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A conserved polylysine motif in CD86 cytoplasmic tail is necessary for cytoskeletal association and effective co-stimulation

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ABSTRACT

T cell activation requires both antigen specific and co-stimulatory signals that include the interaction of CD28 with its ligands CD80 and CD86. These signals are delivered by antigen presenting cells (APC) in the context of the immunological synapse (IS). Reorganization of the cytoskeleton is required for the formation and maintenance of the IS. Our results show that a highly conserved polylysine motif in CD86 cytoplasmic tail, herein referred to as the K4 motif, is responsible for the constitutive association of CD86 to the cytoskeleton in primary human APC as well as in a murine APC model. This motif is not involved in initial APC:T cell conjugate formation but mutation of the K4 motif affects CD86 reorientation at the IS. Importantly, APCs expressing CD86 with mutated K4 motif are severely compromised in their capacity to trigger complete T cell activation upon peptide presentation as measured by IL-2 secretion. Altogether, our results reveal the critical importance of the cytoskeleton-dependent CD86 polarization to the IS and more specifically the K4 motif for effective co-signaling.

1. Introduction

Initiation of adaptive immune response requires the interaction of naïve T cells with antigen presenting cells (APC), dendritic cells, B cells, and macrophages, in the context of an immunological synapse (IS) [1]. Two key signals are required for full activation of naïve T cells. The first signal requires the interaction of the TCR with MHC:peptide and confers antigen specificity to the response. The second signal entails the interaction of a co-signaling receptor at the T cell surface with its counter receptor at the APC surface [2].

Key co-signaling interactions of naïve T cell involve the engagement of its receptors CD28 and CTLA-4 by CD80 and CD86 ligands (B7 proteins; B7-1 and B7-2) expressed on APCs. While CD28-B7 interaction is involved in amplifying the antigen specific signal, CTLA-4-B7 interaction is known to dampen the strength of this signal [3]. The molecular interactions of these molecules require the formation of a contact interface i.e. the IS. Movement of signaling components and cell-surface molecules of T cells and APCs into the IS is not a passive event and requires remodeling of the actin cytoskeleton [4]. Indeed the cytoskeleton regulates molecular and membrane domains movement and also serves as a scaffold for signaling platforms in addition to its role in cell motility and division [5,6]. Increasing evidence shows that the APC cytoskeleton plays an active role in IS formation and its maintenance [7,8] and that CD80 and CD86 are involved in this IS formation process [9]. The intracellular domain of CD80 has been shown to interact with the cytoskeleton and this association impacts its membrane redistribution following T cell interaction and CD28-dependent T cell activation [10,11]. More specifically, both the RRNE region at position 275–278 and serine 284 within CD80 intracytoplasmic domain were identified to be important for its co-stimulatory function [10]. Moreover, Tseng et al. [11] have shown that CD80 deleted of its intracellular domain fails to recruit CD28, CTLA-4 and PKCθ to the synapse.

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Analysis of the human CD86 protein primary sequence led us to identify a conserved positively charged region within the CD86 cytoplasmic domain at residues $Lys^{265-268}$. This sequence as well as its localization is similar to the $RRNE^{275-278}$ region in CD80. This juxtamembrane stretch of four lysine residues in CD86, defined herein as the K4 motif, is conserved in all mammalian CD86 sequences examined. Based on the identification of this conserved motif, we hypothesized that CD86 was associated to the APC cytoskeleton and that this association was of functional relevance to its co-signaling function. We show here that CD86 is indeed associated to the APC cytoskeleton. Importantly, the association of CD86 to the cytoskeleton is critical for T cell activation.

2. Materials and methods

2.1. Recombinant DNA constructs

Cloning of CD80wt, CD80ΔT, CD86wt and CD86ΔT in the eukaryotic expression vector Srα neo was previously described [12]. CD86-K4 was generated by overlap PCR using the following oligonucleotides: K4 forward

GAAATGGGCGGCGGCGGCGGCCTCGCAACTCTTATAAATG, K4 reverse

CATTTATAAGAGTTGCGAGGCCGCGCCGCCGCCGCCCATTTCC, CD86 start

GGCTGACCCGGGTCTGAGCCACCATGGGACTGAGTAACATTCTC and CD86 end

GTATCTTATCATGTCTGGATCC. CD86-K4 was cloned into the Srα neo vector as a *Bam*HI/ *Sma*I fragment. CD80 and CD80ΔT were cloned into the pEYFP-N1 expression vector (Clontech) following amplification with oligonucleotides providing a 5′ *Xho*I and a 3′ *Sac*II restriction site. The sequence of the oligonucleotides were: CD80FwdCTCGAGGCCACCATGGGCCACACACGG and CD80Rev

CCGCGGTACAGGGCGTAC.

2.2. Antibodies and reagents

Antibodies used in this study included PE-conjugated anti-human CD80 and PE-conjugated anti-human CD86 (BD Biosciences) for FACS staining and a polyclonal rabbit antibody against YFP (BD Biosciences) for immunoblotting. NF-κB antibody was obtained from Cell Signaling. All secondary antibodies were from Molecular Probes. Anti-human CD83, anti-human TCR, anti-human CD19 and anti-human CD14 used for DC phenotyping were from BD Biosciences.

2.3. Cell lines

Murine B2D cells are derived from the P815 mastocytoma cell line stably transfected with HLA-DR0101 [13]. Human CD28 negative and positive Jurkat T cells (CD28neg and CD28pos Jurkat T cells) derived from the CH7C17 Jurkat T cell line expressing a V1.2/V3.1 TCR specific for $HA_{306-318}$peptide restricted to HLA-DR0101 have been already described elsewhere [14]. The Daudi B cell line and 293T cells were obtained from ATCC.

2.4. CD28Fc binding assay

Stable cell lines expressing the different mutant forms of CD86 were incubated for 1 h at 4 °C with various concentrations of CD28Fc (R&D Systems). Cells were then washed and incubated for an additional 30 min with a secondary antibody specific for human Fc coupled to Alexa fluor 647. After washing, cells were fixed in 2% paraformaldehyde and analyzed at the cytometer.

2.5. Dendritic cell (DC) generation and culture

Human PBMCs obtained from healthy donors were depleted of $CD3^+$ T cells using the rosetteSep kit (StemCell). $CD14^+$ cells were then isolated by autoMACS (>95% purity). Monocytes were plated at a density of 3×10^6 cells per well in 6-well tissue-culture plates in 3 ml RPMI1640 medium supplemented with 1% human serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, 2 mM L-glutamine, 1% non-essential amino acids, 200 U/ml IL-4 (Sigma) and 1000 U/ml GM-CSF (Cangene). Cells were supplied with 1.5 ml of fresh medium containing the same amounts of IL-4 and GM-CSF on days 2, 4, and 6. Mature DCs (mDCs) were obtained by harvesting non-adherent cells (immature DCs) on day 7 and stimulated with MCM-mimic consisting of 1 ng/ml TNF-α (Invitrogen), 10 ng/ml IL-1β (Invitrogen), 1 g/ml PGE2 (Sigma), and 1000 U/ml IL-6 (Invitrogen) for 48 h. DC purity was assessed by flow cytometry using fluorescently conjugated monoclonal antibodies against CD14, CD19, CD83, CD86, HLA-DR, and TCR. DC purity was consistently higher than 95%.

2.6. Biochemical fractionation

The protocol used for subcellular fractionation was previously described [15]. Briefly 10×10^6 transfected 293T cells, 10×10^6 dendritic cells or 30×10^6 B2D cell lines were pelleted, washed in PBS, resuspended in hypotonic solution (10 mM HEPES pH 6.9, 10 mM KCl, protease inhibitors) and incubated on ice for 20 min. Cells were disrupted by gentle pipetting. Nuclei were pelleted by centrifugation at 3200 rpm for 10 min at 4 °C. Supernatants from pelleted nuclei were further centrifuged at 35000 rpm for 30 minutes at 4 °C. The cytosolic fraction was separated from the pellet (cytoskeletal plus membrane fractions) and was resuspended in NTENT buffer (500 mM NaCl, 10 mM Tris–HCl pH 7.2, 1 mM EDTA, protease inhibitors and 1% Triton X-100). This fraction was centrifuged at 14 000 rpm for 30 min at 4 °C. The resulting pellet, was resuspended again in NTENT buffer and included the cytoskeletal fraction, while the membrane fraction was found in the supernatant.

2.7. Conjugate formation

APC were pre-pulsed with 10 g/ml of $HA_{306-318}$ peptide for 2 h at 37 °C. 2×10^6 cells consisting of 1×10^6 T cells and 1×10^6 HA-loaded B2D cell lines were co-cultured in a 37 °C water bath and then resuspended in PBS supplemented with 2% paraformaldehyde for 20 min. Cells were then washed in PBS and stained with PE-Cy5-conjugated anti-human MHC class I (T cells) and PE-conjugated anti-CD86 (APC) monoclonal antibodies for 1 h at 4 °C. Cells were washed and analyzed using a BD FACS Scan flow cytometer.

2.8. Imaging of immunological synapse

Cells were co-cultured as described above and deposited on poly-L-lysine coated slides for 5 min, spun at 600 rpm for 1 min, fixed in 10% formalin and then stained for CD86. Experiments with DCs were performed using 1×10^6 cells. Images were acquired on a Leica Confocal microscope, using a 63× oil-immersion objective. An average of 10 images per condition were taken for each independent experiment. Synaptic recruitment analysis was performed with the Northern Eclipse software. A fluorescence ratio was obtained by dividing the Mean Fluorescence Intensity (MFI) at the synapse with the total cell MFI. A ratio of 1 indicates that the protein is homogenously distributed at the cell surface (no specific accumulation at the interface) while a ratio greater than 1 indicates a specific accumulation of the protein of interest [16]. Results

are expressed as the fold-increase in the fluorescence ratio since baseline ratio (prior to contact) is equal to one.

3. Results

3.1. CD86 is associated to the cytoskeleton

The cytoplasmic tail of CD80 harbors residues important for CD80 association with the cellular cytoskeleton [10,17]. However, no direct biochemical evidence for CD80 cytoskeletal association has been reported. Therefore, we first verified CD80 subcellular localization by separating the cytoplasmic (Cyt), membrane (M) and cytoskeletal (Sk) subcellular fractions from cells expressing the wild type CD80 molecule. The fractionation procedure involves the removal of nuclei by hypotonic lysis and centrifugation while the other fractions are obtained by detergent solubilization and differential centrifugation. This assay has previously allowed detecting the association of CD95 with the cytoskeleton [15]. 293T cells were transfected with YFP-tagged CD80 wild type (CD80wt) or CD80 deleted of its intracellular domain (CD80ΔT) and then subjected to cellular fractionation. Immunoblotting of the different cellular subfractions using YFP-specific antibodies revealed the presence of CD80wt in both the membrane and cytoskeletal fractions (Fig. 1A, left panel), thereby confirming that CD80 is associated to the cytoskeleton. This association was further confirmed as deletion of CD80 intracellular domain resulted in the absence of any detectable CD80 in the cytoskeletal fraction. As a control, these fractions were subjected to p38 immunoblotting since p38 kinase is significantly enriched in the cytoplasm. As expected, p38 signals were only detected in the cytoplasmic fractions (Fig. 1A, right panel). We then used the same procedure to investigate the potential association of CD86 to the cytoskeleton. Fractionation of the different cellular compartments was performed, as described above, in primary human DCs derived from $CD14^+$ precursors ($n = 2$). CD86 was detected in the membrane and cytoskeletal fractions of both immature (Fig. 1b, left panel) and mature human DCs (Fig. 1b, center panel). As a control, the DCs subcellular fractions were subjected to NF-κB probing. This molecule was either greatly enriched or only present in the cytoplasmic fractions thereby confirming the specificity of our fractionation approach. These results show that CD86 is associated to the cytoskeleton in primary professional APC.

We next transfected the murine mastocytoma B2D cell line with wt CD86, a cell line that has been shown to be a valid APC [13]. As shown in Fig. 1Ab (right panel) CD86 was present in the membrane as well as in the cytoskeletal fractions from these transfected cells (immunoblotting, $n = 3$). All fractions were subjected to NF-κB probing to assess the purity of each fraction and, as expected, NF-κB was either greatly enriched or only present in the cytoplasmic fractions (Fig. 1B).

3.2. A conserved K4 motif in the cytoplasmic tail of CD86 is required for association to the cytoskeleton

The cytoplasmic tail of CD86 encompasses a highly conserved juxtamembrane polylysine motif ($Lys^{265-268}$), or K4 motif (Supplementary Fig. 1A). To assess the role of this motif in CD86 association to the cytoskeleton, constructs encoding either CD86 wild type (CD86wt), CD86 truncated of its intracellular domain (CD86ΔT) or CD86 in which the four-lysine residues of the K4 motif were mutated to alanines (CD86-K4) were generated (Supplementary Fig. 1B). These constructs were transfected into the murine mastocytoma B2D cell line that also stably expresses HLA-DR0101 [13]. Stable cell lines were sorted to obtain clonal populations expressing similar surface levels of CD86, as established by flow cytometry using monoclonal antibodies specific to CD86 (Fig. 2A). Similarly, equal HLA-DR0101 MFI values (1100–1200) were consistently measured with the transfected cells expressing the different CD86 mutants. As shown in Fig. 2B, these cell lines specifically bound to CD28Fc in a dose-dependent manner as assessed by flow cytometry. Non-transfected B2D cells showed a background of 1–5% under non-saturating concentrations of CD28Fc (data not shown).

To assess the requirement for the K4 motif in maintaining CD86 sub-cellular localization integrity, we applied the same fractionation procedure described earlier to the different CD86 stable cell lines. Our data show that CD86 was excluded from the cytoskeletal fraction in CD86-K4-expressing cells while it maintained its presence in the membrane fraction (Fig. 2C, right panel), compared to the CD86wt that partitioned to both membrane and cytoskeleton fractions (Fig. 2C, left panel). As expected, the CD86 molecule truncated of its cytoplasmic tail, CD86ΔT, was detected only in the membrane fraction (Fig. 2C center panel). Altogether, these data clearly show that the K4 motif is a cytoskeletal association motif.

3.3. CD86 cytoskeletal association is important for IS localization but does not affect conjugate formation

The CD80 cytoplasmic tail has been shown to not only be important for early IL-2 production by murine T cells but also for the integrity of CD80 spatial segregation at the IS [11]. We investigated the functional importance of CD86 cytoskeletal association on the

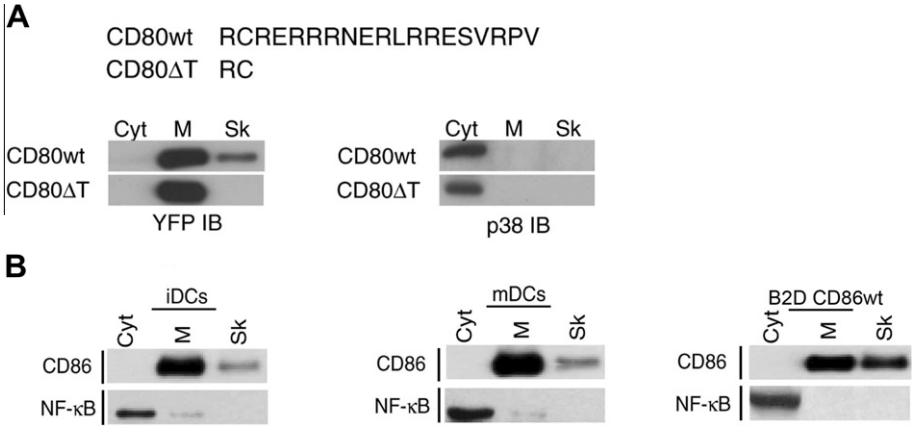

Fig. 1. Sub-cellular distribution of CD80 and CD86. (A) CD80 sub-cellular localization was assessed by sub-cellular fractionation using 293T cells transfected with YFP-tagged CD80 or CD80ΔT ($n = 3$). YFP immunoblotting is shown in left panel and p38 immunoblotting in the right panel. (B) CD86 sub-cellular distribution on natural APCs (DCs) and CD86-transfected B2D cells. CD86 immunoblotting reveals that CD86 is associated to the cytoskeleton in immature (left panel) and mature (center panel) dendritic cells ($n = 2$). NF-κB probing shows enrichment of this molecule in the cytoplasmic fractions (lower blots). Similar to the natural APCs, CD86 immunoblotting from B2D cell line expressing CD86wt reveals that CD86 is associated to the cytoskeleton (right panel, $n = 3$).

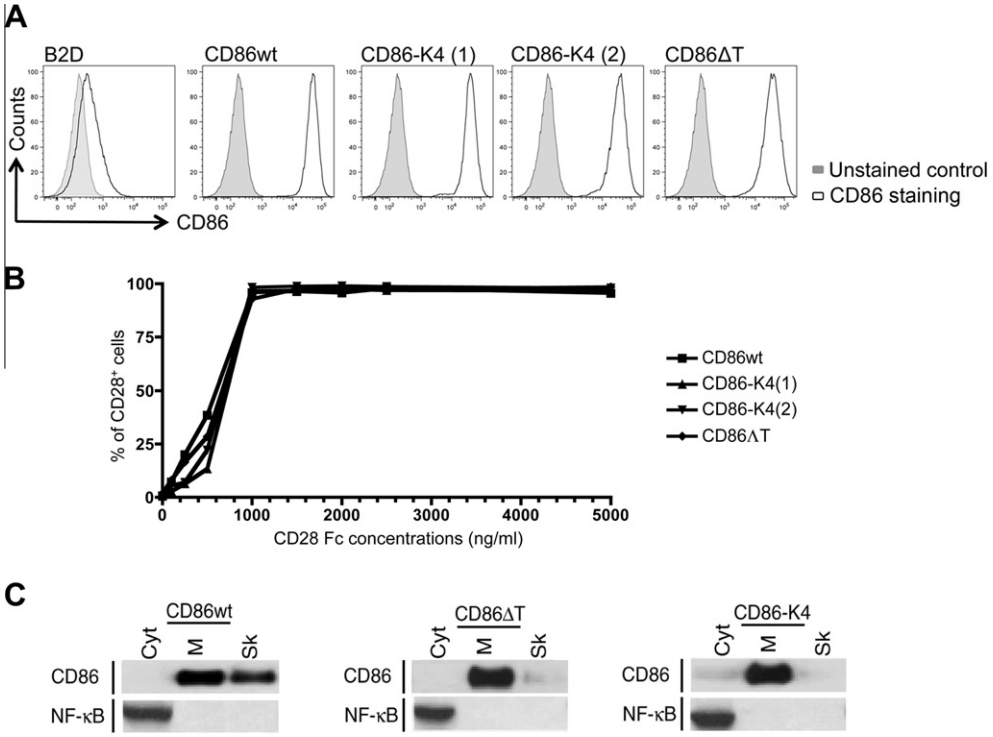

Fig. 2. Expression and binding capacity of the different CD86 clones. (A) Flow cytometry histograms showing CD86 staining of CD86wt cells, two independently derived CD86-K4 clones and CD86ΔT cells. Non-transfected B2D cells were used as a negative control for CD86 staining. (B) Binding of B2D cell lines to soluble CD28 at the various concentrations as indicated. (C) Sub-cellular distribution of CD86wt and mutated clones in B2D cells. NF-κB probing shows enrichment of this molecule in the cytoplasmic fractions of CD86 clones.

early events of T cell activation, i.e. the formation of conjugates between T cells and APCs, by flow cytometry ($n = 3$). CD28 positive or negative (CD28pos or CD28neg) Jurkat T cell lines expressing a Vα1.2/Vβ3.1 TCR recognizing the $HA_{306-318}$ peptide presented by HLA-DR0101 were co-cultured for 0 to 45 min with B2D APC pre-loaded with the $HA_{306-318}$ peptide. Cells were then fixed and T cells were stained with PE-Cy5-conjugated anti-human MHC class I while the B2D cell lines were stained with anti-human PE-conjugated CD86. T cell-APC conjugates were assessed by measuring the number of PE/PE-Cy5 double positive events by flow cytometry (Fig. 3, panel A). A background level of T cell-APC conjugates consistently below 3% was observed when B2D APC were not pre-loaded with HA peptide (data not shown) as well as when CD28neg Jurkat T cells were used (Fig. 3, panel B). For CD28pos Jurkat T cells, kinetic analysis showed that a maximum frequency of conjugates (26.6%) was obtained at 45 min, representing a 12.5-fold difference as compared to CD28neg Jurkat T cells (Fig. 3, panel B). No difference in the efficiency of T cell-APC conjugate formation was observed between CD86wt and CD86-K4 cells (Fig. 3, panel A and B).

3.4. The K4 motif is important for the polarization of CD86 to the IS

Both CD80 and CD86 have been shown to be present at the IS in various experimental models [11,18]. To study the re-localization of CD86 at the IS, HA-pulsed primary mature DCs (Fig. 4, panel A) derived from a HLA-DR0101$^+$ donor were co-cultured with CD28pos Jurkat T cells at a 1:1 ratio for 15 min. Confocal images were analyzed by measuring the ratio between the mean fluorescence intensity (MFI) of CD86 at the interaction interface and that of the whole cell [16]. Two independent experiments in mDCs showed CD86 to be polarized towards the interaction interface with a fluorescence ratio of 1.85 ± 0.33 (Fig. 4, panel A and Supplementary Table 1).

In three independent experiments using B2D cell lines as APC, CD86 was strongly polarized at the IS interaction interface after 30 min of interaction between CD86wt and CD28pos Jurkat T cells, with a fluorescence ratio of 2.58 ± 0.38 (Fig. 4, panel B and supplementary Table 1). Polarization in CD86-K4 cells was much less significant, with a fluorescence ratio of 1.29 ± 0.21. These data clearly show that the integrity of the K4 motif was required for successful re-localization of CD86 to the IS.

3.5. The K4 motif of CD86 is critical for IL-2 production by T cells upon activation

The physiological relevance of CD86 cytoskeleton association through the K4 motif in late events of T cell activation was then assessed. IL-2 secretion of CD28pos Jurkat T cells in response to antigen specific TCR triggering was measured by ELISA following an overnight co-culture with APC expressing highly similar levels of CD86wt, CD86-K4 or CD86ΔT (as shown in Fig. 2A) loaded with varying HA peptide concentrations (Fig. 4C). The system used herein is highly dependent on co-stimulation; only HLA-DR0101$^+$ CD86wt cells triggered IL-2 production by T cells while HLA-DR0101$^+$ CD86$^-$ B2D cells failed to induce cytokine production even in the presence of optimal concentrations of HA peptide ($p < 0.05$). Deletion of CD86 intracellular domain almost completely abrogated IL-2 production while two independently derived CD86-K4 clones induced drastically lower IL-2 levels when compared to CD86wt at all tested concentrations of HA peptide (Fig. 4C).

4. Discussion

The goal of this study was to establish if CD86 was associated to the cytoskeleton and to determine the functional relevance of such association. Using a biochemical fractionation approach allowing

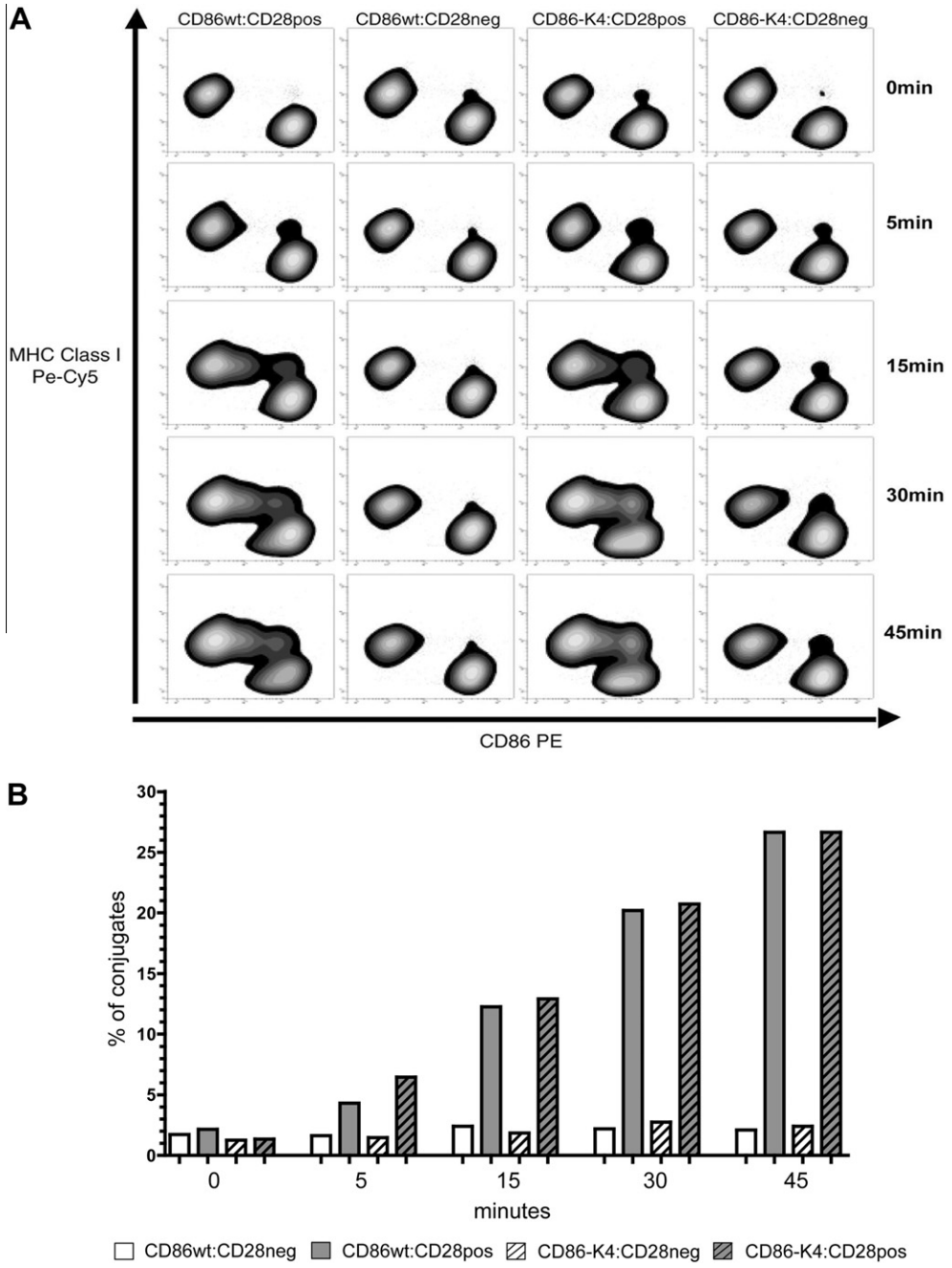

Fig. 3. CD86 association to the cytoskeleton does not affect conjugate formation. (A) B2D cell lines pre-pulsed with 10 mg/ml $HA_{306-318}$ peptide for 2 h at 37 °C were co-cultured with CD28pos and CD28neg Jurkat T cells for 0 to 45 min and then stained with PE-conjugated anti-human CD86 and PE-Cy5-conjugated anti-human MHC Class I. Double positive ($PE^+/PE\text{-}Cy5^+$) events represent conjugates. (B) Bar graph representation of the average results of 3 independent experiments as performed in A.

the distinction between cytoplasmic, membrane and cytoskeletal fractions [15] we showed that CD86, as previously shown for CD80, was associated to the cytoskeleton. This association requires the intracellular domain of CD86 and more specifically the K4 motif thereby identifying this sequence stretch as a cytoskeleton association motif in CD86.

Having established the association of CD86 to the cytoskeleton and having delineated the involved motif, we aimed at demonstrating the functional relevance of such findings. We first showed that CD86 association to the cytoskeleton was not involved in the initial T cell-APC conjugate formation since no difference in the formation efficiency was observed between CD86wt and CD86-K4 cells. These results are in agreement with the suggested adhesion role mediated by CD86/CD28 interactions in the earliest events of T cell-APC contacts [19]. On the other hand, CD86 association to the cytoskeleton is important for CD86 re-localization at the interaction interface when contacting T cells as shown by our imaging experiments. Our results also indicate that CD86 is actively recruited and/or retained at the IS in a cytoskeleton-dependent fashion since CD86 mutated in its K4 motif (CD86-K4), which prevents its interaction with the cytoskeleton, does not accumulate at the IS following T cell interaction. Our findings are thus in agreement with previous studies showing the importance of the APC cytoskeleton in the formation of peptide-dependent IS [7,20]. Of note, CD86 cytoskeletal association is functionally relevant since IL-2 production by T cells is almost completely abrogated following

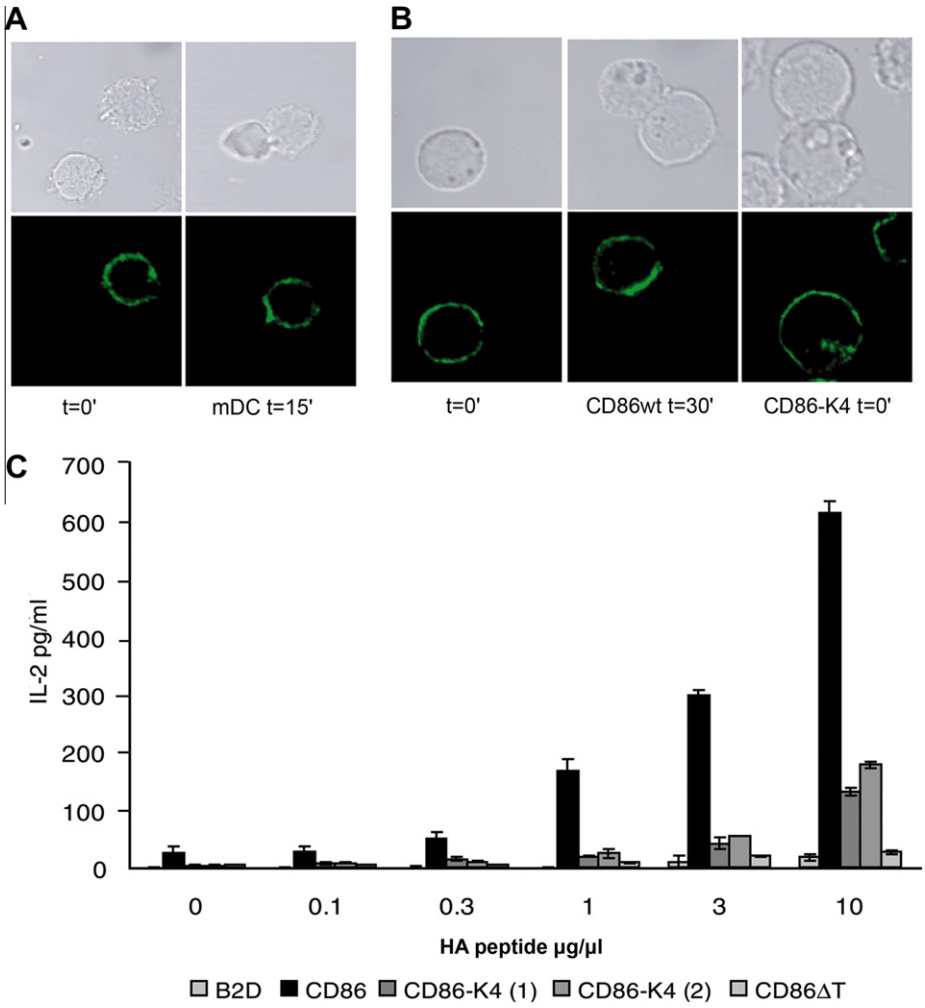

Fig. 4. CD86 localization to the IS requires the K4 motif. (A) Human mature dendritic cells derived from a HLA-DR0101^{+} donor were pre-pulsed with $HA_{306-318}$ peptide for 2 h at 37 °C and co-cultured with CD28pos Jurkat T cells for 15 min at 37 °C. Cells were then spun onto a slide, fixed and stained for CD86 (shown in green). (B) CD86wt cells also present relocalisation of CD86 at the interface after 30 min of interaction with CD28pos Jurkat T cells whereas CD86-K4 cells do not show such relocalisation. Representative images of two (A) and three (B) independent experiments are shown. (C) CD86wt and two different CD86-K4 cellular clones expressing comparable levels of CD86 were co-cultured overnight with CD28pos Jurkat T cells at a 1:1 ratio with various concentrations of $HA_{306-318}$ peptide as indicated and IL-2 production was measured by ELISA.

stimulation by APC expressing the CD86 molecule mutated in its cytoskeletal association motif.

Altogether, we suggest a reciprocal two-signal model in APC that would involve a first signal triggered through adhesion or MHC molecules [21,22] inducing the cytoskeleton-dependent recruitment or retention of co-signaling molecules at the immunological synapse. Signaling through adhesion or MHC molecules would require initial conjugate formation through cytoskeletal relaxation [23] and CD28-CD86 dependent adhesion as inferred by our data and others [14]. The second signal would involve the stable interaction between CD80/CD86 molecules with their counter-receptors for proper and sustained co-signaling. This reciprocal two-signal model highlights the bi-directional nature of signaling in the IS [24] that leads to cytoskeletal rearrangements thereby creating an optimal environment for CD28-B7 interaction and function.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.116.

References

[1] A. Grakoui, S.K. Bromley, C. Sumen, M.M. Davis, A.S. Shaw, P.M. Allen, M.L. Dustin, The immunological synapse: a molecular machine controlling T cell activation, Science 285 (1999) 221–227.
[2] R.M. Steinman, J.W. Young, Signals arising from antigen-presenting cells, Curr. Opin. Immunol. 3 (1991) 361–372.
[3] R.J. Greenwald, G.J. Freeman, A.H. Sharpe, The B7 family revisited, Annu. Rev. Immunol. 23 (2005) 515–548.
[4] M.L. Dustin, J.A. Cooper, The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling, Nat. Immunol. 1 (2000) 23–29.

[5] J.K. Burkhardt, E. Carrizosa, M.H. Shaffer, The actin cytoskeleton in T cell activation, Annu. Rev. Immunol. 26 (2008) 233–259.
[6] T.D. Pollard, J.A. Cooper, Actin, a central player in cell shape and movement, Science 326 (2009) 1208–1212.
[7] M.M. Al-Alwan, R.S. Liwski, S.M. Haeryfar, W.H. Baldridge, D.W. Hoskin, G. Rowden, K.A. West, Cutting edge: dendritic cell actin cytoskeletal polarization during immunological synapse formation is highly antigen-dependent, J. Immunol. 171 (2003) 4479–4483.
[8] M.M. Al-Alwan, G. Rowden, T.D. Lee, K.A. West, The dendritic cell cytoskeleton is critical for the formation of the immunological synapse, J. Immunol. 166 (2001) 1452–1456.
[9] S.A. Wetzel, T.W. McKeithan, D.C. Parker, Live-cell dynamics and the role of costimulation in immunological synapse formation, J. Immunol. 169 (2002) 6092–6101.
[10] R.T. Doty, E.A. Clark, Two regions in the CD80 cytoplasmic tail regulate CD80 redistribution and T cell costimulation, J. Immunol. 161 (1998) 2700–2707.
[11] S.Y. Tseng, M. Liu, M.L. Dustin, CD80 cytoplasmic domain controls localization of CD28, CTLA-4, and protein kinase C theta in the immunological synapse, J. Immunol. 175 (2005) 7829–7836.
[12] M.G. Agadjanyan, M.A. Chattergoon, M.J. Holterman, B. Monzavi-Karbassi, J.J. Kim, T. Dentchev, D. Wilson, V. Ayyavoo, L.J. Montaner, T. Kieber-Emmons, R.P. Sekaly, D.B. Weiner, Costimulatory molecule immune enhancement in a plasmid vaccine model is regulated in part through the Ig constant-like domain of CD80/86, J. Immunol. 171 (2003) 4311–4319.
[13] D. Gay, P. Maddon, R. Sekaly, M.A. Talle, M. Godfrey, E. Long, G. Goldstein, L. Chess, R. Axel, J. Kappler, et al., Functional interaction between human T-cell protein CD4 and the major histocompatibility complex HLA–DR antigen, Nature 328 (1987) 626–629.
[14] F. Michel, G. Attal-Bonnefoy, G. Mangino, S. Mise-Omata, O. Acuto, CD28 as a molecular amplifier extending TCR ligation and signaling capabilities, Immunity 15 (2001) 935–945.
[15] S. Parlato, A.M. Giammarioli, M. Logozzi, F. Lozupone, P. Matarrese, F. Luciani, M. Falchi, W. Malorni, S. Fais, CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway, EMBO J. 19 (2000) 5123–5134.
[16] C. Brossard, M. Semichon, A. Trautmann, G. Bismuth, CD5 inhibits signaling at the immunological synapse without impairing its formation, J. Immunol. 170 (2003) 4623–4629.
[17] R.T. Doty, E.A. Clark, Subcellular localization of CD80 receptors is dependent on an intact cytoplasmic tail and is required for CD28-dependent T cell costimulation, J. Immunol. 157 (1996) 3270–3279.
[18] T. Pentcheva-Hoang, J.G. Egen, K. Wojnoonski, J.P. Allison, B7–1 and B7–2 selectively recruit CTLA-4 and CD28 to the immunological synapse, Immunity 21 (2004) 401–413.
[19] O. Acuto, S. Mise-Omata, G. Mangino, F. Michel, Molecular modifiers of T cell antigen receptor triggering threshold: the mechanism of CD28 costimulatory receptor, Immunol. Rev. 192 (2003) 21–31.
[20] G. Bouma, A. Mendoza-Naranjo, M.P. Blundell, E. de Falco, K.L. Parsley, S.O. Burns, A.J. Thrasher, Cytoskeletal remodeling mediated by WASp in dendritic cells is necessary for normal immune synapse formation and T cell priming, Blood (2011).
[21] R. Al-Daccak, N. Mooney, D. Charron, MHC class II signaling in antigen-presenting cells, Curr. Opin. Immunol. 16 (2004) 108–113.
[22] T.N. Sims, M.L. Dustin, The immunological synapse: integrins take the stage, Immunol. Rev. 186 (2002) 100–117.
[23] S. Faure, L.I. Salazar-Fontana, M. Semichon, V.L. Tybulewicz, G. Bismuth, A. Trautmann, R.N. Germain, J. Delon, ERM proteins regulate cytoskeleton relaxation promoting T cell-APC conjugation, Nat. Immunol. 5 (2004) 272–279.
[24] C. Orabona, U. Grohmann, M.L. Belladonna, F. Fallarino, C. Vacca, R. Bianchi, S. Bozza, C. Volpi, B.L. Salomon, M.C. Fioretti, L. Romani, P. Puccetti, CD28 induces immunostimulatory signals in dendritic cells via CD80 and CD86, Nat. Immunol. 5 (2004) 1134–1142.