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Cutting Edge: Cognate CD4 Help Promotes Recruitment of Antigen-Specific CD8 T Cells around Dendritic Cells

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The cellular orchestration underlying help provided by CD4 T lymphocytes to CD8 T cell responses is not fully understood. We documented that the formation of three-cell clusters occurred as soon as day 1 and relied on long-lasting CD4 and CD8 T cell interactions with dendritic cells (DCs). The influence of CD4 help on CD8 T cell differentiation could be observed as early as the second round of cell division. Importantly, our results identify a new facet to the phenomenon of CD4 help in which DCs, upon cognate interactions with CD4 T cells, increase their ability to attract and retain Ag-specific CD8 T cells. Our results support a model in which CD4 help operates rapidly, in part by favoring CD8 T cells recruitment around those DCs that are the most competent for priming. The Journal of Immunology, 2006, 177: 1406–1410.

In many instances, CD4 helper T cells promote the quality of CD8 T cell responses (reviewed in Ref. 1, 2). How CD4 T lymphocytes communicate with other cells during this process continues to be the subject of intense investigation. The initial model postulated that, by bringing together Ag-specific DCs and CD8 T cells, APCs served as a platform for differentiating CD4 T cells, increase their ability to attract and retain Ag-specific CD8 T cells. The Jour-

nal of Immunology, 2006, 177: 1406–1410.

3 Abbreviations used in this paper: DC, dendritic cell; LN, lymph node; WT, wild type; MFI, mean fluorescence intensity.

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Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from Charles River Laboratories. Female Marylin TCR transgenic RAG-2−/− mice and CD40-deficient mice were obtained from the Centre de Distribution Typage et Archivage animal. OT-I TCR transgenic mice and mice expressing the GFP under the chicken β-actin promoter were bred in our animal facility. All animal experiments were performed in accordance with the institutional guidelines for animal care.

Cell preparation and transfer

Splenec DCs were purified as described (17) and pulsed with the indicated concentration of S8L (SIINFEKL) and/or with 1 μM N155 (NAGENSRRANS SRSS) peptide (NeoMPS) for 1 h at RT. CD4 T cells were collected from LNs of Marylin TCR RAG-2−/− mice. OT-I CD8 T cells were purified using the CD8 T cell isolation kit (Miltenyi Biotec). Cells were labeled with the indicated concentration of SNARF and/or CFSE dyes (Invitrogen Life Technologies).

FACS analysis

Lymph nodes (LN) were harvested and incubated at 37°C for 15 min in RPMI 1640 containing 1 mg/ml collagenase. Cells were stained with a combination of the following Abs: allophycocyanin-labeled anti-CD8, PE-labeled anti-CD25, anti-IFN-γ (BD Biosciences), allophycocyanin-labeled anti-CD11c, PE-labeled anti-I-A, PE-labeled anti-K, PE-labeled anti-CD80, and PE-labeled anti-CD86 (BioLegend) mAbs. Intracellular IFN-γ staining of LN cells was performed after a 4-h restimulation in the presence of 1 μM S8L peptide and 1 μg/ml brefeldin A.

Confocal and two-photon microscopy

LNs sections were analyzed by confocal microscopy as described (18). Two-photon imaging was performed using an upright Axioscope 2 FS microscope (Zeiss) and a Ti:sapphire laser (Coherent) tuned at 780 nm as described (18). POP swell LNs were maintained at 37°C and superfused with medium bubbled with 95% O2 and 5% CO2. Images were acquired at >100 μm below the LN surface every 15 s.

Results and Discussion

Visualizing DCs interacting with Ag-specific CD4 and CD8 T cells in intact LNs

To gain some insight into the cellular orchestration underlying CD4 T cell help, we visualized interactions between Ag-bearing DCs and CD4 and CD8 T lymphocytes. DCs pulsed with the S8L peptide (recognized by the OT-I TCR) and the N155 peptide (recognized by the Marylin TCR) were labeled with vital
FIGURE 1. Three-cell clusters occur as stable CD4 and CD8 T cell interactions with DCs. DCs (3 × 10⁶) were pulsed with S8L (0.1 μM) and N15S (1 μM) peptides, labeled with a mixture of SNARF (5 μM) and CFSE (2.5 μM), and injected in the footpad. Marilyn CD4 T cells (10 × 10⁶) labeled with SNARF (10 μM) and OT-I CD8 T cells (20 × 10⁶) labeled with CFSE (10 μM) were injected i.v. Popliteal LNs were subjected to two-photon imaging at 20 h. a, Most (17 of 24) clusters around DCs (yellow) contained both Ag-specific CD8 T cells (green) and CD4 T cells (red). b, Time-frame images showing that CD4 and CD8 T cells established long-lasting interactions with DCs. Time is indicated as minutes/seconds. Right panels depict trajectories of CD8 T cells (green), Ag-bearing DCs (yellow), and CD4 T cells (red). Two examples of clusters containing DCs and CD4 and CD8 T cells are shown together with one example of cluster containing DCs and CD8 T cells only. Stable interactions were observed whether or not CD4 T cells were present in the cluster. Scale bar, 10 μm. c, Graph shows the percentage of CD4 T cell–DC interactions (n = 15) and CD8 T–DC interactions (n = 47) maintained at the indicated time points.

FIGURE 2. Cognate CD4 help impacts on the early stages of CD8 T cell activation. a, DCs (1.5 × 10⁶) were pulsed with 0.1 nM S8L peptide alone (DC⁵⁷⁶⁵) or in conjunction with 1 μM N15S peptide (DC⁺S⁵⁷⁺N), and were injected in the footpad. Recipients were adoptively transferred with Marilyn CD4 T cells (2 × 10⁶), and 16 h later, with CFSE-labeled OT-I CD8 T cells (3 × 10⁶). b, DC⁺S⁵⁷⁺N, but not DC⁺S, promote CD25 expression on OT-I CD8 T cells. Numbers indicate the percentage of divided cells falling into the indicated region. c, The percentage of CD25⁺ OT-I CD8 T cells is graphed as a function of the number of cell division. d and e, OT-I CD8 T cells were assayed for IFN-γ production by intracellular staining. f, MFI of IFN-γ–producing OT-I CD8 T cells in the presence or absence of cognate CD4 help. Results are representative of four independent experiments.

to real-time two-photon imaging. Both Marylin CD4 T cells and OT-I CD8 T cells were found to establish interactions with DCs, and the majority of clusters (71%) contained the three cell types (DCs and CD4 and CD8 T cells). Most CD4 T cell–DC contacts (80%) as well as CD8 T cell–DC contacts (87%) were maintained during imaging periods of 20 min (Fig. 1, b and c; and Movies 1 and 2), indicating that these interactions were in the range of hours. Time-lapse movies suggested that CD8 T cells were generally retained within clusters through contact with DCs rather than through interactions with CD4 T cells (Fig. 1, b and c; and Movies 1 and 2). Our results suggest that information exchange between DCs and CD4 and CD8 T cells can occur in the early phases (<1 day) of the immune response prompting us to evaluate the role of CD4 T cell help on the initial stages of CD8 T cell activation.

Early imprinting of CD4 T cell help on CD8 T cell differentiation

We established an in vivo model to study the impact of CD4 help on CD8 T cell responses. Mice were transferred with Marylin CD4 T cells and immunized with DCs pulsed with S8L peptide (referred to as DC⁵⁷⁶⁵) or with DCs pulsed with both S8L and N15S peptides (DC⁺S⁵⁷⁺N). After 16 h, CFSE-labeled OT-I T cells were transferred in recipients (Fig. 2a). In this system, DC⁺S⁺N can stimulate OT-I CD8 T cells, but cognate CD4 help could only be delivered during immunization with DC⁺S⁺N⁺N. Proliferation and expression of CD25 were assessed as several studies have linked sustained CD25 expression with efficient T cell activation (19–21). Although immunization with DC⁺S⁺N and DC⁺S⁺N⁺N induces a similar level of OT-I T cell proliferation, DC⁺S⁺N⁺N but not DC⁺S⁺N⁺N⁺N promoted expression of the CD25 molecule (Fig. 2, b and c). In addition, OT-I T cells stimulated by DC⁺S⁺N⁺N were approximately two times more likely to produce IFN-γ at 48 h than OT-I T cells stimulated by DC⁺S⁺N (Fig. 2, d and e) and produced more of this cytokine on a per-cell basis (mean fluorescence intensity (MFI) 2153 ± 81 vs 817 ± 34, respectively). These results support the idea that CD4 T cells rapidly instruct the differentiation of naive CD8 T cells.

* The online version of this article contains supplemental material.
Role of DC maturation during CD4 T cell help

Next, we assessed whether helper T cells were enhancing the quality of the CD8 T cell response by increasing expression of CD80/CD86 costimulation molecules on DCs. We examined the phenotype of GFP-expressing DCs that migrated to the LN following footpad injection, in the presence or absence of cognate interactions with CD4 T cells. As previously reported (17), DCs that had migrated to the draining LN displayed a mature phenotype with high level of MHC class II and CD80 and CD86 molecules (Fig. 3). Interestingly, cognate interactions with CD4 T cells did not result in additional changes in the level of expression of these markers (Fig. 3). Thus, in our system, CD4 help did not translate into increased CD80/CD86 costimulation molecules on DCs. Based on our results and those from other studies (22, 23), we conclude that, in some experimental systems, signals provided by CD4 help and those responsible for DC maturation are at least partly distinct.

Preferential recruitment of CD8 T cells by DCs that engage in cognate interactions with Ag-specific CD4 T cells

Although previous studies have indicated that cognate helper T cells are not required for the formation of stable CD8 T cell–DC contacts (17, 24, 25), it was still possible that CD8 T cell–DC interactions were influenced by the presence of CD4 help. To test this possibility, we compared the clustering of CD8 T cells around DCs that were capable or incapable of establishing interactions with cognate CD4 T cells. Dye-labeled DCs and CD80 and CD86 molecules were analyzed by flow cytometry, as were DCs before injection. Expression of MHC class II (I-Ab), MHC class I (Kb), CD80 and CD86 molecules at the surface of DCs was graphed for DCS8L and DCS8L *N155* (Fig. 3). Interestingly, cognate interactions with CD4 T cells did not result in additional changes in the level of expression of these markers (Fig. 3). Thus, in our system, CD4 help did not translate into increased CD80/CD86 costimulation molecules on DCs. Based on our results and those from other studies (22, 23), we conclude that, in some experimental systems, signals provided by CD4 help and those responsible for DC maturation are at least partly distinct.

Cognate CD4 help promotes recruitment of Ag-specific CD8 T cells

To establish interactions with cognate CD4 T cells, DCS8L or DCS8L *N155* were mixed and injected in female B6 mice. Recipients were then adoptively transferred with unlabeled Marylin CD4 T cells and after 16 h, with dye-labeled OT-I CD8 T cells. After 3h, LNs were sectioned and analyzed by confocal microscopy (Fig. 4a). Clustering of CD8 T cells around DCs was quantified using two different methods (Fig. 4). First, we counted for each DC the number of OT-I T cells for which a zone of interaction with the DC was detected (contact method). We also quantified cell clustering by counting the number of OT-I T cells included within a 30-μm diameter circle centered around each DC (circle method), a more sensitive
method that can however occasionally include OT-I T cells that were not truly in contact with the DC. For each image, we recorded and plotted the mean number of OT-I T cells clustered around each DC8L against the number of OT-I T cells clustered around each DC8L+N15S. We repeatedly found that DC8L+N15S recruited significantly more OT-I T cells than DC8L in a 3-hour period (p < 0.01, Wilcoxon paired test). In the same image, there were on average 1.4–2.2 (depending on the quantitation method and the experiment) more CD8 T cells around DC8L+N15S than around DC8L (Fig. 4d, left and center panels, and Table I). As a control, CD8 T-cell recruitment was virtually equivalent between two populations of dye-labeled DC8L (Table I). The difference in recruitment was also observed when we averaged clustering values for each individual DCs (Fig. 4d, right panel) compiled from the different images of the same lymph node. In addition, DC8L+N15S were again more competent than DC8L to recruit CD8 T cells when clusters were analyzed at 24 h after CD8 T-cell transfer or when we used a lower concentration of S8L peptide (0.1 nM instead of 10 nM) to pulse DCs (data not shown). Altogether, these results suggest that following interactions with CD4 T cells, DCs increase their ability to recruit CD8 T cells. The difference in clustering around DC8L+N15S and DC8L illustrates the importance for CD4 and CD8 T cells to recognize Ag on the same APCs.

To assess whether CD40 expression on DCs was implicated in the observed regulation of CD8 T-cell recruitment, we compared the clustering of OT-I T cells around DC8L+N15S prepared from wild-type (WT) and CD40−/− mice. As shown in Fig. 4e, WT DC8L+N15S recruited significantly more OT-I T cells than CD40−/− DC8L+N15S in a 3-hour period (p < 0.01, Wilcoxon paired test). In the same image, there were on average 1.5 (respectively 1.8) more CD8 T cells around WT DCs than around CD40−/− DCs using the contact method (respectively, circle method) of cluster quantification. This result strongly suggests that the DCs ability to recruit CD8 T cells is regulated by engagement of CD40 molecule expressed by DCs.

Conclusion

Three important conclusions can be drawn from the present study. First, CD4 help can impact on CD8 T-cell differentiation very rapidly resulting in sustained CD25 expression and improved ability to produce IFN-γ. Second, three-cell clusters form as early as day 1 and rely on long-lasting CD4 and CD8 T-cell contacts with DCs. Third, CD4 help increases the ability of DCs to recruit CD8 T cells, likely reflecting an enhanced ability of DCs engaging helper CD4 T cells to attract and/or retain CD8 T cells. In this respect, it will be interesting to investigate the role of chemokines and/or adhesion molecules. This phenomenon likely reflects that CD4 help promotes a net gain of stimulation for CD8 T cells by favoring T-cell arrest on DC (25), and/or by increasing the duration of CD8 T cell–DC contacts. Interestingly, the preferential association between CD8 T cells and DCs establishing cognate interaction with helper T cells also increases the opportunity for CD8 T cells to benefit from IL-12 produced by licensed DCs (13, 14, 26), or from IL-2 produced by neighboring CD4 T lymphocytes. We propose that, by controlling the duration and the location of CD8 T-cell engagement on DCs, helper T cells optimize the set of signals received by CD8 T lymphocytes.

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Disclosures

The authors have no financial conflict of interest.

References


Table I. Cognate CD4 help promotes CD8 T-cell–DC interactions

<table>
<thead>
<tr>
<th>CFSE-labeled DCs</th>
<th>DCN15S</th>
<th>DC8L</th>
<th>DC8L+N15S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clustering Ratio (CFSE/SNARF)</td>
<td>1.24 ± 0.37</td>
<td>1.05 ± 0.16</td>
<td>2.27 ± 0.60</td>
</tr>
<tr>
<td>No. of DCs analyzed</td>
<td>n = 285 CFSE</td>
<td>n = 399 CFSE</td>
<td>n = 299 CFSE</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>n = 295 SNARF</td>
<td>n = 339 SNARF</td>
<td>n = 422 SNARF</td>
</tr>
</tbody>
</table>

* The indicated DC populations were labeled with either SNARF or CFSE, mixed, and injected in the footpad. Unlabeled Marilyn CD4 T cells were injected i.v., and 16 h later, OT-I CD8 T cells labeled with a mixture of SNARF and CFSE, were injected i.v. Popliteal LNs were collected after 3 h and processed for confocal microscopy.

* Overall clustering of CD8 T cells around DCs was reduced = 3-fold in the absence of S8L peptide.

* The clustering ratio is calculated for each individual image as the mean number of CD8 T cells clustered around CFSE-labeled DCs divided by the same value for SNARF-labeled DCs using the contact method. Values are mean ± SEM for all analyzed images.


