Regulation of T-cell migration by co-stimulatory molecules

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Abstract
Migration of primed T-cells to the antigenic site is an essential event in the development of effective immunity. This process is tightly regulated in order to ensure efficient and specific responses. Most studies have focused on non-specific mediators of T-cell migration, including integrins and chemokines. However, recent studies have highlighted the key role of the T-cell receptor and co-stimulatory molecules in guiding T-cell access to antigenic tissue. Here, we review the experimental evidence for an essential contribution of co-stimulation-mediated molecular interactions regulating T-cell migration in the development of T-cell immunity and tolerance.

Introduction
During an inflammatory response, leucocytes, including primed T-cells, need to cross the endothelial barrier in order to enter non-lymphoid tissue. This process is known as leucocyte extravasation and is mediated by selectins, integrins, chemokines and other cell-surface receptors [1,2]. T-cell trafficking is also regulated by homing receptors expressed on their surface, which direct different T-cell subsets to specific tissues by interacting with tissue-selective endothelial ligands [3].

In addition to these non-specific mechanisms, T-cell migration to antigenic sites is promoted by the recognition of antigen presented by the endothelium [4,5] and subsequent TCR (T-cell receptor)-induced cytoskeletal rearrangements necessary for T-cell migration [6].

Positive and negative co-stimulation
In addition to TCR-induced signalling, co-stimulation has also recently been shown to be instrumental to the regulation of T-cell migration during immunity. The best-characterized co-stimulatory molecule is CD28, which is expressed by both naïve and memory T-cells. CD28 binds to CD80 or CD86 on the surface of conventional APC (antigen-presenting cell), initiating various signalling cascades, which lead to increased cytokine production, IL (interleukin)-2 mRNA stability and expression of the anti-apoptotic protein Bcl-XL [7]. CD28 signalling is essential to sustain proliferation and survival of T-cells; in the absence of this signal, T-cells become anergic or apoptotic. CD28 is also thought to lower the threshold of T-cell activation by reducing the number of engaged TCRs required for full signalling [8,9].

Upon activation, T-cells also transiently express the negative co-stimulatory molecule CTLA-4 (cytotoxic T-lymphocyte-associated protein-4; CD152). The latter has 30% homology with CD28, and binds to the same ligands, but with higher affinity [8]. CTLA-4 ligation inhibits IL-2 transcription and progression through the cell cycle, thus antagonizing the co-stimulatory signals induced by CD28 [10]. CTLA-4 mRNA is first detected 1 h post-activation [11]. CTLA-4 protein increases steadily and is stored in vesicles, which are transported to the sites of TCR activation and the immunological synapse [12,13]. The accumulation of the CTLA-4-containing vesicles is proportional to the strength of the TCR signal, suggesting the existence of a feedback loop, whereby the strength of the stimulus regulates the recruitment of the inhibitory molecule CTLA-4 [14]. CTLA-4 is subsequently endocytosed in clathrin-coated vesicles, mediated by the clathrin-associated AP2 adaptor protein complex [15,16].

Regulation of adhesion and cytoskeleton rearrangements mediated by co-stimulatory molecules
The regulation of migratory events is associated with the activation of signalling pathways that mediate cell adhesion and cytoskeleton rearrangements. It is becoming clear that both CD28 and CTLA-4 signalling can engage these pathways.

Adhesion
Both CD28- and CTLA-4-mediated signals have been involved in the regulation of T-cell adhesion. CD28 cross-linking increased adhesion of human CD4⁺ T-cells to fibronectin, ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule 1), in a process...
Figure 1 | Regulation of T-cell migration by co-stimulatory molecules

(A) In lymphoid tissue, CD28 signalling induces CXCR5 expression in primed T-cells, which drives their migration to the germinal centre (1). In addition, CD28 triggering is required for migration of primed T-cells to target tissue (2). In contrast, CTLA-4-mediated signals prevent sustained interactions with the APC (3). (B) After priming, CD28 enhances TCR-dependent T-cell transendothelial migration and localization to non-lymphoid target tissue (1). In addition, CD28 engagement by resident APCs results in increased adhesion and retention into the antigenic tissue (2). Dominant CD28 signalling due to either the lack of CTLA-4-mediated signals or extensive antibody triggering leads to uncontrolled antigen-independent T-cell extravasation (3) resulting in tissue damage [19]. In contrast, CTLA-4 signals prevent T-cell recruitment by antigen-presenting endothelial cells (4).

involving PKC (protein kinase C) [17]. Similarly, CD28 was shown to enhance LFA-1 (lymphocyte function-associated antigen 1)–ICAM-1-mediated interactions between human T-cells and CD80-expressing melanoma cells via PKC activation [18]. Further evidence for the contribution of CD28-mediated signals to integrin function came from the observation that β1-integrin-mediated adhesion of a myelomonocytic cell line was enhanced by CD28 activation [19]. This event involves an interaction of its SH2 domain (Src homology 2 domain) with the p85 catalytic subunit of PI3K (phosphoinositide 3-kinase), a process regulated by the adaptor molecule Cbl-b [19]. Interestingly, CTLA-4 has also been shown to increase integrin-mediated adhesion in pre-activated murine CD4+ T-cells in a Rap-1 (repressor activator protein 1)-dependent manner by enhancing LFA-1 clustering, thus increasing adhesion to APCs [20].

Cytoskeleton reorganization

CD28 has also been implicated in the regulation of cytoskeletal reorganization. CD28 triggering increases F-actin levels in mouse T-cells, in a process involving the Ras homologue GTPases Rac1 and Cdc42 (cell division cycle 42) [21,22]. Cdc42 activation upon CD28 engagement is thought to be mediated by Src kinases independently of ZAP-70 (ζ-chain (TCR)-associated protein kinase of 70 kDa) activation [23]. CD28 can also activate the guanine-nucleotide-exchange factor for Rho GTPases Vav1 independently of the TCR [24]. In addition to activating Rho GTPases, CD28 engagement has also been found to induce actin polymerization in a WASP (Wiskott–Aldrich syndrome protein)-dependent manner [25]. The latter molecule plays a major role in coupling TCR signals with cytoskeletal rearrangements, in a process mediated by Vav1 and Rho GTPases. WASP interacts with the Arp2/3 complex (actin-related protein 2/3 complex), and together they induce actin filament nucleation [6]. CD28 is thought to interact with WASP via PI3K and Snx9 (sorting nexin 9) activation [25].

Differential regulation of T-cell migration by CD28 and CTLA-4

A direct involvement of CD28 and CTLA-4 in the control of T-cell migration has been suggested by recent studies (Figure 1 and Table 1). CD28-deficient murine T-cells stimulated
Table 1 | Effects of co-stimulatory signals on T-cell migration

A summary of all the experimental evidence of the effect of co-stimulatory molecules on T-cell migration.

<table>
<thead>
<tr>
<th>Signal</th>
<th>Effect on motility</th>
<th>Experimental model</th>
<th>Key observations</th>
<th>Mechanism</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD28</td>
<td>Adhesion</td>
<td>Human/mouse T-cells, myelomonocytic line (in vitro)</td>
<td>Increased adhesion to fibronectin, VCAM, LFA-1 clustering</td>
<td>PI3K, PKC</td>
<td>[17–19]</td>
</tr>
<tr>
<td></td>
<td>Cytoskeleton</td>
<td>Mouse T-cells (in vitro)</td>
<td>Increased F-actin, actin polymerization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemokines</td>
<td>Localization to</td>
<td>CD28−/− T-cells, T-cells (in vitro)</td>
<td>Decreased secretion of MIP1α, control of CXCR5 expression</td>
<td>Induction of OX40 expression</td>
<td>[26,27]</td>
</tr>
<tr>
<td>antigenic sites</td>
<td></td>
<td>EAE model in CD80/CD86−/−, CD28−/− mice, antibody blockade (in vivo)</td>
<td>Decreased tissue infiltration</td>
<td>CD80, CD86, PI3K</td>
<td>[28,29]</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Adhesion</td>
<td>Pre-activated mouse CD4+ T-cells (in vitro)</td>
<td>Increased adhesion, LFA-1 clustering</td>
<td>Rap-1</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>Migration</td>
<td>Pre-activated mouse CTLA-4−/- T-cells (in vitro)</td>
<td>Enhanced migration due to short-lived adhesion to APCs</td>
<td>Unknown</td>
<td>[37]</td>
</tr>
<tr>
<td>Localization to</td>
<td>Localization to</td>
<td>CTLA-4 ligation in mouse memory T-cells (in vivo)</td>
<td>Decreased TCR-dependent recruitment</td>
<td>Unknown</td>
<td>[31]</td>
</tr>
<tr>
<td>antigenic sites</td>
<td>antigenic sites</td>
<td>CTLA-4−/- (in vivo)</td>
<td>Aberrant T-cell migration</td>
<td></td>
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<tr>
<td>OX40</td>
<td>Migration</td>
<td>CD28−/− mice (in vivo)</td>
<td>Migration of T-cells to follicles, germinal centre formation</td>
<td>CXCR5, CD28</td>
<td>[27]</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Antibody blockade,</td>
<td>Activated human T-cells</td>
<td>Cell polarization, membrane protrusions</td>
<td>PI3K, PKC</td>
<td>[45]</td>
</tr>
<tr>
<td>ICOS</td>
<td>Cytoskeleton</td>
<td>OX40−/- mice</td>
<td>Block of Th2 cytokine and chemokine expression</td>
<td>Unknown</td>
<td>[46,47]</td>
</tr>
<tr>
<td></td>
<td>Antibody blockade,</td>
<td>Activated human T-cells</td>
<td>Cell polarization, membrane protrusions</td>
<td>PI3K, PKC</td>
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</tr>
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with APCs or peptide secrete significantly less CC chemokine MIP1α (macrophage inflammatory protein-1α) [26]. In addition, CD28-dependent up-regulation of OX40 is instrumental to CXCR5 (CXC chemokine receptor 5) expression and helper T-cell migration to germinal centres in mice [27].

Furthermore, experimental models of autoimmunity have highlighted a role for CD28 co-stimulation in the localization of memory T-cell to antigenic sites. Resistance to EAE (experimental autoimmune encephalomyelitis) in CD80/CD86− and CD28-deficient mice correlated with reduced development of parenchymal T-cell infiltrates of the central nervous system following immunization with MOG35−55 peptide, despite the occurrence of efficient T-cell priming [28]. Selective deficiency of CD80 or CD86 molecule expression led to disease development comparable with wild-type mice, suggesting a redundant role for CD28 ligands in the development of T-cell-mediated inflammation [28].

Similarly, in an EAE model induced by PLP56−70 immunization of female NOD (non-obese diabetic) mice, antibody blockade of CD80/CD86 abrogated tissue infiltration, inflammation and demyelination. Notably, the complete lack of histological damage was only observed when CD80 and CD86 monoclonal antibodies were used in conjunction [29].

We have recently reported that CD28 co-stimulatory signals are essential for primed T-cells to reach their antigenic sites. CD28Y170F mice carry a mutation in the cytoplasmic tail of CD28 that abrogates PI3K recruitment without leading to defects in clonal expansion [30]. OT-II (ovalbumin-specific, MHC class II restricted TCR-transgenic) and OT-II/CD28Y170F double transgenic mice were generated and their T-cells were shown to proliferate equally following immunization with ovalbumin. However, only OT-II T-cells were able to localize to ovalbumin-expressing peritoneal membrane, suggesting that CD28-induced, PI3K-mediated signals are necessary for the recruitment of T-cells to antigenic sites [31].

In addition, we observed that dominant CD28 signals were also able to overcome the requirement for TCR engagement to sustain T-cell localization to non-lymphoid tissue. Optimal antibody activation of CD28 in HY-specific H2-Dk-restricted CD8+ T-cells led to unregulated infiltration of kidney, liver, spleen, heart and ‘homing-privileged’ gut tissue in syngeneic female mice. Enhanced trafficking was specifically induced in memory, but not in naïve, T-cells. Abrogation of PI3K activation in CD28-activated CD28Y170F HY-specific CD8+ T-cells prevented this effect [31].

Aberrant migration of primed T-cells into non-lymphoid tissue due to dominant CD28 signalling may explain the multiple organ failure described in CTLA-4-deficient mice [32−34] and in healthy human subjects recently treated with a CD28 superagonist (TGN1412; Tegenero). This compound was found in the preclinical trials to stimulate and expand...
T-cells independently of the TCR triggering, in particular Th2 (helper T-cells) and CD4+CD25+ regulatory T-cells [35]. Despite the lack of pro-inflammatory effects in the preclinical trials, when administered in six healthy male individuals, the compound led to multiorgan failure, severe lymphopenia and a cytokine storm, with increased levels of the pro-inflammatory cytokines TNFα (tumour necrosis factor α), IL-2, IL-10 and IFN-γ (interferon-γ) [36]. Given that the human T-cell repertoire comprises approx. 50% memory T-lymphocytes, the CD28 superagonist may have induced uncontrollable extravasation of memory T-cells into non-lymphoid tissue, and subsequent T-cell-mediated tissue damage. The specific effect on memory T-cells may have been overlooked in experimental models where the T-cell repertoire is mainly composed by naïve T-lymphocytes.

The effects of CTLA-4 on T-cell migration appear to be more complex. CTLA-4 has been shown to induce LFA-1 clustering and T-cell adhesion [20]. The same group examined the clustering and T-cell adhesion [20]. The specific effect on memory T-cells may have been overlooked in experimental models where the T-cell repertoire is mainly composed by naïve T-lymphocytes.

We have analysed the effects of CTLA-4 signals on antigen-dependent migration of a non-regulatory HY-specific, CD8+ T-cell clone [31]. CTLA-4 triggering prevented TCR-induced T-cell recruitment to antigenic non-lymphoid tissue in vivo, suggesting that CTLA-4-mediated signalling may prevent excessive T-cell recruitment following priming. This ‘regulatory’ role of CTLA-4 on memory T-cell trafficking is also indirectly suggested by the lethal, uncontrolled infiltration of non-lymphoid tissue observed in CTLA-4-deficient mice [32–34].

The mechanism by which CD28 and CTLA-4 differentially affect T-cell migration is at present unclear. Although both have been shown to increase adhesion, CD28 and CTLA-4 may differentially regulate cell orientation and de-adhesion. Differential activation of Rho GTPases involved in the control of cell adhesion, orientation, direction and de-adhesion may underlie these antagonistic effects [38].

Regulation of T-cell migration by other co-stimulatory molecules

OX40–OX40L (OX40 ligand)

OX40 (CD134) is a member of the TNF family of molecules. OX40 is not constitutively expressed in T-cells but is induced after activation [39]. OX40 mRNA and surface expression in murine T-cells is dependent on CD28 co-stimulation [39].

OX40-mediated interactions have been shown to be instrumental to germinal centre formation. Transgenic constitutive expression of OX40L by dendritic cells led to higher numbers of CD4+ T-cells localization in B-cell follicles [40]. CD28-dependent ligation of OX40 results in up-regulation of CXCR5 receptor, which directs T-cells to the B-cell follicles, thus promoting germinal centre formation [27]. OX40 engagement following priming also skews the T-cell profile to a primarily Th2, which promotes their migration to the B-cell follicles [41]. As OX40 signalling seems to operate on a positive feedback loop, it has been hypothesized that high-affinity T-cells co-stimulate dendritic cells through CD40 more effectively, thus receiving a stronger OX40 signal, which directs them to the B-cell follicles and results in high expansion of antigen-specific T-cells [39].

In addition to germinal centre formation, OX40 has also been implicated in the development of T-cell-mediated inflammation. OX40 antibody blockade ameliorates EAE in mice immunized with PLP139–151 [42] and OX40−/− mice were shown to be less susceptible to inflammatory lung disease [43]. The role of OX40 in inflammation is linked to PKB (protein kinase B) signalling, as expression of PKB by OX40−/− T-cells rescued the phenotype, inducing high levels of Th2 cytokines and promoting inflammation [43].

ICOS (inducible co-stimulator)–ICOSL (ICOS ligand)

ICOS is a member of the CD28 superfamily of molecules, which binds to ICOSL on the surface of B-cells and macrophages and can induce CD28-dependent expansion and cytokine production by T-cells [44]. Although it acts in a similar way as CD28, it does not result in high levels of IL-2 secretion due to differences in the SH2 binding domain [7]. ICOS has been shown to induce morphological changes that are important in migration. ICOS signalling induces cell polarization and the development of membrane protrusions in activated human T-cells. This effect is mediated through tyrosine phosphorylation of PI3K. PKB, which is downstream of PI3K, is also involved in the T-cell elongation mediated by ICOS, possibly by activating Rho GTPases [45].

ICOS-mediated signals have been shown to contribute to T-cell-mediated inflammatory processes, including lung disease and inflammatory neuropathies [46,47], suggesting that it may play a role in the localization of primed T-cells. In addition, blocking of ICOS with a neutralizing antibody has been shown to block Th2 cytokine expression, such as IL-4 and IL-10, as well as chemokine receptor expression, such as CCR (CC chemokine receptor) 3, CCR4 and CCR8 in murine T-cells [46].

Conclusions

The aforementioned studies (summarized in Table 1) suggest that the regulation of immune response by positive and negative co-stimulators relies not only on their ability to regulate T-cell activation and effector function. The integration of these signals also determines the outcome of T-cell responses by orchestrating their anatomy.

The relevance of the observations described above applies to the therapeutic manipulation of co-stimulatory signals in human diseases. CTLA-4-Ig has been used in clinical trials for the prevention of xenograft rejection [48], as well as the
treatment of autoimmune diseases, including rheumatoid arthritis [49]. Similarly, CTLA-4 blockade has been employed to enhance antitumour immune responses with side effect related to the development of T-cell-mediated inflammation [50]. The newly identified role of these molecules in the physiological regulation of T-cell migration and the development of T-cell-mediated inflammation as a result of their imbalance must clearly be taken into account in the clinical application of these strategies.

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We declare no conflict of interest.

References