Immune Intervention with Monoclonal Antibodies Targeting CD152 (CTLA-4) for Autoimmune and Malignant Diseases

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CD152 or cytotoxic T lymphocyte antigen-4 (CTLA-4) is an essential receptor involved in the negative regulation of T cell activation. Because of its profound inhibitory role, CD152 has been considered a sound susceptible candidate in autoimmunity and a persuasive target for cancer immunotherapy for over a decade. However, the precise roles played by this molecule continue to emerge. In particular, recent evidence suggests that CD152 is also important in the homeostasis and function of a population of suppressive cells, termed regulatory T cells (Treg). In this review, we discuss the recent progress and main features of monoclonal antibodies (mAbs) targeting CD152 and examine how each mAb prepared to a distinct epitope may impact differently upon CD152 modulation depending on its demonstrated regulatory role acting as an agonist, antagonist, or inverse agonist. (Chang Gung Med J 2008;31:1-15)

Key words: anti-CD152 antibodies, Treg, autoimmunity, cancer, immunotherapy

Ongoing studies of collaboration scenarios between T cells and antigen-presenting cells (APCs) have led to anticipation of dramatic changes in immunotherapy and revealed some therapeutic targets and medicinal potentials in this area. For example, it has been shown that the specialized and dynamic molecular machinery present in the tight junction between a T cell and an APC regulates immunological responses.¹⁻³ It has also been inferred that the machinery, termed the immunological synapse (Fig. 1), correlates with a high degree of intercellular communication controlling disparate biological processes.³⁻⁵ A number of molecules have been confined at the immunological synapse to ensure their interim expression and interaction at the right time and place. Thus the sum and integration of signals are relevant to evoke appropriate T cell responses. This limited, micrometer-sized area is full of interacting molecules, of which CD152 has been identified to be responsible for inhibiting T cell responses in a T cell receptor (TCR)-dependent manner.⁴⁻⁵ Human CD152 was mapped to band q33 of chromosome 2⁶ and was classified into a group of immunomodulating receptors collectively termed the CD28 superfamily.⁷ It is well established that two members of this superfamily, CD28 and CD152, have opposing functions and that CD152 represents one of the major inhibitory receptors involved in co-

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stimulatory pathways regulating both humoral and cellular immune responses. A majority of studies indicate that CD28 provides direct enhancement signals, including up-regulation/stabilization of cytokine gene transcription, improved cell survival, lowered threshold for activation, and cytoskeletal effects. However, information on the function of CD152 is much less clear. Thus far, the most compelling evidence for the inhibitory role of CD152 is derived from deficient knockout mice (CTLA-4-/-). These mice suffer from a fatal T-cell lymphoproliferative disorder with splenomegaly, lymphadenopathy and hyper-responsive infiltration in several organs, including the heart. This disorder becomes apparent by four weeks after birth. This fatal disorder is presumably due to reactivities to multiple self-antigens, since the expression of a single transgenic TCR prevents this disease. The TCR-dependent activation in these knockout mice appears to require CD28 costimulation, because CTLA-4-/- CD28+ mice do not suffer this lymphoproliferative disease. Likewise, treatment of these mice perinatally with soluble CTLA-4-Ig, which competes with ligand access of cell surface CD152, effectively prevents this disease. Nonetheless, the mechanism of CD152 action is still unclear, with no obvious central theme.

Receptors and ligands in the B7-CD28 pathway

Conceptually, the interaction of CD28 on the lymphocyte with B7 proteins on the APC provides a necessary costimulatory second signal for a T cell to be able to fully respond to an antigen. The original family members in the pathway consist of two B7 ligands - CD80 (B7-1) and CD86 (B7-2), which have specificities towards the two receptors - CD28 and CD152. CD86 is constitutively expressed on interdigitating dendritic cells, Langerhans cells, peripheral blood dendritic cells, memory B cells and germinal center B cells. Furthermore, CD86 is expressed at low levels on monocytes, but its rapid up-regulation through IFN-γ stimulation has led to the hypothesis that CD86 functions primarily in initiating an immune response. On the other hand, CD80, being expressed later, may serve to amplify or regulate the response. Newly identified family members of related molecules include the inducible costimulatory molecule (ICOS), program death 1 (PD-1) receptor, B and T lymphocyte attenuator (BTLA), B7-H1, B7-H2, B7-H3, B7-H4, PD-1 ligand 1 (PD-L1) and PD-L2. The novel interactions among these new family members underscore additional complexity of this costimulatory pathway in mounting an appropriate immune response (Fig. 2).

Monoclonal antibody (mAb) interventions targeted at CD152

Studies of the physiological function of CD152...
became possible with the isolation of anti-CD152 mAbs, leading to the first indication of a negative regulatory role for CD152. However, these studies were controversial, since not all preparations of anti-CD152 mAbs exhibited identical effects. For example, in murine models, anti-CD152 mAb administration frequently exacerbated autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE)(17) and diabetes.(18) While in vivo animal studies predicted the nature of an enhancing effect of a mAb regimen, the results of in vitro crosslinked mAbs showed inhibited T-cell activation.(9,19) Moreover, characterization of mAbs targeted at human CD152 revealed similarly diverse features. Study with the first mouse anti-human CD152 mAb (clone 11D4) suggested that CD152 might deliver a positive signal synergized with that delivered by CD28.(20) On the contrary, cross-linking of CD152 by another clone 14D3 mAb in the presence of optimal costimulation appears to induce negative regulation of T-cell activation.(19)

Subsequent analyses that may reconcile these findings have shown that the CD152 protein is composed of disulfide-linked homodimers of extracellular immunoglobulin variable (IgV) domains, with each domain consisting of two layered β-sheets with ten strands (A, A’, B, C, C’, D, E, F and G) (Fig. 3).(21,22) Together with one mutational study, these

**Fig. 2** The yin and yang of co-stimulatory molecules. Pairs of ligands and receptors are responsible for providing positive and negative costimulation. Abbreviations used are L: ligand; R: receptor; ICOS: inducible costimulatory molecule; PD-1: programmed death 1; BTLA: B and T lymphocyte attenuator.

**Fig. 3** Protein organization of human CD152. Upper panel: Three-dimensional biomolecular structures of the extracellular domain with critical amino acids corresponding to CDR1, 2 and 3 indicated. The original structure was obtained from the Molecular Modeling Database (MMDB) of National Center for Biotechnology Information’s structure database under the accession number 1A1H and can be retrieved from http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?form=6&db=t&Dopt=s&uid=7506. Lower panel: The complete protein sequence of human CD152 isoforms A and B with denoted functional motifs. Asterisks indicate amino acid identity. Abbreviation used is CDR: complementarity-determining region.
two structural studies have independently pointed out that CDR1-like (the B-C loop) and CDR3-like (the F-G loop) regions in CD152 directly bind endogenous B7 ligands, whereas CDR2’s responsibility is very trivial if there is any. Therefore, although initial studies found no definite information to describe the CD152 epitope on which the blocking mAbs bind, antagonistic effects and the subsequent enhancement on T-cell activation may be mediated by mAb competition that results from specific binding with amino acid residues on or close to CDR1 and/or CDR3.

The idea of negative regulation of CD152 in T-cell activation has been further substantiated by the demonstration that CD152 promotes clonal anergy development by limiting cell cycle progression during the primary response in vivo. In addition, anti-CD152 mAb treatment in primary antigen exposure increases cell cycle progression and enhances recall antigen responsiveness. The immune-enhancing nature of CD152 antagonism has thus opened the possibility for a readily applicable tumor immunotherapy by temporary removal of CD152-mediated inhibition using antagonistic Abs.

Although the mechanisms by which CD152 regulates T cell responses are not completely understood, blocking its activity with an antagonistic mAb indeed induces IFN-γ secretion by T cells and tumor regression, thus offering a novel approach that holds promise for cancer immunotherapy. A corresponding human mAb derived from transgenic mice, MDX-010, shows promising results in cancer patients as a reagent for monotherapy and adjuvant therapy, despite the accumulating evidence that the behavior of an antagonistic mAb to individual T cells may be clonally heterogeneous. The approach of interrupting CD152-agonist interactions (CTLA-4 blockade) to strongly enhance antitumor responses has been highly regarded for potential treatment. This enthusiasm has escalated in recent years as a result of success in animal and pre-clinical studies, translating this promising concept into tangible therapeutic reality for cancers.

In addition to antagonistic Abs, mouse anti-human CD152 mAbs with agonist-like activities, i.e., confirming suppression upon engagement of these particular mAbs, were obtained from mice immunized with recombinant receptor or mitogen-activated human peripheral blood mononuclear cells (PBMCs). In both cases, attempts were made to identify the epitope responsible for the functional activity of the Abs. This analysis has led to the identification of two CD152 epitopes: (i) the CDR2-adjacent epitope called 60LTFLDD65 and (ii) the conformational epitope or CDR3 epitope called 102PPYYL106. These two epitopes are responsible for agonist- and antagonist-like activities, respectively. Furthermore, this study demonstrated that CD152 contributes different sites for interactions required for antagonism or agonism which can be interpreted in terms of inhibiting T cell proliferation or IL-2 production by activated T cells, respectively.

The list of mAbs to CD152 with diverse pharmacodynamical effects has not come to an end, because there is another class of mAbs proven to be inverse agonists, i.e., they have the capability to trigger the distinct and reverse functions of a receptor without disturbing the binding of a natural agonist. Using a recombinant bispecific tandem single-chain variable fragment (scFv) recognizing 9LTFLD6 and 66SICT90 (Fig. 3), Madrenas et al. revealed a possible

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**Box**

**Agonist:** Pharmacodynamically speaking, agonists bind to cellular receptors, produce various effects and initiate changes in cell function. Endogenous agonists are generally naturally-occurring ligands such as neurotransmitters. Exogenous agonists are usually drugs.

**Antagonist:** Pharmacological antagonists bind to receptors but do not activate signal transduction mechanisms. The biological effects of a given antagonist are derived from preventing agonist binding and receptor activation.

**Inverse agonist:** An inverse agonist is a ligand that binds to the same receptor at a site distinct from that of an agonist. However, an inverse agonist produces an effect opposite to that of an agonist.
CD152 inverse agonist with the nature of an Ab. Importantly, they also observed that upon scFv binding to CD152, an lck-dependent signaling cascade was assembled by increased recruitment of the serine/threonine phosphatase 2A to the cytoplasmic tail of CD152, and that IL-2 production was induced subsequently.

ELT and SICT, respectively termed M10 and M11 epitopes, are localized between the C" and D strands downstream of Met 55 (Fig. 3) characterized in the C"-C" loop by co-crystallographic studies to be CDR2. However, in the mutational model, the extracellular AATYM motif was recognized to be CDR2. Consequently, in contrast to the harmonized results obtained on the relative contributions of individual CDR1 and CDR3, a severe discrepancy exists in the span of CDR2. The finding of a possible CD152 inverse agonist suggests additional domains may be involved in some functions of CD152. The impression of a valuable contribution in this area defined by the Met 55 core (AATYMGNELT-FLDDICT) is further strengthened by observing a considerable conservation across all identified CD152, with six of the 19 amino acids having identical residues to the human sequence.

To explore the effect of this Met 55-cored sequence, we have recently developed a complete human monoclonal IgG4 targeting this particular stretch. Under the condition that the binding of a natural agonist was not interrupted, this mAb is in fact capable of triggering a distinct reduction of CD80-induced FasL expression and is also capable of inducing a subtle increase in the number of CD3+, thus symbolizing an inverse agonist (Fig. 4). We have also shown that the resultant human IgG4, mAb, known as γ4hu152IA, acts as an inverse agonist in the immunological synapse. These observations suggest an important role for the CDR2-encompassed Met 55-cored region in inhibiting immune responses.

CD152 mechanisms of action

As described above, although the precise mechanism is unclear and remains incompletely defined, CD152 may directly dampen T-cell responses via multiple suggested pathways. Several possibilities that may be considered for its negative regulation are reviewed below.

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**Fig. 4** Inverse agonism of a complete human anti-CD152. A, γ4hu152IA mAb increases CD3+ T cell numbers in vitro. PBMCs were activated with anti-CD3 + IL-2 alone and with immobilized or soluble γ4hu152IA. The number of CD3+ T cells was accessed after 72 h by flow cytometry. Bars represent mean ± SEM. γ4hu152IA exhibits specific binding towards human CD152 expressed both as a recombinant protein and a cell surface receptor. An asterisk indicates a statistically significant difference (p < 0.05) in cell number when adding γ4hu152IA compared to anti-CD3 and IL-2 alone. B and C, visible and fluorescent images of mitogen-activated PBMCs illustrating that γ4hu152IA exhibits specific binding towards human CD152 expressed both as a recombinant protein and a cell surface receptor. An asterisk indicates a statistically significant difference (p < 0.05) in cell number when adding γ4hu152IA compared to anti-CD3 and IL-2 alone. B and C, visible and fluorescent images of mitogen-activated PBMCs illustrating that γ4hu152IA exhibits specific binding towards human CD152 without interfering with the interaction of the natural agonist, CD80. Both anti-CD152 and anti-CD80 appear to co-localize at the T cell-APC interface framed by an 8.67 µm × 7.14 µm square, indicating that CD80 and γ4hu152IA converged toward the cellular junction. Abbreviation used is PBMCs: peripheral blood mononuclear cells.
**Ligand competition**

Since CD152 shares its ligands with CD28, the inhibitory effects are due at least in part to a higher avidity of binding by the same endogenous agonists, CD80 and CD86, compared with its stimulatory homologue, CD28. Ligation of CD152 to these agonists thereby deprives T cells of CD28 costimulatory signals and reduces T cell proliferation and cytokine production, resulting in attenuated immune responses, and thus mediates immune tolerance and/or anergy. The ligand competition model, which suggests the engagement delivers an inhibitory signal through the membrane proximal region irrespective of the cytoplasmic tail, gains support from the observation that transgenic expression of CD152 lacking a cytoplasmic domain is still functional and capable of ameliorating the fatal lymphoproliferative disease seen in CTLA-4 knockouts. Additionally, several mutagenesis studies of the cytoplasmic domain demonstrate that removing tyrosine residues also fails to completely abrogate CD152 inhibition. However, recent data reveal that CD28 mice still show effects following CTLA4 blockade, and that agonistic antibodies to CD152 are also inhibitory, implying that the dominant negative regulation is likely to be dependent on the delivery of a negative signal. Thus, while it is theoretically possible for CD152 to negatively regulate T-cell activation by restricting the access of CD28 to its ligands, this model is useful in indicating that not all functions of CD152 are likely to be dependent on the delivery of a negative signal to the T cell. It remains to be determined to what extent it actually occurs normally.

**Soluble CD152**

A shorter soluble form of CD152 lacking the transmembrane region has been produced from RT-PCR cloning of non-activated T cells in animals as well as humans. Soluble CD152 (sCD152, CD152 isoform B) seems to be a fully functional CD80 and CD86 receptor, thus likely to affect T-cell responses in a paracrine manner. Furthermore, immunoreactive sCD152 could be detected in the serum of 14 of 64 healthy subjects. Recently, it has been observed that the level of sCD152 mRNA from the homozygous autoimmunity-protective haplotype was higher than that from heterozygous individuals, whereas the lowest level was observed in homozygous-susceptible subjects. As activated T cells suppress sCD152 mRNA expression and have also been shown to be associated with a preferential expression of membrane-bound, full-length CD152 (flCD152) mRNA, discrepancies in the ratio of sCD152 to flCD152 may play an important role in the regulation of immune homeostasis. Nonetheless, further studies in this area are needed before a definitive conclusion can be drawn. However, if this hypothesis is generally true, the scheme of CTLA4 blockade could act preferentially on sCD152 instead of membrane-bound flCD152, as the latter localizes in the confined immunological synapse which may prevent access of whole IgG1 molecules.

**Negative signaling**

CD152 appears to be involved in negative signaling, because the proliferative disorders of CTLA-4-/- mice can be prevented by transgenic expression of flCD152 and knockout mice show a reduction in solid organ infiltration but not lymphoproliferation when a truncated CD152 lacking a cytoplasmic tail is used in transfection. This has become the most intuitive and popular model of CD152 inhibition, which inhibits T-cell activation, most likely acting at the level of TCR or CD28 signaling. However, CD152 lacks intrinsic catalytic activity, and thus it needs to recruit adapter and/or enzyme molecules. A role for phosphatases has been suggested by the finding that fyn, lck, Zap-70 and the Ras pathway are persistently activated in T cells derived from CTLA-4-/- mice. Moreover, CD152 has a short cytoplasmic tail, which is completely conserved in the mouse, rabbit and human and 94% conserved in the rat, with two invariant tyrosine-containing motifs (YVKM and YFIP) which presumably play a pivotal role in the interaction with key signaling molecules.

Indeed, SHP-2 phosphatase has been described to bind the phosphorylated YVKM motif and dephosphorylate p52h, a molecule that, together with Grb2 and Sos, forms a regulatory complex of the Ras pathway. It has also been shown that the binding of SHP-2 to CD152 leads to form a complex with the T cell receptor complex ζ (TCRζ) chain, eventually inhibiting the phosphorylation of TCRζ, and thus being responsible for suppression. It should be noted that the above evidence comes mostly from cross-linked agonistic mAbs, while the role of ligand engagements is less clear. Whether this observation of negative signaling is also applied to CD80 and...
CD86 is yet to be determined, especially as it has been shown that expression of CD152 molecules lacking any extracellular ligand-binding domains can be functionally significant leading to diminishing TCR phosphorylation events.\(^{58}\)

**Limitation of cell surface components**

A number of experiments failed to confirm direct interaction of the phosphorylated YVKM motif and SHP-2 in negative signaling.\(^{49,56}\) Instead, researchers identified that AP-50 (\(\mu 2\)), the medium chain of the clathrin-associated adaptor (AP-2) complex, is a genuine intracellular YVKM-binding molecule.\(^{60-63}\) As AP-2 bridges the clathrin complex and CD152, it induces rapid endocytosis of CD152 into endosomes and eventually into lysosomes. However, once the YVKM motif is phosphorylated, CD152 no longer binds to AP-2. Furthermore, the mutation of tyrosine within this AP-50 binding site results in high expression of cell surface CD152. These results are taken as evidence that CD152-based negative signals may interfere with TCR proximal events by interacting with and possibly removing critical signaling partners from the TCR complex.\(^{53}\) Along a similar line, one study has shown that CD152 inhibits the surface expression of lipid rafts, which is important for T-cell signaling, again diminishing the ability of the TCR to signal.\(^{64}\) The finding that ligand-independent CD152 appears to be functional\(^{58}\) represents yet another highly relevant and supportive indication. Collectively, these results provide evidence that cycling CD152 may be responsible for limiting the amounts of TCR\(\zeta\) and lipid rafts, and this limitation would have the effect of diminishing the ability of the TCR to signal, thereby mediating a negative effect.

**Altered adhesion**

Based on fluorescence microscope images of T-cell interaction with agonist MHC-peptide complexes and intercellular adhesion molecule-1 (ICAM-1), it has been shown that a central supramolecular activation cluster (cSMAC) of engaged TCRs surrounded by a ring of peripheral supramolecular activation clusters (pSMAC) composed of lymphocyte function-associated antigen 1 (LFA-1) adhesion to ICAM-1 forms and becomes stable over a period of minutes.\(^{11}\) This supramolecular organization, which is present in the contact area, is initiated by LFA-1/ICAM-1 interactions in the center after triggering of TCR signaling (Fig. 1). Intriguingly, CD3/LFA-1 stimulation has been shown to increase CD152 expression,\(^{65,66}\) indicating that this very stimulatory pathway also contributes to the preparation of the signaling machinery for down-regulating T-cell activation. Studies of crystal structures also document that each CD152 dimer may bind two B7 ligands, forming a repetitive organization that is reminiscent of a zipper at the T cell-APC interface.\(^{21,22}\) The CD152-B7 zipper may thus provide significant adhesion. More importantly, it has been observed that CD152 engagement by soluble antibody or CD80 potently up-regulates LFA-1/ICAM-1 interaction and receptor clustering concurrent with IL-2 inhibition.\(^{67}\) Accordingly, CD152\(^+\) cells adhere much more strongly than CD152-deficient cells to ICAM-1 substrates. Additionally, the authors demonstrated that anti-CD152 could increase adhesion, thus adding a new perspective to the issue of “CTLA-4 blockade” to augment the antitumor responses mentioned previously. Increased LFA-1 adhesion may facilitate increased cell-cell contact and/or frequency of interaction with target cells. The co-receptor will also alter T cell motility, intravascular migration, and migration to peripheral organs induced by chemokines. The altered localization of CD152-bearing cells will in turn affect the microenvironment with different surrounding cells, possibly affecting activation and cytokine production.

**Induction of negative regulatory molecules**

Evidence indicates that CD152 may negatively regulate T cell activation through the induction of other inhibitory molecules. One of the most compelling candidates for this hypothesis has been transforming growth factor \(\beta\) (TGF-\(\beta\)) which is known to have pleiotropic, immunosuppressive activity. For instance, it has been shown that cross-linking of CD152 induces TGF-\(\beta\) production by murine CD4\(^+\) T cells of the Th1, Th2, and Th0 subtypes.\(^{68}\) Moreover, addition of anti-TGF-\(\beta\) partially reverses this T cell suppression and CD152 cross-linking induces only slight T-cell suppression in mice of the TGF-\(\beta\) null phenotype compared with a 95% reduction in wild-type mice. This indicates that induction of TGF-\(\beta\) by CD152 signaling represents a ubiquitous feature of murine CD4\(^+\) T cells. The finding that the removal of TGF-\(\beta\) in mice (TGF-\(\beta\)^-/) results in
autoimmunity and rapidly fatal multi-organ inflammatory syndromes similar to the ones found in CD152−/− mice also supports this view. On the contrary, using TCR transgenic cells, Sullivan et al. observed that CD152 engagement does not result in increased production of TGF-β by CD4+ T cells and equally inhibits proliferation of wild-type or TGF-β−/− T cells, thus concluding that CD152 and TGF-β represent distinct mechanisms for regulation of T cell responses. This discrepancy may be related to the fact that the two pathways are at least partly independent. Additionally, murine models of defective apoptosis provide another strong candidate that links CD152 to its inhibitory effect on T cell activation. For example, mice with a deficiency involving Fas or Fas ligand (FasL) developed systemic autoimmune disorders characterized by T cell and B cell hyperplasia, increased inflammatory cytokine production, polyclonal hypergamma globulinemia, autoantibody production, and immune complex disease. Moreover, upon phytohemagglutinin (PHA) mitogenic stimulation, microvesicles containing bioactive FasL have been shown to be released from Jurkat T cell leukemia cells and from normal human T cell blasts. Although a direct relation between CD152 signaling and Fas was not established in this particular study, these investigators suggested an efficient mechanism for the rapid autocrine or paracrine control of cell death via FasL during immune regulation.

**Back signaling via B7 to up-regulate the enzyme IDO**

CTLA-4 immunoglobulin fusion protein (CTLA4-Ig) is a biological agent consisting of the extracellular domain of CD152 fused to the Fc region of IgG1. As CTLA4-Ig potentially promotes tolerance through costimulatory blockade, it has been explored extensively in conditions such as transplantation. It was later found in mice that administration of CTLA4-Ig results in the induction of indoleamine 2,3-dioxygenase (IDO) in professional APCs like dendritic cells. IDO is induced during inflammation by IFN-γ and other pro-inflammatory cytokines and acts to deplete the local microenvironment of the essential amino acid, tryptophan. The resulting low levels of extracellular tryptophan act as a signal to inhibit T-cell proliferation. Therefore, stimulation of IDO activity is dominantly immunosuppressive. This assumption is consistent with the observation that, whereas CTLA4-Ig treatment did not block T cell clonal expansion in IDO-deficient recipients, induction of IDO completely blocked clonal expansion of T cells from TCR transgenic mice following adoptive transfer of T cells. Accordingly, rather than acting as a simple blockade of CD80 and CD86 to induce tolerance, CTLA4-Ig signaled the upregulation of IDO activity within APCs via CD80 and CD86. This study demonstrates that IDO expression is an inducible feature of specific subsets of DCs (professional APCs). The observation that CD152 and possibly CD28 can ‘back signal’ via CD80 and CD86 into the DCs with a resulting upregulation of the enzyme IDO provides a potential mechanistic explanation for T cell regulatory properties.

**Different conformational states of CD152**

From the pharmacological perspective, the identification of inverse agonists to a given receptor often demonstrates the ability of that particular receptor to have different conformational states. While CD152 has been one of the most investigated members of the Ig superfamily, little is known regarding the existence of conformational states generally or its role in immune responses. However, alternate transcripts or spliced variants, previously described as sCD152, which lack the transmembrane encoding regions, were first deposited in the GenBank Sequence Database in humans, mice, and rats (accession numbers U90273, U90270, and U90271) in 1997, followed by a description of the same transcript in humans being expressed by non-stimulated human T cells. The 174-aa soluble form, designated as isoform b, can be retrieved under the accession number NP_001032720. Subsequently, a Swedish group deposited several short versions of human CD152 with the original signal peptide directly linked to the carboxyl end of either isoform (accession numbers AAY00166, AAV66331, and ABG85285). Our preliminary study has confirmed the universal presence of sCD152 transcripts in activated human PBMCs (Chu C and Chin L-T, unpublished data). These findings were consistent with CD152 isoforms, and thus different conformational states, being present and probably immunologically active, as opposed to merely representing degraded or shed polypeptides. It may be possible that the ratio of CD152 isoforms could be translated into biochemical differences
capable of modulating immune responsiveness. Furthermore, a physical association of the phospho-rylated form of TCRζ with CD152 has been established and accounts for selective decreases in the amount of TCRζ that accumulates in the immunological synapse once CD152 is occupied by endogenous B7 agonists.(77) This, together with the identification of mAbs with the activities of an inverse agonist,(39,41) suggests that a likely explanation is that the Met-cored CDR2 region of CD152 is responsible for TCRζ association where binding of an inverse agonist abolishes such an association and thus the inherited down-regulation. Clearly, additional experiments are required to dissect these possibilities.

**Regulatory T cells (Tregs)**

It is known that immune reactivity is further controlled by various types of regulatory T cells (Tregs). Tregs can be broadly divided into two subsets, i.e., the natural Treg cells of the CD4⁺CD25⁺ phenotype, which constitute 5-10% of peripheral T cells, and the stimulation-induced (or adaptive) Treg cells identified in various models of inflammation, alloreactivity, autoimmunity, and chronic viral infection.(76,78) In the latter study, over 16 functional HLA class II-restricted peptide epitopes on HBcAg overlapping with HBeAg have been identified using the SYFPEITHI system to measure CD4⁺CD25⁺ Treg cell frequencies, which are then used to correlate with pathological changes in the liver, immune response, other hepatitis B virus-related infection and clinical parameters.(80) Consequently, two significant findings derived are that HBcAg-specific Treg cells modulate the immune tolerance phase and that the decline of these Treg cells may account for the spontaneous acute exacerbation on the natural histo-
cal interactions between APCs and T cells (Fig. 1)

Together, these results indicate a strong correlation between CD152 expression and suppressive regulatory function, supportive of the concept that CD152 is functionally relevant to Tregs.

At present, we know little of the exact mechanism by which CD152 controls the negative signaling. However, possibilities described in the preceding section have been implied. Notably, the upregulation of CD80(90,91) and CD86(92) on activated human T cells, Tregs constitutively express CD152,(87) and CD152 expression. Unusually for non-activated T cells, Tregs constitutively express CD152,(87) and CTLA-4 blockade on the Treg by a specific mAb can attenuate their suppressive activity, leading to the development of autoimmune disease in vivo.(88) Furthermore, it has been observed that CD4⁺CD25⁺ cells purified on the basis of recycling CD152 are much more potent with regard to suppression.(89) Together, these results indicate a strong correlation between CD152 expression and suppressive regulatory function, supportive of the concept that CD152 is functionally relevant to Tregs.

Because Tregs are involved in preventing allograft rejection and graft versus host disease (GVHD), and exert a dominant effect in controlling autoimmunity and maintaining peripheral tolerance, specific immune therapies designed to expand them may improve the clinical course of various T-cell mediated pathologies. The application of T cell vaccination with a dual altered peptide ligand has proven successful in mice in ameliorating myasthenia gravis.(84) As expected, the peptide analog acts by up-regulating composite NF-AT/AP-1 sites in cytokine gene promoters and the region responsible for NF-AT inhibition was mapped to the amino terminus.(85,86)

Of recent interest is the association and potential synergism between the suppressive function of Tregs and CD152 expression. Unusually for non-activated T cells, Tregs constitutively express CD152,(87) and CTLA-4 blockade on the Treg by a specific mAb can attenuate their suppressive activity, leading to the development of autoimmune disease in vivo.(88) Furthermore, it has been observed that CD4⁺CD25⁺ cells purified on the basis of recycling CD152 are much more potent with regard to suppression.(89) Together, these results indicate a strong correlation between CD152 expression and suppressive regulatory function, supportive of the concept that CD152 is functionally relevant to Tregs.

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Intracellular and membrane-bound forms. Moreover, the authors showed an association between the levels of TGF-β and JNK activity. The JNK protein is known to activate the transcription of the FasL gene and thus initiates activation-induced cell death. The results also indicate an increase in the apoptotic rate. A phase I clinical trial also revealed that T cell vaccination with modified myelin basic protein (MBP) induces the specific regulatory T cell network and thus depletes circulating MBP-reactive T cells in a clonotype-specific fashion. Conversely, encouraging data on vaccines administered with antagonistic CD152 mAbs designed to interfere with normal Treg suppression showed an objective tumor regression rate, representing a novel method for enhancing a patient’s immune response to fight cancer. Evidently, approaches such as anti-CD152 mAbs to manipulate CD25^+^CD4^+^ Treg will enable their use to modulate specific immune responses if a better understanding of the mechanisms of suppression is achieved.

The use of various pharmacodynamic mAbs to modulate Treg function has not yet become a general practice. However, removal of costimulatory signals to suppress immune responses was pioneered with CTLA4-Ig, a soluble fully humanized fusion protein consisting of the ligand binding domain of CD152 and the Fc portion of IgG1 which works by binding B7 on DCs. This binding prevents the engagement of surface CD28, thus blocking the subsequent costimulatory events required for optimal activation of T cells. The process is important for the maintenance of the inflammatory response in autoimmune diseases such as rheumatoid arthritis (RA). A successful trial with a monthly infusion of 10 mg/kg CTLA4-Ig has proven safe and effective for reducing the signs and symptoms of active RA. It is conceivable that more humanized (including chimeric) and fully human mAbs to CD125 will be constructed and tested in preclinical studies when mAbs with agonist, antagonist or inverse agonist in nature can be generated in the next ten years. Potential therapeutic antibodies must be further tested in humans to reveal their true clinical utility. Hopefully, some of these biologicals will be used for the effective treatment of autoimmune and/or malignant diseases.

Concluding remarks

In conclusion, it is becoming clear that the actions of CD152 can not be explained by a single mechanism and evidence exists for a number of mechanisms that might all act simultaneously. These observations represent a step forward in beginning to understand the complexity and importance of this particular receptor. Furthermore, there are very few other examples, if any, where immunity can be manipulated in such a dramatic way in humans by maneuvering the activity of a single receptor. As the results of different mAbs targeted at human CD152 are diverse, the goal of selective immunotherapy in various diseases now seems attainable, with many possible points of engagement using mAbs with the activities of an agonist, antagonist and inverse agonist. These endeavors should prove to be an exciting area of immunotherapy as the science expands.

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利用各種抗 CD152 單株抗體進行自體免疫及惡性腫瘤
免疫治療的探討

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被稱為毒殺性 T 細胞第四抗原 (cytotoxic T lymphocyte antigen-4, CTLA-4) 的 CD152 分子，實際上是參與負向調控 T 細胞活化之重要生理受體。雖然 CD152 受體之詳細作用機轉仍持續地被發現中，但是由於其廣泛的免疫抑制效應，特別是近年來有足夠的證據指出：CD152 分子在 CD4+CD25+ 調控性 T 細胞上的測定及功能上亦扮演重要角色，使得 CD152 分子在過去十幾年來都被認為是治療自體免疫疾病及惡性腫瘤的理想標的。本文主要討論近期對 CD152 單株抗體的進展與特徵，並檢視每一種具獨特「抗原決定位」的單株抗體如何在作爲協同劑、拮抗劑或反向拮抗劑的同時影響 CD152 之調控作用。(長庚醫誌 2008;31:1-15)

關鍵詞：抗 CD152 抗體，調控性 T 細胞，自體免疫，癌症，免疫治療

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