

Class II MHC-independent suppressive adhesion of dendritic cells by regulatory T cells in vivo

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Regulatory T (T reg) cells are essential for peripheral homeostasis and known to target and suppress dendritic cells (DCs). One important mechanism is through prolonged interaction between antigen-specific T reg cells and DCs that down-regulates the co-stimulatory capacity of DCs. However, the dynamics and TCR specificities of such T reg cell–DC interaction and its relevance to the suppressive outcomes for individual DCs have not been clarified. To gain insights into the underlying cellular events in vivo, we analyzed individual T reg cell–DC interaction events in lymph nodes by intravital microscopy. Our results show that, upon exposure to interleukin-2, T reg cells formed prolonged adhesive contact with DCs, independent of antigen or MHC recognition, which significantly suppressed the contemporaneous interaction of the same DCs with antigen-specific conventional T cells and impaired T cell priming. Therefore, T reg cells may function in part as feedback regulators in inflammatory milieu, by suppressing local DCs and interrupting immune activation in a contact-dependent and class II MHC-independent manner.

INTRODUCTION

Regulatory T (T reg) cells play an essential role in maintaining homeostasis by suppressing activation of self-reactive T cells and by limiting the magnitude of immune activation in response to pathogens (Wing and Sakaguchi, 2010; Germain, 2012; Josefowicz et al., 2012). DCs are an important target for T reg cell-mediated suppression, and down-modulation of their expression of co-stimulatory molecules is one suppressive mechanism (Cederbom et al., 2000; Huang et al., 2004; Onishi et al., 2008; Wing et al., 2008). However, cellular events underlying T reg cell-mediated DC suppression remain elusive, particularly with regard to the role of individual physical T reg cell–DC interactions in vivo. Previous imaging work in situ reveals that interactions between antigen-specific conventional CD4⁺ T cells (T conv) and DCs are prolonged in the absence of the entire Foxp3⁺CD25⁺ T reg cell population (Tadokoro et al., 2006; Tang et al., 2006), suggesting that T reg cells may interfere with T conv–DC interactions. However, these studies have not established whether such interference is based on soluble factors or requires T reg cell–DC physical interactions. It is also not clear if recognition of foreign or self-antigen presented by the DCs is a prerequisite for T reg cells to suppress these same DCs. Genetic evidence indicates that TCR expression in mature T reg cells is essential for maintaining peripheral tolerance at the organismal level (Levine et al., 2014; Vahl et al., 2014). However, these results cannot differentiate the possibility that TCR signaling is required to activate T reg cells into a suppressive state in which they can inhibit DC functions by soluble or contact-dependent factors from the possibility that T reg cells suppress DCs in a

contact-dependent manner and the TCR signaling must take place at the moment of suppression.

In this study, we first visualized behaviors of endogenous T reg cells and found these cells exhibiting enhanced adhesion to antigen-presenting DCs that mediated T conv cell activation in the draining LNs. Subsequent experiments using adoptive transfer of T reg cells and class II MHC-deficient DCs demonstrate the increased T reg cell–DC adhesion can be promoted by exposure to IL-2, while requiring no antigen or MHC recognition. Importantly, physical contacts by polyclonal T reg cells significantly reduce the ability of DCs to form stable conjugates with cognate T conv cells in vivo. Our results suggest that T reg cells of any TCR specificity can suppress DCs in a contact-dependent and class II MHC-independent manner.

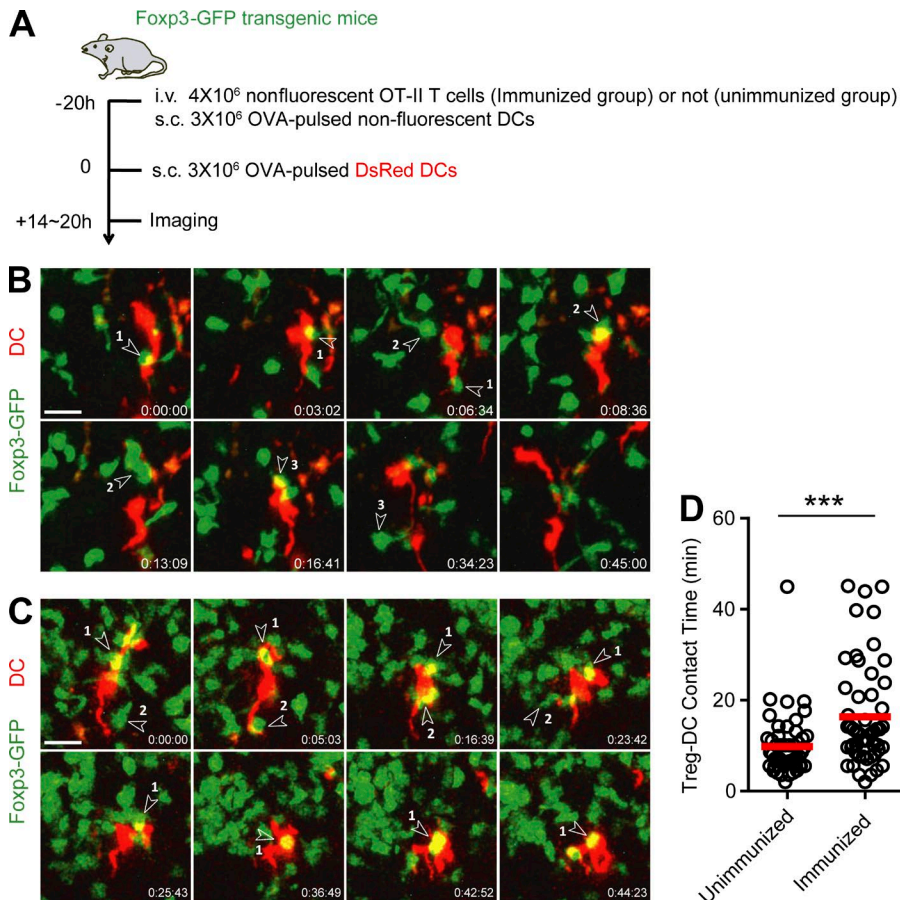
RESULTS AND DISCUSSION

Prolonged T reg cell–DC interactions during immune activation

To explore how physical T reg cell–DC interactions play a role in regulating T conv activation by DCs, we conducted two-photon intravital microscopy to examine endogenous T reg cell interactions with DCs in inflamed LNs, where OT-II T cells that bear the TCR-recognizing OVA epitope (OVA_{323–339}) complexed with the I-A^b MHC class II molecule were being activated by OVA-pulsed DCs (Fig. 1 A). Interestingly, T reg cells undertook significantly more prolonged contacts with DCs in these LNs than in control LNs, where no overt

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T cell activation was induced (Fig. 1, B–D; and Videos 1 and 2), suggesting that factors produced during T conv activation in the inflamed LN may condition T reg cells to interact with DCs in a feedback manner.

IL-2 conditions T reg cells for DC adhesion

IL-2 plays a crucial role in the maintenance and survival of T reg cells in vivo (Fontenot et al., 2005; Vang et al., 2008) and promotes T reg cell-mediated suppression in vitro (de la Rosa et al., 2004; Thornton et al., 2004). OT-II T cells made ample IL-2 at the time of imaging analyses described above (Fig. 2 A). Given that T reg cells rapidly respond to IL-2 produced by primed T conv cells in vivo (O’Gorman et al., 2009), we tested whether IL-2 would be able to condition T reg cells for more extensive interactions with and functional suppression of DCs in vivo. Polyclonal T reg or control CD25⁺ T conv cells were treated with IL-2, transferred into B6 mice that were s.c. injected with LPS-activated DCs, and intravitaly imaged in the draining LNs 14–20 h later (Fig. 2 B). As expected, IL-2 treatment did not appreciably alter behaviors of CD25⁺ T conv cells (Fig. 2, C–E; and Video 3), whose duration of contacts with DCs was relatively short (mean \pm SEM contact time of treated vs. untreated, 5.7 ± 0.6 vs. 5.8 ± 0.5 min; $P = 0.9$), comparable to what was previously reported for antigen nonspecific T cell–DC

Figure 1. Endogenous T reg cells form prolonged contacts with DCs after immunization. (A) Protocol used for visualizing interactions between endogenous T reg cells and OVA-pulsed DCs in the presence or absence of transferred OT-II T cells in vivo. (B and C) Snapshots of interactions between DCs and Foxp3-expressing T reg cells visualized in quiescent (B) or immunized (C) draining LNs where OT-II were being activated by LPS-stimulated, OVA-pulsed DCs (see corresponding Videos 1 and 2). Arrowheads and corresponding numbers trace different T reg cells interacting with the DCs sequentially, covering from the beginning to the end of each T reg cell–DC interaction incidence. Time code, hour:min:sec. Bar, 15 μ m. (D) Duration of contacts quantitated for 50 and 52 T reg cell–DC conjugates with or without overt T cell activation. Data are pooled from three independent experiments. ***, $P < 0.001$, Student’s t test.

contacts (Mempel et al., 2004). T reg cells tended to engage DCs for a longer period (mean \pm SEM contact time, 9.0 ± 0.9 min) than the T conv cells. Importantly, the engagement was significantly extended by IL-2 treatment (14.4 ± 1.4 vs. 9.0 ± 0.9 min; $P < 0.001$; Fig. 2, D and E; and Video 4). On the other hand, when recently activated CD25⁺ T conv cells were examined, their contacts with DCs presenting no antigen were not prolonged by the same IL-2 treatment (Fig. S1 and Video 5). Therefore, IL-2 stimulation promotes physical T reg cell interactions with DCs in vivo.

T reg cells suppress the antigen-specific T conv–DC interactions in a contact-dependent manner

Given that IL-2-conditioned T reg cells can strongly adhere to DCs, we wanted to examine whether such adhering T reg cells exert any suppressive effects on DCs at the single cell–cell contact level. This question cannot be addressed by comparing T conv–DC interactions in the full presence or complete absence of the endogenous T reg cell population, the protocol used in previous studies of T reg cell-mediated functional DC suppression at the population level (Tadokoro et al., 2006; Tang et al., 2006). Instead, we visualized behaviors of antigen-specific T conv cells interacting with individual DCs that were free of or engaged by co-transferred polyclonal T reg cells in the same LN (Fig. 3 A). As shown in Fig. 3

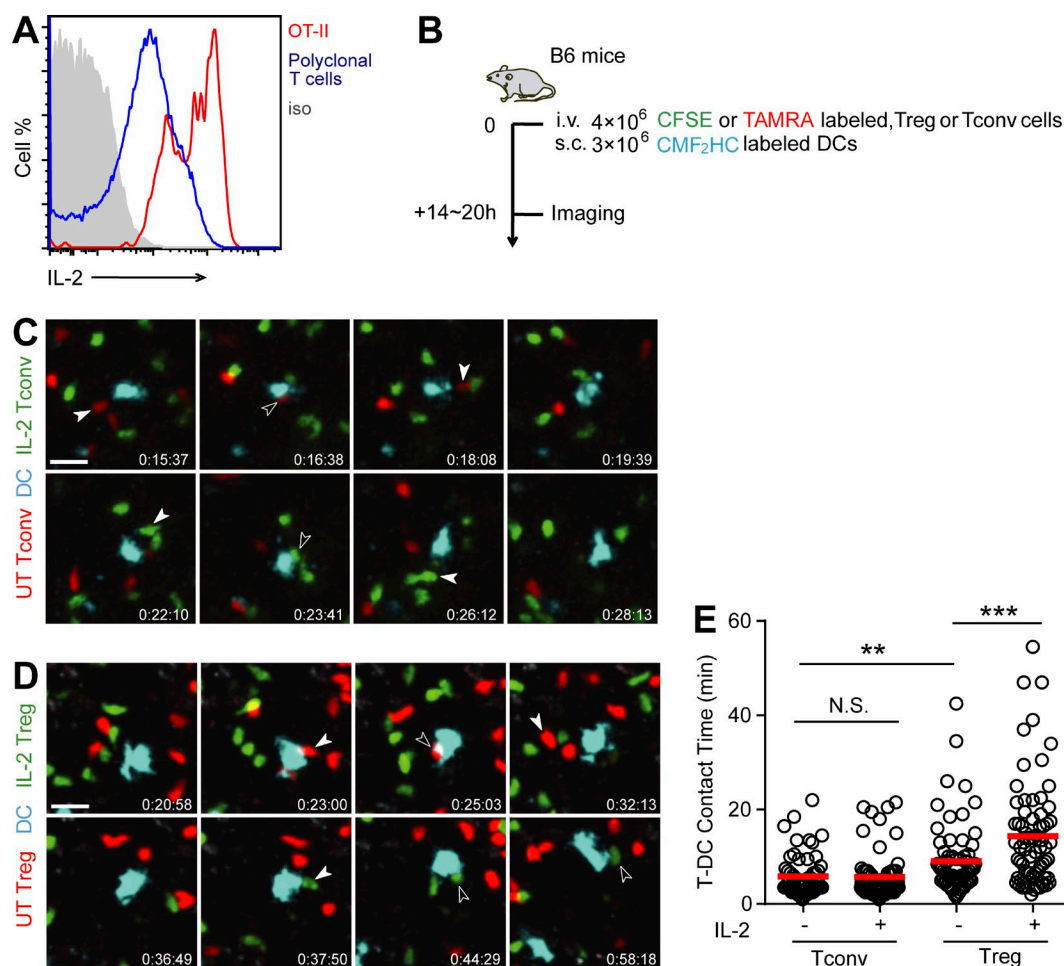


Figure 2. Prolonged interactions between DCs and T reg cells. (A) Intracellular IL-2 staining of OT-II T cells and polyclonal T cells from draining LNs after brief stimulation with PMA and ionomycin, ~20 h after s.c. injection of OVA-pulsed DCs, coinciding with the time point when T reg cell–DC interactions were visualized. (B) The protocol for imaging T cell–DC interactions in vivo. (C and D) Snapshot for T conv cells (C) or T reg cells (D) and DCs interaction analysis. UT, untreated; IL-2, pre-treated with IL-2. (E) Duration of contacts quantitated for T cell–DC conjugates under different conditions. For CD25⁺ T conv cells 63 untreated and 67 IL-2 pretreated T cells are analyzed; for CD25⁺ T reg cells, 72 untreated and 70 treated cells are analyzed. Data are pooled from four independent experiments (see Videos 3 and 4). **, $P < 0.01$; ***, $P < 0.001$; N.S., not significant, Student's t test.

(B and D) and Video 6, OVA-pulsed DCs that were contacted by the IL-2-treated T reg cells tended to support shorter contacts by OT-II T cells (mean \pm SEM contact duration, 26.8 ± 3.9 min), as compared with those DCs that were free of contacts by transferred T reg cells (mean \pm SEM contact duration, 44.6 ± 3.7 min; $P < 0.001$; Fig. 3, C and D; and Videos 7 and 8). Furthermore, antigen-specific T conv–DC contact times were negatively correlated with durations of contacts between these same DCs and T reg cells (Fig. 3 E). However, contacts by T conv cells had no effect on the stable interactions between antigen-specific T cells and antigen-presenting DCs (Fig. 3, F and G; and Video 9). These data suggest that IL-2-conditioned T reg cells impair the ability of DCs being engaged to simultaneously form durable conjugates with cognate T conv cells in vivo. Consistent with this notion, antigen-specific OT-II proliferation stimulated by OVA-

pulsed DCs in vitro could be significantly suppressed by polyclonal T reg cells present in the same culture that were not agonistically stimulated through the TCR (Fig. S2, A and B). This polyclonal T reg cell-mediated suppression was enhanced when the T reg cells were preconditioned by IL-2 (Fig. S2 C).

T reg cell-mediated suppression of DCs does not require concomitant antigen recognition

Because natural T reg cells tend to carry high-affinity self-reactive TCRs (Wing and Sakaguchi, 2010), and strong T cell adhesion to DCs is typically orchestrated by inside-out signaling from TCR activation to integrins during cognate antigen recognition (Abram and Lowell, 2009), it seems plausible that the strong T reg cell–DC interactions promoted by IL-2 would also be based on MHC-restricted self-antigen recognition, as proposed by a recent study (Matheu et al., 2015).

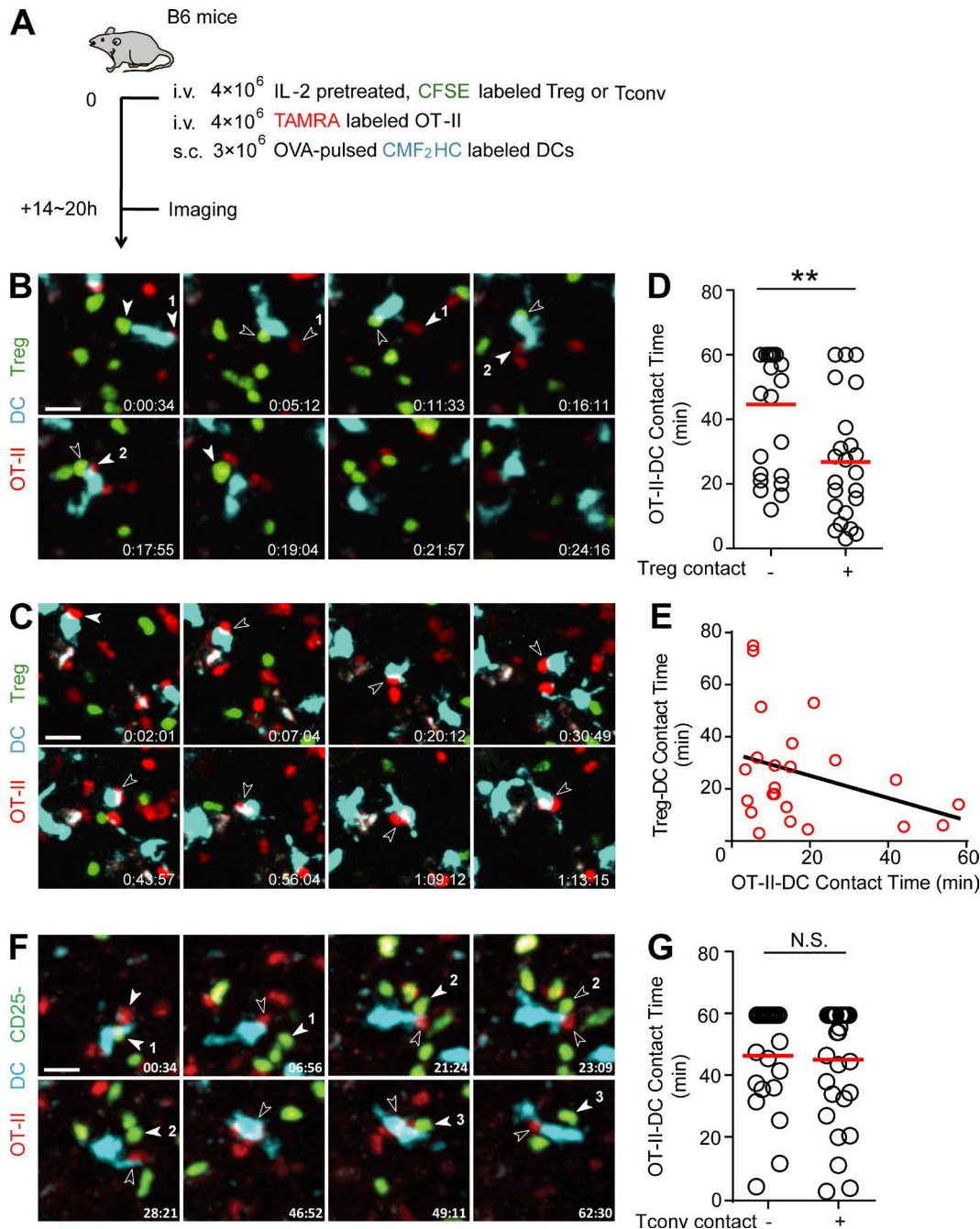


Figure 3. T reg cell contacts with DCs suppress contemporaneous engagement by antigen-specific T conv cells in vivo. (A) The protocol for visualizing interactions between OT-II T cells and OVA₃₂₃₋₃₃₉-presenting DCs that were exposed to T reg cells in the same LN. (B–G) Interactions between OT-II T cells and OVA₃₂₃₋₃₃₉-presenting DCs that were exposed to transferred T reg cells in the LN. (B–D) Typical behaviors of OT-II T cells interacting with DCs visibly contacted (+; B) or untouched (–; C) by the transferred T reg cells. Arrowheads in B trace one T reg cell and two OT-II T cells (number 1 and 2) making contacts with the same DC. Arrowheads in C trace one OT-II T cell interacting with a DC free from transferred T reg cells. Closed arrowheads mark the beginning and end of an interaction incidence (see Videos 6 and 7). (D) Duration of contacts between OT-II T cells and DCs with ($n = 25$) or without ($n = 23$) T reg cell contacts. (E) Contact durations with T reg cell and OT-II cells by the same individual DCs analyzed in Fig. 3 (B–D). Each symbol represents one DC, and the trend line denotes the negative correlation. (F) Typical behaviors of OT-II T cells interacting with DCs visibly contacted by transferred T conv cells. Arrowheads in F trace three T conv cells (number 1, 2, and 3) and one OT-II T cell making contacts with the DC. (G) Duration of contacts between OT-II T cells and DCs with ($n = 22$) or without ($n = 30$) T conv cell contacts (see Video 9). Data are pooled from six imaging sessions. **, $P < 0.01$; N.S., not significant, Student's t test.

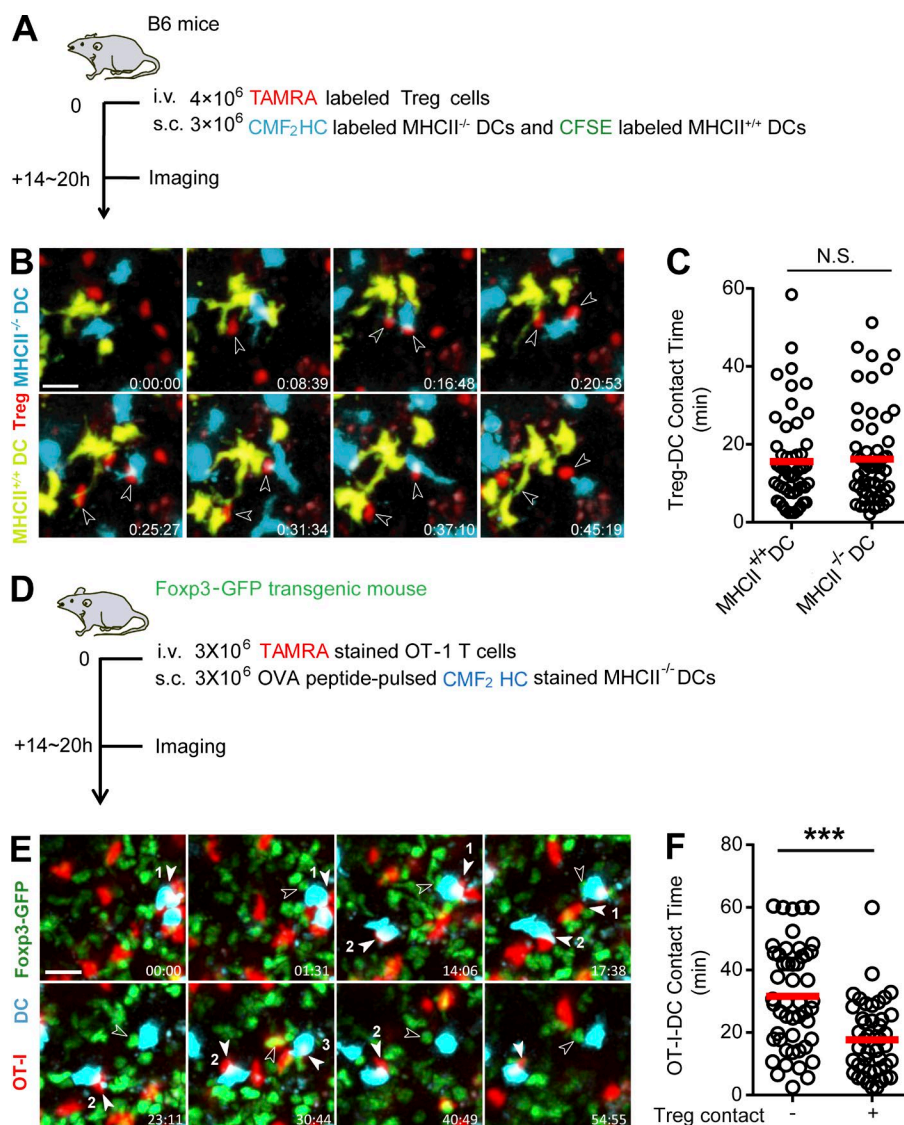


Figure 4. Class II MHC-independent contacts and suppression of DCs by T reg cells. (A) The protocol for visualizing T reg cells interact with MHC class II sufficient or deficient DCs in vivo. (B) Interactions between T reg cells and DCs deficient in MHC class II (I-A^b) expression (MHCII^{-/-}) or WT DCs (MHCII^{+/+}) visualized in the same draining LN (see Video 10) for the corresponding image sequence. Arrowheads trace two T reg cells comparably interacting with two DCs of the two genotypes, respectively, with closed arrowheads specifically denoting the beginning and end of an interaction incidence. Time code, hour:min:sec. Bar, 15 μ m. (C) Duration of T reg cell contacts with DCs of indicated types (52 MHC class II^{+/+} and 47 MHC class II^{-/-}) pooled from two experiments. (D) The protocol for visualizing interactions between OT-I T cells and OVA₂₅₇₋₂₆₄-presenting DCs that in LN of Foxp3-GFP mouse. (E and F) Typical behaviors of OT-I T cells interacting with DCs visibly contacted or untouched by the Foxp3⁺ T reg cells. Closed arrowheads trace three OT-I T cells making contacts with DCs. Two (cell #1 and #3) exhibit shorter contacts with a DC touched by a T reg cell (open arrowhead), and one (cell #2) was engaged in long-lasting contact with a DC not touched by any visible T reg cells during the session. (F) Duration of contacts between OT-I T cells and DCs with ($n = 52$) or without ($n = 50$) T reg cell contacts. Data are pooled from two independent experiments (see Video 11) for the corresponding image sequence. ***, $P < 0.001$; N.S., not significant, Student's t test.

To test this possibility directly, interactions of IL-2-treated T reg cells with wild-type or class II MHC-deficient DCs were imaged intravitaly in LNs (Fig. 4 A). Interestingly, as shown in Fig. 4 (B and C), and Video 10, these T reg cells engaged in prolonged contacts with both types of DCs in a comparable manner in vivo (mean \pm SEM contact time, 16.8 ± 2.0 vs. 15.0 ± 2.4 ; $P = 0.6$), indicating that intense T reg cell-DC interactions conditioned by IL-2 do not require concomitant antigen recognition. To test whether the same holds true for contact-dependent suppression of T conv-DC interactions, we measured class I MHC-restricted CD8 T cell activation induced by antigen-pulsed class II MHC-deficient DCs. In such a system, recognition of DC-presented self-antigen by T reg cells can be ruled out, and any contact-dependent suppression of CD8 T cell priming would have to be antigen and class II MHC independent. We s.c. injected OVA-pulsed class II MHC-deficient DCs into FoxP3-GFP reporter mice

to activate transferred OT-I CD8 T cells (Fig. 4 D). As shown in Fig. 4 (E and F) and Video 11, class II MHC-deficient DCs engaged by endogenous T reg cells exhibited significantly reduced contact durations with OT-I T cells, as compared with those unengaged DCs (mean \pm SEM contact duration, 19.4 ± 2.7 min vs. 33.2 ± 3.6 min; $P < 0.001$). These data indicate that T reg cells can suppress, in a contact-dependent and class II MHC-independent manner, the ability of individual DCs being engaged to simultaneously form durable conjugates with cognate T conv cells. Consistent with this, activation of OVA-specific CD8⁺ OT-I T cells by class II MHC-deficient DCs can also be suppressed by polyclonal T reg cells in vitro (Fig. S3).

Our results reveal a novel DC-targeted, T reg cell-mediated suppression mechanism that is promoted by IL-2, dependent on individual cell-cell adhesion but independent of concomitant antigen recognition. We speculate that rele-

vant IL-2 can be produced by pathogen-specific T cells or by inflammatory DCs (Zelante et al., 2012) during infection, and possibly by autoantigen-reactive T cells after sporadic activation under the steady state (Setoguchi et al., 2005; Liu et al., 2015). IL-2 could condition T reg cells in the vicinity to interact with DCs and suppress these same DCs for activating antigen-specific T conv cells. Because this mode of suppression does not require contemporaneous TCR signaling into the T reg cells, all T reg cells irrespective of TCR specificities can participate. Whereas we could not measure local IL-2 concentrations around T conv DC clusters in situ, contact duration between DCs and T reg cells conditioned with IL-2 in vitro (Fig. 2) are comparable to those between DCs and endogenous T reg cells observed during OT-II activation (Fig. 1). Therefore, to the extent that we can detect by a functional readout, in vitro IL-2 conditioning did not overtly exaggerate T reg cell–DC interactions that did not involve specific ligand recognition. Importantly, one suppressive T reg cell contact is unlikely to render a DC completely inert; it appears that individual T reg cell contacts lead a suppressed DC state that lasts as long as the particular T reg cell remains in contact, and DC recovers from the suppressed state thereafter (unpublished observations). The fact that contacts between transferred T reg cells and DCs exhibited suppressive effects in the presence of intact and full complement of endogenous T reg cells, which certainly constituted the overwhelming majority of all T reg cells in our experimental system, strongly support that the T reg cell–mediated suppression of DCs is not only contact-dependent but also short-lived after T reg cell disengagement at the level of individual contacts. This is because, otherwise, the effect of endogenous T reg cells would obscure the transferred T reg cells. Because all T reg cells presumably can effectuate this type of reversible suppressive contacts and DCs are subjected to repeated contacts by surrounding T reg cells, such suppression reduces the spatiotemporal availability of DCs that are otherwise functionally competent to form long-lasting synapses with cognate T cells (Celli et al., 2007). At the molecular level, T reg cell contacts can cause acute and reversible cytoskeletal reorganization in DCs, reducing their ability to form a productive synapse with cognate T conv cells (see Chen et al. in this issue).

It is well established that the integrity of mature T reg cells, as reflected by the maintenance of immune homeostasis at the organismal level, requires TCR-mediated functions in the periphery (Levine et al., 2014; Vahl et al., 2014). However, it is not necessary for T reg cells to be triggered via TCR all the time in vivo to be functionally suppressive. A plausible scenario is that periodic signaling from the TCR and other accessory receptors maintains suppressive capabilities of individual T reg cells and helps localize these cells to the most relevant tissue sites, whereas suppressive contacts with DCs do not necessarily involve contemporaneous TCR triggering. By allowing spatiotemporal separation of TCR-dependent maintenance of T reg cell functional capacity and the delivery of their suppressive activities, at least to the extent that can be

regulated by exposure to IL-2, the mode of contact-dependent but class II MHC-independent suppression would maximize flexibility and utility of T reg cells of all specificities, permitting an immune response to proceed in check without being overtly exaggerated. At the same time, when T reg cells recognize TCR ligands presented on any given DC, either cross-reactive foreign antigens or self-epitopes, suppression of such DCs likely becomes more intense as a result of additional mechanisms that may be uniquely responsive to TCR triggering (Wing et al., 2008; Qureshi et al., 2011).

MATERIALS AND METHODS

Mice

DsRed-transgenic mice, class II MHC-deficient *H2^{dAb1-Ea}* mice, Foxp3-IRES-GFP mice (Qureshi et al., 2011), OT-I and OT-II transgenic mice are maintained on the C57BL/6 background at Tsinghua University Animal Facilities. All mice were maintained under specific pathogen-free conditions, and all animal experiments were conducted in accordance of governmental and institutional guidelines for animal welfare and approved by the IACUC at the Tsinghua University.

Antibodies, reagents, and cell isolation

Anti-IL-2, anti-CD4, anti-CD11c, and anti-CD25 were purchased from eBioscience; and anti-I-A/I-E and anti-FOXP3 were purchased from BD. CFSE, CMF₂HC, TAMRA, CellTrace Violet, and PMA (phorbol 12-myristate 13-acetate) were purchased from Invitrogen. The OVA_{323–339} peptide and ‘T4’ OT-I OVA_{257–264} (SIITFEKL) were synthesized by ChinaPeptides. Murine CD4⁺CD25⁺T reg cell and CD4⁺CD25[–]T conv cells were isolated from spleens using mouse regulatory T cell isolation kit (STEMCELL Technology). Murine DCs were isolated with CD11c Microbeads (Miltenyi Biotec) from the spleen after digestion with 400 µg/ml liberase CI and 20 µg/ml DNase I for 30 min (Roche). OT-II/OT-I T cells were isolated using the CD4/CD8 T cell isolation kits (Miltenyi Biotec).

Flow cytometry

Cells were stained with appropriate primary and secondary antibodies for 30 min on ice with PBS washes in between. Stained cells were analyzed directly or after being fixed with 1% PFA using an LSR II flow cytometer. Data were analyzed using FlowJo. To detect IL-2 production by CFSE-labeled OT-II T cells, single-cell suspension of draining LN cells was stimulated with 50 ng/ml PMA and 500 ng/ml ionomycin for 4 h before being subjected to the standard intracellular cytokine staining procedure.

Assays of polyclonal T reg cell-mediated suppression

For in vitro suppression, 2×10^4 OT-II T cells or OT-I cells labeled with 5 µM CellTrace Violet and 10^4 splenic DCs pulsed with 0.5 µM OT-II OVA_{323–339} peptide or 0.5 nM T4 OT-I OVA_{257–264} (SIITFEKL) were co-cultured together with or without 2×10^4 T reg or T conv cells in a U-bottom

96-well plate, as previously described (Pace et al., 2012). The division profile of responder OT-II T cells was analyzed after 60 h; OT-I was measured after 96 h.

Intravital imaging of T cell–DC interactions

To visualize endogenous T reg cell–DC interactions, each Foxp3–GFP mouse was s.c. injected with 3×10^6 nonfluorescent DCs that were pulsed with 1 μ M OVA_{323–339} peptide in the presence of 0.2 μ g/ml LPS for 2 h at 37°C. Some recipients were also i.v. transferred with 4×10^6 OT-II cells. After ~24 h, each mouse was s.c. injected with 3×10^6 DsRed-transgenic splenic DCs pulsed with 1 μ M OVA_{323–339} peptide in the presence of 0.2 μ g/ml LPS. Intravital imaging of the draining LN was performed 14–20 h after the DC injection, as previously described (Xu et al., 2013). To visualize interactions of T reg cells, OT-II T cells, and OVA-presenting DCs, each mouse was s.c. injected with 3×10^6 BMDCs that were pulsed with 1 μ M OVA_{323–339} peptide in the presence of 0.2 μ g/ml LPS for 2 h at 37°C, and then labeled with 100 μ M CMF₂HC. Each recipient was also i.v. transferred with 4×10^6 OT-II cells (labeled with 10 μ M TAMRA) together with 4×10^6 CD25⁺ T reg or CD25[−] T conv cells (labeled with 5 μ M CFSE) that had been treated or not with 200 U/ml IL-2 overnight. CD25⁺ T conv cells were generated by stimulation of plate-bound anti-CD3 and anti-CD28 (both at 8 μ g/ml) for >20 h in the presence of at least 40 U/ml IL-2. Intravital imaging of the draining LN was performed 14–20 h after the T cell transfer. In brief, the microscopic system was composed of a MaiTaiDeepSee laser (Spectra-Physics) and an Olympus FV1000 upright microscope equipped with the XLPlan 25 \times water immersion lens (NA 1.05; Olympus). A typical XYZ voxel size of $\sim 0.5 \times 0.5 \times 3 \mu$ m, a time resolution of 30 s per frame, and a typical tissue volume of $512 \times 512 \times 63 \mu$ m³ were used. After acquisition, 4-D datasets were analyzed using the Imaris software (Bitplane), and duration of contact was scored manually in a blinded manner. For unbiased analyses of the contact-dependent T reg cell suppression on OT-II/OT-I–DC interactions, our quantitation requires that DCs were continuously trackable in the imaging field for at least an hour and that the beginning and the end of an OT-II/OT-I–DC contact were visually identifiable in the same imaging session. Adobe Photoshop and Adobe AfterEffect were used to prepare time-lapse videos.

Statistical analyses

Student's *t* tests were used to compare endpoint means, and data were always presented as mean \pm SEM, unless indicated otherwise. Calculation and graphing were done with Prism (GraphPad).

Online supplemental material

Fig. S1 show brief contacts between CD25⁺ T conv cells and DCs in vivo. Fig. S2 shows suppression of OT-II T cells division by IL-2–conditioned polyclonal T reg cells in vitro. Fig. S3 shows polyclonal T reg cells suppress OT-I T cells division

triggered by MHCII-deficient DC in vitro. Video 1 shows interactions between endogenous Foxp3–GFP⁺ T reg cells and DCs in LNs without overt T cell activation. Video 2 shows interactions between endogenous Foxp3–GFP⁺ T reg cells and DCs in LNs in which OT-II T cells are activated. Video 3 shows transient interactions between IL-2–treated polyclonal CD4⁺CD25[−] T conv cells and transferred DCs in the LN. Video 4 shows prolonged interactions between IL-2–treated polyclonal CD4⁺CD25⁺ T reg cells and transferred DCs in the LN. Video 5 shows comparable interaction time with DCs by CD4⁺CD25⁺ (activated) and CD4⁺CD25[−] (resting) T conv cells in the LN. Video 6 shows IL-2–treated polyclonal T reg cells suppress antigen-specific T–DC interactions. Video 7 shows prolonged antigen-specific OT-II cell interactions with OVA-pulsed DCs that are not contacted by IL-2–treated T reg cells. Video 8 shows another example of T reg cell contact-dependent suppression of OVA-pulsed DC interactions with OT-II T cells. Video 9 shows IL-2–treated CD4⁺CD25[−] T cells do not affect the duration of antigen-specific T–DC interactions. Video 10 shows Comparable polyclonal T reg cell interaction time with WT or class II MHC-deficient DCs in the LN. Video 11 shows Endogenous T reg cells suppress OT-I interactions with OVA-presenting MHC class II^{−/−} DCs.

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