Molecular Immunology 49 (2011) 527–536

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Quantitative analysis predicts the relative therapeutic efficacy of different forms of CTLA4Ig

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ARTICLE INFO

Article history:
Received 12 July 2011
Received in revised form 30 August 2011
Accepted 29 September 2011
Available online 27 October 2011

Keywords:
Abatacept
Belatacept
LEA29Y
Simulation
Computational

ABSTRACT

Modulating the activities of costimulatory molecules controlling immune responses holds considerable promise for immunotherapy. CTLA4Ig (abatacept), a soluble version of the T cell-expressed membrane receptor CTLA-4, is approved for the treatment of rheumatoid arthritis. Like natural CTLA-4 molecules, CTLA4Ig ligates B7-1 and B7-2 on antigen presenting cells, preventing CD28-mediated costimulation of T cells. However, CTLA4Ig can also prevent ligation of CTLA-4, potentially blocking vital inhibitory signals, thereby augmenting immunity. There have been no quantitative analyses of the likely effects of CTLA4Ig on costimulatory interactions at the immunological synapse. We present a mathematical model, based on rigorous biophysical and expression data, for simulating the effects of abatacept and a mutated derivative, LEA29Y, on the synaptic interactions of CD28 and CTLA-4. The simulations reveal an unexpectedly large window within which CD28, but not CTLA-4, ligation is blocked by CTLA4Ig, perhaps explaining the efficacy of abatacept at the recommended therapeutic dose (10 mg/kg) and its relative safety. However, the simulations suggest that the present dosing regimen is close to the maximum theoretically safe dose. The simulations also show that, within the therapeutic window, LEA29Y enhances the interaction of CTLA-4 with the more potent of its two native ligands, B7-1. They also suggest that CTLA-4 ligation by B7-1 could, in principle, be enhanced by further decreasing the off-rate of CTLA4Ig for binding to B7-2. Our findings therefore offer molecular explanations for why LEA29Y might prove to be more effective than abatacept in a clinical setting, and suggest ways in which its therapeutic efficacy could be further optimised.

1. Introduction

Since CD28 and CTLA-4 have crucial, opposing functions during T cell activation they hold considerable promise for immunotherapy. Whereas CD28 induces activation signals, CTLA-4 plays a pivotal role in downregulating T cell responses and has been shown to have important functions in modifying the course of autoimmunity and transplantation tolerance (Bayry, 2009; Linsley and Nadler, 2009). Understanding the molecular properties of therapeutic agents that block these molecules is crucial and is, therefore, an appealing target for mathematical modeling.

T-cell activation is dependent on close contacts with antigen-presenting cells (APCs). This cell-to-cell contact, the immunological synapse, enables surface receptors to interact with their ligands on the opposing membrane (Grakoui et al., 1999; Monks et al., 1998). The current understanding is that optimal T-cell activation requires two signals. The first is triggered by the specific interaction of the T-cell receptor (TCR) with major histocompatibility complex (MHC)-bound peptide, whereas the interactions of costimulatory molecules provide the second signal. An interaction between the B7 family members B7-1 (CD80) and B7-2 (CD86) on the surface of the APC, and their ligand CD28, expressed on the T cell, provides the major costimulatory signal for the activation of naïve and resting T cells (Frauwirth and Thompson, 2002; Sharpe, 2009). CTLA-4 is a second receptor for B7-1 and B7-2 that is expressed on activated T cells, which delivers a signal that inhibits T-cell activation (Bayry, 2009; Linsley and Nadler, 2009).

CTLA-4 molecules are stored in intracellular vesicles and injected into the synapse area upon TCR signalling late in activation (Egen and Allison, 2002; Linsley et al., 1996). CD28, on the other hand, is expressed at a relatively high level at the cell surface and is not directed to the synapse during TCR signalling in the absence of B7 ligands (Bromley et al., 2001; Linsley et al., 1996). Also, CD28 is not associated with lipid rafts suggested to be responsible for active redistribution of molecules to the synapse (Brown and London, 2000; Xavier et al., 1998; Yashiro-Ohtani et al., 2000). This

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doi:10.1016/j.molimm.2011.09.021

indicates that the accumulation of CD28 into the synapse is governed by passive diffusion. However, only 30% of surface expressed CD28 molecules are mobile (Bromley et al., 2001) and the expression of CD28 during activation remains essentially unchanged (Jansson et al., 2005). Immature dendritic cells (DCs) express ~20,000 B7-2 and ~2000 B7-1 molecules, and the expression of both molecules doubles on mature DCs (Jansson et al., 2005).

The solution affinity of B7-1 for CTLA-4 is among the highest described for interacting cell surface molecules. The affinity of this interaction is 13-fold higher than that of B7-2 binding to CTLA-4, 20-fold higher than that of the B7-1/CD28 interaction and 100-fold higher than that of B7-2/CD28 binding (Collins et al., 2002; Ikemizu et al., 2000; van der Merwe et al., 1997). In addition, CTLA-4 and B7-1 share the property of binding bivalently, whereas CD28 and B7-2 are monovalent (Collins et al., 2002). Bivalent CTLA-4 binding is estimated to generate complexes that increase their half-life approximately 100-fold, compared to monovalent interactions (Collins et al., 2002). Recent work has shown that B7-1 has a third ligand, programmed cell death ligand 1 (PD-L1) expressed on T cells. It is proposed that B7-1 and PD-L1 interact with an affinity ~3 fold higher than that of the monovalent B7-1/CD28 interaction, and that B7-1-induced signaling by PD-L1 is inhibitory (Butte et al., 2007, 2008).

Both *in vivo* and *in vitro* studies demonstrate that blocking T cell costimulation by CD28 inhibits a variety of immune responses (Linsley and Nadler, 2009). Abatacept was the first costimulation-blocking agent licensed for clinical use in patients with rheumatoid arthritis (RA) and has recently been approved for treatment of juvenile idiopathic arthritis (Bluestone et al., 2006; Linsley and Nadler, 2009). There are also on-going clinical trials with abatacept for indications including lupus nephritis and systemic lupus erythematosus (Linsley and Nadler, 2009). For adult patients with RA, abatacept is administered by intravenous infusion (10 mg/kg) fortnightly for the first month, and monthly thereafter. Abatacept has a half-life of approximately 14 h, and the recommended dose treatment generates an average serum level of ~65 μg/mL (Ma et al., 2009). For treatment of RA, abatacept is either used as a monotherapy or concomitantly with disease-modifying anti-rheumatic drugs other than TNF antagonists (see www.orencia.com). Abatacept is a soluble fusion protein that consists of the extracellular domain of CTLA-4 coupled to a modified form of the Fc domain of human immunoglobulin G1 (IgG1) that is unable to initiate complement activation or antibody dependent cellular cytotoxicity (Davis et al., 2007). Like naturally occurring CTLA-4, abatacept binds B7-1 and B7-2 on APCs, reducing the number of ligands available to CD28 and selectively inhibiting activation of T cells. Abatacept only showed modest inhibitory effects on allograft rejection (Kirk et al., 1997; Levisetti et al., 1997), leading to the development of LEA29Y (belatacept), a mutant derivative with a four-fold smaller off-rate for B7-2 and a two-fold smaller off-rate for B7-1 (Larsen et al., 2005). LEA29Y has exhibited considerable promise in phase III studies of renal transplant recipients with graft- and patient-survival rates being similar to those observed with cyclosporine treatment (Lucchese, 2010; Vincenti et al., 2010). Two recent reports indicated that abatacept and LEA29Y do not induce signaling in APCs, but rather modulate CD28 costimulation on T cells (Carman et al., 2009; Davis et al., 2008).

Although the affinity and kinetic data are suggestive that LEA29Y may have advantages over abatacept (Larsen et al., 2005; Roy et al., 2007), their potential effects on CD28 complex formation in the context of the immunological synapse have not been systematically compared. We previously used *in silico* simulations of costimulatory interactions at the immunological synapse to illustrate the importance of system effects on signalling by cell surface receptors (Jansson et al., 2005). Our analysis of the effects of abatacept and LEA29Y on CD28 and CTLA-4 ligation within this framework suggests reasons why LEA29Y may have important clinical advantages over abatacept that are not easily predicted from the affinity and kinetic data alone.

2. Methods

2.1. Description of the model

We use our previously established theoretical framework based on rigorous biophysical and expression data obtained for costimulatory interactions at the synaptic interface between a naïve/activated T cell and an immature/mature DC (Jansson et al., 2005), and integrated within this framework the blocking effects of soluble CTLA4Ig molecules (abatacept or LEA29Y). A two-compartment model utilising a system of nonlinear ordinary differential equations (ODE), incorporating precise stoichiometric, affinity and expression data are used; the mathematical model is described in detail in the Appendix. A simplified scheme of the model is presented in Fig. 1. We model the costimulatory interactions taking place within the central supramolecular activation cluster (c-SMAC) between a dendritic cell and a T cell. For the purposes of this study, "synapse" and "c-SMAC" are used synonymously. Membrane bound molecules are only allowed to bind their ligands once they are located within the synapse. Unbound CD28, B7-1 and B7-2 molecules diffuse freely on the cell surface and are recruited to the synapse due to the ligation of unbound molecules within the synapse, whereas the CTLA-4 molecules are injected into the synapse from intracellular compartments in activated T cells. To allow for the observation that only a certain fraction of molecules

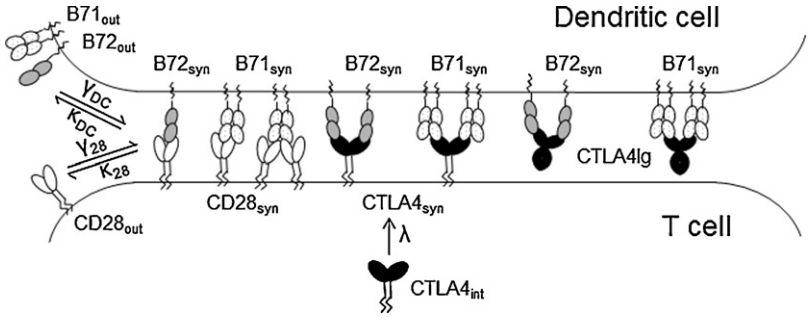

Fig. 1. A simplified scheme of the model. The scheme shows the different types of complexes that can form within the synapse between an activated T cell and a dendritic cell. Mobile B7-1 and B7-2 molecules diffuse in and out from the synapse at rates, γ_{DC} and κ_{DC}, respectively. Mobile CD28 on the T cell diffuse in the same manner at rates γ_{28} and κ_{28}, whereas intracellular CTLA-4 molecules are injected into the synapse at rate λ. Free CD28 and CTLA-4 molecules inside the synapse may ligate either B7-1 or B7-2 on the opposing membrane. The soluble CTLA-4 molecules (CTLA4Ig) ligate to B7-1 and B7-2 both inside and outside the synapse, which reduces the available ligands for CD28 and CTLA-4 interactions. All formed complexes dissociate at a given rate (see Table 1).