with TCR recognition of the appropriate antigen presented in the context of an MHC molecule. CD4 participates in this process, and the molecular complex CD3 is important for the signal transduction. **(B)** Signal 2. A consequence of signal 1 is the expression of CD40L by the T cell. The engagement of CD40L with CD40 drives the expression of the co-stimulatory molecules CD80 and CD86 by the DC. CD80 and CD86 bind CD28 on the T cell delivering potent stimulatory signals to the T cell. Activated T cells start expressing CTLA-4. As this molecule binds CD80 and CD86 with greater affinity than CD28, CTLA-4 contributes to the termination of the positive stimuli delivered by CD28. In addition, CTLA-4 delivers inhibitory stimuli to the T cell. **(C)** Signal 3. The T cell produces a high affinity IL-2 receptor, as well as IL-2 essential for its own proliferation. In addition, the DC releases cytokines able to influence the cell fate of the T cell.

by signal 1 and signal 2 is the induction of a gene expression program on T cells leading, among other things, to factors important for T cell prolifera-

tion such as the production of interleukin-2 (IL-2) and the IL-2 receptor  $\alpha$ -chain (CD25). A clear demonstration of the importance of IL-2 signaling is the fact that several of the most common immunosuppressive drugs, such as cyclosporin-A (CsA), tacrolimus, and sirolimus (rapamycin), target different components of the IL-2 signaling pathway therefore preventing the proliferation of the T cell.<sup>15</sup>

Naive CD4<sup>+</sup> T cells, once activated can acquire different functional properties – such as Th1, Th2, or Th17.<sup>16</sup> It has been described that some of such functional subsets of terminally differentiated T cells, such as the IL-10 producing Tr1 cells or the Foxp3<sup>+</sup> Treg cells, may be specialized in the suppression of excessive immune responses.<sup>17</sup> The decision between the different types of specialized T cells differentiating from naive precursors is determined, to a large extent, by the nature of cytokines present at the site of T cell activation. These cytokines, produced by the APC or the T cells, are generally referred as signal 3 (Figure 1C).

For instance, activation in the presence of IL-12 derived from the DC favors the T cell differentiation in Th1, a subset specialized in the production of interferon- $\gamma$  (IFN- $\gamma$ ), associated with macrophage activation and pro-inflammatory manifestations characteristic of several autoimmune diseases (Figure 2). However, if T cell activation takes place in an IL-4 rich environment, the resulting activated T cells assume a Th2 phenotype characterized by the secretion of IL-4, IL-5, IL-9, and IL-13 molecules associated, for example, with the differentiation of B cells into IgE-secreting plasma cells, and recruitment of eosinophils. These Th2 cytokines are important in parasite infections and also in allergy. More recently, it was described that T cell



**Figure 2.** The functional specialization of T cells is determined by the cytokine environment at the time of activation. T cell activation in conditions leading to the expression of IL-12 by the DC will trigger the differentiation of the naive CD4<sup>+</sup> T cell towards an IFN- $\gamma$  producing Th1 cell. IFN- $\gamma$  will then reinforce the polarization towards Th1 type, while directly inhibiting the differentiation of other functional T cell subsets (Th2, Th17). If T cell activation takes place in an environment containing TGF- $\beta$ , the T cell will differentiate towards a Treg - Th17 pathway. The decision between these two subsets is due to the presence of DC-derived IL-6 that inhibits Treg polarization while inducing the acquisition of a Th17 phenotype. In addition, IL-23 favors the survival of Th17 cells. Finally, peripheral tissues, such as epithelial cells, can produce a cytokine named TSLP able to induce the acquisition of a Th2-polarizing phenotype by the DC. Such TSLP-conditioned DCs do not produce Th1 or Th17-polarizing cytokines, but instead produce TARC and MDC known to promote a Th2 immune response. This type of Th2 polarization is further reinforced in the presence of IL-4 - a cytokine produced by Th2 cells that further stimulates Th2 differentiation while directly inhibiting Th1 polarization.

activation in the presence of IL-6 and transforming growth factor- $\beta$  (TGF- $\beta$ ) promotes the acquisition of a Th17 phenotype, characterized by the production of IL-17. Strikingly it appears there is a subtle balance between the differentiation of anti-inflammatory Treg cells and pro-inflammatory Th17. In fact the activation in the presence of TGF- $\beta$  is required in both cases, and it is the concomitant presence of IL-6 that inhibits Treg polarization while promoting the acquisition of a Th17 fate. Recent studies have suggested an important role for Th17

and IL-17 in RA.<sup>18</sup> This cytokine has an important role on neutrophil activation and strong pro-inflammatory action.<sup>19</sup> Importantly, cytokines produced by each type of activated T cell inhibit the differentiation of naive T cells towards different types of activated T cells thus creating a feed-forward mechanism that further amplifies immune responses with the same characteristics (Figure 2).

### CTLA-4 and the termination of immune responses

The termination of a normal immune response is not known in the same detail as its initiation. However, negative co-stimulatory molecules such as CTLA-4 appear to have an important role in switching off an immune response,<sup>20</sup> thus limiting the feed-forward loop associated with T cell activation described above.<sup>21</sup> A good illustration of this role of CTLA-4 in terminating immune responses derives from studies in cancer immunotherapy, where it has been shown that blockade of CTLA-4 allows the persistence of anti-tumor immune responses.<sup>22</sup> Another indication concerning the critical role of CTLA-4 in the regulation of pathological immune responses derives from observations that a number of autoimmune diseases (such as RA, type-1 diabetes, multiple sclerosis, Grave's disease, Addison's disease, and Hashimoto's thyroiditis) have shown linkage with the CTLA-4 locus. Furthermore, different splice variants of CTLA-4 are associated with greater risk for the development of autoimmune diseases.<sup>23</sup>

CTLA-4 is expressed by activated T cells and has the capacity to bind to CD80 and CD86 with greater affinity than CD28. Moreover, and contrary to the stimulatory signals delivered to the T cell following CD28 engagement, CTLA-4 binding to CD80/CD86 triggers the delivery of inhibitory signals that counter the activation effect of co-stimulatory molecules.<sup>24,25</sup> In addition, CTLA-4 binding of CD80/CD86 on DCs can influence DC function. It was shown that such interactions (for instance following exposure to CTLA4Ig) stimulate an intracellular signaling pathway on the DC ultimately driving the production of indoleamine 2,3-dioxygenase (IDO), an enzyme with immunosuppressive function by promoting the local degradation of tryptophan and therefore preventing T cell proliferation.<sup>26</sup> These observations were more recently confirmed in human DCs.<sup>27</sup> Furthermore,

CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>Treg cells constitutively express CTLA-4. An interaction between Treg cell and DC was also shown *in vitro* to lead to IDO production by the DC following CTLA-4 engagement.<sup>28</sup> Given this requirement of CTLA-4 interactions for the function of Treg cells, it is not surprising that in some conditions CTLA4Ig treatment may compromise immune tolerance by acting on the development and function of Treg cells.<sup>29</sup>

As a consequence, CTLA-4 appears to have three different actions in limiting T cell activation: first, by competing with CD28 for binding to CD80 and CD86, where CTLA-4 is in great advantage due to a much greater affinity; second, by delivering inhibitory signals to the T cell that counter the activation signals delivered by CD28; and finally by signaling the DC to acquire characteristics that no longer support T cell activation and directly inhibit T cell proliferation (through the expression of IDO).

Other co-stimulatory molecules, with stimulatory and inhibitory effects have been described (Table I). Several of those molecules have become promising targets for the development of pharmacological compounds for the modulation of immune responses.

## Immunomodulation by co-stimulation blockade

Given the central role of co-stimulation in the regulation of immune responses, several strategies have been developed for co-stimulation blockade as a way to prevent undesired or excessive immune responses in autoimmunity, transplantation and allergy.<sup>30,31</sup>

One way to block co-stimulation is through antibodies binding to CD40L and thus preventing CD40/CD40L interactions.<sup>32</sup> This strategy has been successfully used in several animal models of autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE), and lupus, both in preventing the onset of the disease or in treating the disease progression after its onset.<sup>33-36</sup> It was recently shown that treatment with anti-CD40L mAbs can prevent allergic sensitization in mice, but cannot prevent the disease in pre-sensitized animals.<sup>37</sup> Besides autoimmunity, CD40L-blockade was shown effective in preventing allograft rejection leading to long-term transplantation tolerance in mice, rats, and non-human primates.<sup>38-42</sup> Unfortunately, the presence of CD40L on human pla-

**Table I. T cell co-stimulatory molecules.**

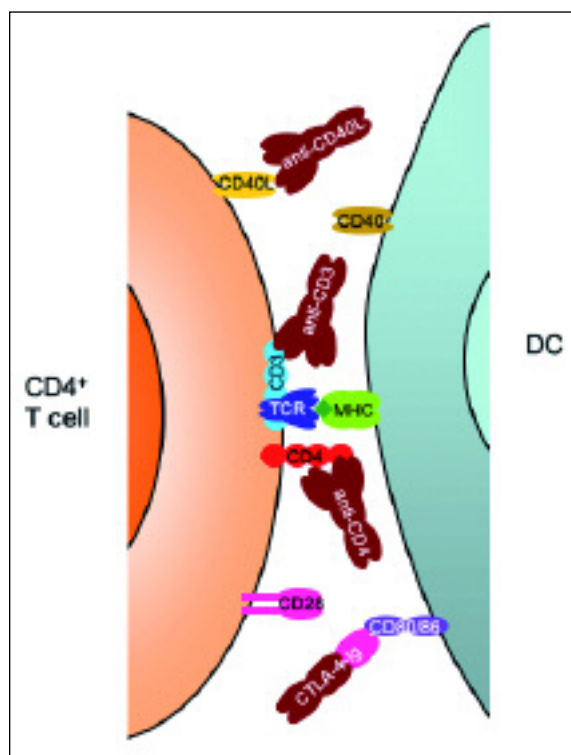
T cell	APC	Effect
CD28	CD80	
CD86	+++	
CTLA-4	CD80	
CD86	- -	
ICOS	ICOSL	+
PD-1	PDL1	
PDL2	-	
???	B7-H3	+
CD40L	CD40	+
4-1BB	4-1BBL	+
OX40	OX40L	+
GITR	GITRL	+/-
CD27	CD70	+
LFA-1	ICAM-1,2,3	0/+
CD2	LFA-3	0/+
CD47	???	0/+

A large number of co-stimulatory molecules with different effects have been described. The table details the molecular pairs that establish interactions between the T cell and the APC, as well as the outcome in terms of T cell stimulatory signals: +++, strong activation; - inhibition; +/-, can drive stimulatory or inhibitory signals; ???, currently unknown.

telets has hampered the translation of these pre-clinical results into clinical treatments as in human patients the use of CD40L mAbs may lead to thromboembolic complications.<sup>35,43</sup>

CTLA4Ig (abatacept) is the only drug blocking co-stimulation that has been licensed for clinical use.<sup>44</sup> CTLA4Ig has been shown effective in inhibiting T cell activation not only by preventing CD28 interaction with CD80 and CD86 on the DC, but also by inducing the expression of IDO by the DC, as discussed above. The initial pre-clinical studies concerning therapeutic modulation of immune pathology with CTLA4Ig were done in organ transplantation,<sup>45,46</sup> where it was shown able to induce long term graft survival. These results were extended to autoimmunity, where CTLA4Ig was able to prevent the onset or treat several animal models of autoimmune diseases, such as murine lupus,<sup>47</sup> EAE,<sup>48</sup> and diabetes.<sup>49</sup>

Several of the above mentioned pre-clinical studies with CTLA4Ig suggested the induction of long-term tolerance, as the treatment could be stopped after a short time, with the persistence of its beneficial effects.



**Figure 3.** The therapeutic targeting of the immune synapse. Several biological drugs tested for their ability to prevent immune pathology have been suggested to induce immune tolerance. These molecules include non-depleting monoclonal antibodies targeting the co-receptor molecules CD3 and CD4; co-stimulation blockade with anti-CD40L; and co-stimulation blockade with CTLA4Ig.

### Co-stimulation blockade as a strategy for tolerance induction

It has been a long held assumption, known as the «two-signal model for T cell activation», proposed by Bretschner and Cohn almost 40 years ago,<sup>50</sup> that T cell activation in the absence of co-stimulation would lead to T cell deletion or functional inactivation (anergy). Recent studies in transplantation have indeed confirmed that tolerance induction mediated by co-stimulation blockade required activation-induced cell death (AICD).<sup>51,52</sup> In these reports, the administration of CsA at the time of co-stimulation blockade was shown to prevent tolerance induction as the drug prevents T cell activation and, consequently, AICD. In the same study, the immune suppressive drug rapamycin did not abrogate tolerance induction, as it targets T cell proliferation without interfering with the initial steps of T cell activation, and thus allows AICD. A

report has also shown that the activation of complement by CD40L-blocking mAbs can also contribute to the elimination of T cells.<sup>53</sup>

However, T cell deletion is only half the story. Subsequent studies have demonstrated that co-stimulation blockade can lead to the emergence of regulatory T cells (Treg cells) and a state of dominant tolerance.<sup>54</sup> One of the characteristics of such tolerance state is its robustness: the Treg cells while co-existing with potentially aggressive T cells can recruit some of those aggressive T cells into becoming regulatory, a phenomenon named «infectious tolerance» and initially described for tolerance induced with mAbs targeting CD4 and CD8.<sup>55</sup>

It is now generally accepted that the outcome of co-stimulation blockade is to tip the balance of the immune system towards tolerance by reducing the number of potentially aggressive T cells while expanding suppressive Treg cells.<sup>56</sup> The Treg cells can be found not only in lymphoid organs, such as the spleen and lymph nodes, but also infiltrating the tissues harboring the antigens they recognize, such as tolerated allografts.<sup>57</sup> In addition, the induction of IDO expression by DCs, following direct interaction with Treg cells or CTLA4Ig, or other local tolerogenic mediators further reinforce the state of dominant tolerance.<sup>58</sup> Moreover, it has been suggested that co-stimulation blockade may contribute to the induction of tolerance by altering the threshold for T cell activation leading to peripheral Treg cell expansion.<sup>59</sup>

Currently a number of other T cell and DC molecules are being targeted by mAbs as a way to achieve immune tolerance (Figure 3). It is the case of CD3,<sup>60</sup> CD4,<sup>61,62</sup> OX40L,<sup>63</sup> ICAM-1,<sup>64</sup> LFA-1.<sup>65</sup>

### Co-stimulatory blockade in the clinical arena

The initial pre-clinical studies of CTLA4Ig in non-human primates were conducted in transplantation. In those studies CTLA4Ig did not prove efficient in achieving long-term transplant survival except if given continuously, or combined with CD40L blockade.<sup>38,66</sup> For this reason, treatment with CD40L blockade appeared more appealing than with CTLA4Ig. However, the identification of unanticipated side effects in anti-CD40L phase II clinical trials,<sup>67</sup> together with encouraging results in terms of efficacy and safety from an open-label dose-escalation phase I trial of CTLA4Ig in psoriasis,<sup>68,69</sup> revived the enthusiasm for CTLA4Ig as a pro-

mising drug for immune pathology.

The low efficacy of CTLA4Ig in transplantation is being addressed through the development of a modified molecule with greater affinity for CD80 and CD86 named belatacept (also known as LEA29Y).<sup>70</sup> This molecule has a mutation in two amino acids from the extracellular CTLA-4 domain. Belatacept was shown significantly more effective than abatacept (CTLA4Ig) in inducing long-term transplant acceptance in non-human primates, particularly when combined with other immune modulators.<sup>70</sup> In a recent renal transplantation phase II clinical trial of belatacept combined with basiliximab (anti-CD25), mycophenolate mofetil, and corticosteroids, the rejection rate was similar to the control group where belatacept was replaced with CsA and the renal function at 12 months was significantly better in belatacept treated patients.<sup>71</sup>

Initial clinical trials of abatacept in RA have shown the efficacy of this drug in patients with refractory disease.<sup>72</sup> These results were extended through large, multicenter, randomized, controlled clinical trials that confirmed the efficacy of abatacept in RA, with response rates similar to the ones reported for TNF- $\alpha$  antagonists.<sup>73</sup>

Importantly, opportunistic infections (including *Mycobacterium tuberculosis*) were not reported in the clinical trials.<sup>74,75</sup> But the combination of abatacept with TNF- $\alpha$  blockade has been associated with a greater risk of serious infection.<sup>76</sup> Therefore, a combination of abatacept with TNF- $\alpha$  inhibitors is not recommended. Given the importance of CTLA-4 in tumor immunity, even leading to the use of anti-CTLA-4 antibodies in clinical trials to boost anti-tumor immune responses,<sup>22</sup> the incidence of malignant disease in CTLA4Ig-treated patients has always been a concern. In any case, although several cases of lung cancer and lymphomas have been reported in abatacept clinical trials, the frequency of those cases is within what is expected in RA patients with long-standing active disease.<sup>73</sup>

## Conclusions

In spite of the recent advances that made co-stimulation blockade a clinical reality, several issues remain to be addressed as they can potentially lead to more effective strategies:

– *The long-term beneficial effect of CTLA4Ig (aba-*

*tacept) treatment in RA and other autoimmune diseases.* It is possible the achievement of prolonged disease-free periods if tolerance can indeed be induced in certain patients. Can immune tolerance be attained?

- *The most adequate combination therapy, when required.* Transplantation studies have suggested certain immunosuppressive agents (such as CsA) can abrogate tolerance induction, while other drugs (such as rapamycin) can support the establishment of long-term tolerance. It will be important to assess which drugs do not hamper, and even facilitate, the beneficial long-term effects of co-stimulatory blockade.
- *The most efficient way to monitor tolerance induction and maintenance.* A diagnostic test for the tolerant state would allow a more efficient follow up of patients, by potentially allowing early intervention in advance of clinical exacerbation of the disease.

At this time, we are likely to be simply observing the beginning of a new approach for the treatment of autoimmune diseases, such as RA. In the same way that biological drugs targeting TNF- $\alpha$  have paved the way for the development of several interventions aiming the control of inflammation by targeting cytokines, CTLA4Ig may be the starting point towards immunomodulation by directly targeting the immune synapse.

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