Dendritic Cells Matured by Inflammation Induce CD86-Dependent Priming of Naive CD8+ T Cells in the Absence of Their Cognate Peptide Antigen

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Dendritic Cells Matured by Inflammation Induce CD86-Dependent Priming of Naive CD8+ T Cells in the Absence of Their Cognate Peptide Antigen1

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Dendritic cells (DC)1 are highly potent APCs crucial for the innate and adaptive immune response and for maintaining immune tolerance toward self-Ags (1, 2). To maximize the efficiency of naive T cell activation, Ag-bearing DC undergo a complex process of maturation, guided by signals transmitted through a broad range of pattern recognition receptors (PRR), including Toll-like receptors (3, 4), C-type lectin receptors (5), and NOD-like receptors (6) that interact with cognate ligands derived from pathogens. Maturation mediated through PRRs has also been shown, at least for CD4+ T cells, to assist in the process of selecting peptides for MHC class II-dependent Ag presentation (7). However, an increasing body of literature suggests that DC can also be indirectly stimulated to mature by endogenous danger signals such as uric acid (8), kinins (9, 10), and proinflammatory cytokines and chemokines (11–14). Although these signals may induce levels of DC maturation sufficient to induce Ag-dependent CD4+ T cell proliferation, it has been argued that in the absence of “licensing” by cognate engagement of pattern recognition receptors, mature DC are unable to induce full CD4+ effector T cell differentiation (12). Recent evidence suggests that extension of the half life of MHC-peptide complexes, allowing increased serial triggering of TCRs, may provide a molecular basis for licensing by PRRs (15).

Although DC maturation and the multiplicity of signals required for optimal naive T cell activation combine to promote specificity, a large body of evidence indicates that, under certain conditions, Ag-independent activation of T lymphocytes can occur after infection. For example, early studies by Ehle and colleagues (16) demonstrated that a low frequency of LCMV-specific TCR transgenic CD8+ T cells became activated after vaccinia virus infection, and a number of subsequent reports have shown proliferation and cytokine production by “bystander” CD4+ and CD8+ T cells in mice with ongoing infection, for example with Mycobacterium avium (17), Burkholderia pseudomallei (18), and Leishmania donovani (19). In most of these systems, bystander activation for cytokine production, as opposed to bystander proliferation, has been attributed to high levels of infection-associated proinflammatory cytokine production by mononuclear phagocytes, notably of IL-12 and IL-18 in the case of IFN-γ production by CD8+ T cells (18). In contrast, the contribution of DC maturation to the induction of bystander proliferation has not been examined in detail.

In this study, we have re-examined the issue of bystander CD8+ T cell activation, focusing on whether DC maturation independent of pathogen uptake and largely resulting from inflammation is sufficient to drive this process. Using Leishmania donovani infection as a model to induce systemic inflammation, we have shown that inflammation-induced maturation of DC is sufficient to confer on DC the capacity to induce proliferation of OT-I cells in the absence of their cognate Ag. Both in vitro and in vivo, this process was largely attributable to enhanced CD86-dependent costimulation.
Although OT-I cells proliferating in this way did not produce IFN-γ, they were nevertheless primed to do so upon exposure to otherwise ineffective weak TCR agonist peptides. Using *Streptococcus pneumoniae* as a model infection where DC maturation can be restricted to distinct lymphoid organs, we also showed that OT-I cells primed in the absence of their cognate Ag-seeded sites distal from that of their initial activation. Collectively, our data suggest that CD86-dependent but cognate peptide-independent priming of CD8⁺ T cells induced by mature DC may be a common mechanism to increase the efficiency of immune surveillance against systemic pathogen spread.

### Materials and Methods

#### Mice and infection

C57BL6 (Charles River Laboratories) and OT-I RAG1⁻/⁻ and F5 RAG1⁻/⁻ (a gift from Drs. B. Seddon and D. Kiossis, National Institute for Medical Research, London, U.K.) mice were used. All mice were housed under specific pathogen free conditions and used at 6 to 8 wk of age. Amastigotes of *Leishmania donovani* (LV9) were isolated (20), labeled (5 μM CFSE, 37°C for 10 min), and injected (5 × 10⁴ i.v.) into mice. In some experiments LPS was adsorbed onto fluorescent microspheres (2 μm) for 24 h (100 μg/ml/10⁶ microspheres) and then washed extensively in PBS before injection (5 × 10⁴ i.v.). All animal care and procedures were in accord with U.K. Home Office requirements and performed with local ethical approval.

#### DC isolation and enrichment

Conventional DC (CD11chigh MHCIlo) from naive and infected mice were enriched by digesting the spleens or lymph nodes in RPMI 1640 supplemented with 0.25 mg/ml collagenase (5 ml per spleen) for 25 min at room temperature. All subsequent steps were done between 0 and 4°C. Following collagenase digestion, 5 ml of 50 mM EDTA/PBS solution was added and the digest was passed through a 100 μm strainer to make a single cell suspension. To enrich DC, dead cells and highly phagocytic cells were first depleted by magnetic cell sorting using basic microbeads (Miltenyi Biotec) following the manufacturer’s protocol. In brief, digested splenocytes were incubated with basic magnetic microbeads for 10 min on ice. After washing, cells that had nonspecifically bound or phagocytosed microbeads were trapped on a separation column. CD11c⁺ cells were then enriched by incubating with anti-CD11c microbeads for 30 min on ice and then passed twice over a separation column. CD11c⁺ cells (85–98% pure) were stained with CD11c-PE, MHCI allophycocyanin and isotopically (99% purity) using a MoFlo (Beckman Coulter). Cytospots were Giemsa-stained and visualized by light microscopy. For immunofluorescence, cytospots were fixed with 1% paraformaldehyde for 15 min. Fixed DC were blocked with 1% BSA and 0.5% saponin (saponin buffer). Cells were then stained with Pacific blue-conjugated anti-IFN-γ (XMG 1.2) or allophycocyanin-conjugated anti-IL-12 (IFN-γ) and then washed in ice-cold PBS before fixation (5 × 10⁴ i.v.). All animal care and procedures were in accord with U.K. Home Office requirements and performed with local ethical approval.

#### Flow cytometry

Cells were incubated with 10 μg/ml 2.4G2 anti-Fc receptor mAb (American Type Culture Collection) followed by staining with directly conjugated mAbs, including: FITC-conjugated anti-CD8α (53-6.7), anti-CD8δ (GL1); PE-conjugated anti-MHC class II (M5/114.15.2), anti-CD11c (HL3), anti-CD62L (MEL-14), PE-Cy7-conjugated, CD11c (N418); allophycocyanin-conjugated anti-CD4 (RM4-5), anti-CD44 (IM7), anti-CD86 (GL1); allophycocyanin-Cy7-conjugated, anti-CD25 (PC61.5), anti-CD8α (53-6.7) (all from eBioscience). Minimal background staining was observed using appropriate fluorochrome-labeled isotype controls. Flow cytometric analysis was performed with a Cyan ADP (Beckman Coulter) and analyzed using Summit software (Beckman Coulter). Absolute cell numbers were quantitated by spiking samples with beads of known amount and gating on cells of interest.

#### Adoptive transfer

Naive CFSE-labeled CD45.2 WT polyclonal or transgenic monoclonal OT-1 cells were injected (2 × 10⁶ i.v.) into CD45.1 mice 1 day before infection with *L. donovani* i.v. or *S. pneumoniae* i.v or i.n. –1 × 10⁷ CFU. After 5 days, proliferation of OT-1 cells in spleens and mediastinal LNs was assessed. Blocking mAbs against CD80 (16-10A1) and CD86 (GL1) or control mAbs were administered (200 μg) 12 h before infection as required.

#### Statistics

Statistical analysis was performed using a paired Student t test. *p* < 0.05 was considered significant. All experiments were performed independently at least twice and with three technical replicates performed for each sample.

#### Results

**Bystander maturation of DC**

To obtain DC that had been activated by inflammation, we injected mice with CFSE-labeled *L. donovani* and sorted splenic DC into parasitized or nonparasitized populations (Fig. 1). As anticipated from other studies (22), only a minor population of DC (here referred to as DCint) contained parasites (3 ± 1.41%, following injection of 5 × 10⁷ parasites; Fig. 1A). By light and confocal microscopy, sorted CFSEhigh DCint contained *Leishmania* whereas neither intact parasites nor CFSE-labeled cellular debris was associated with CFSE⁻/⁻ DC (Fig. 1A). Within the CFSEhigh and CFSE⁻/⁻ DC, the major subsets of splenic DC (CD4⁺/CD45⁺) were equally represented (data not shown), indicating that each subpopulation of splenic DC was equally susceptible to infection with *L. donovani* in vivo.
We then compared CFSE<sup>−</sup> and CFSE<sup>high</sup> DC for their expression of costimulatory molecules associated with DC maturation. As anticipated, compared with DC in naive mice (referred to as DC<sub>naive</sub>), DC<sub>inf</sub> expressed high levels of CD86 (increased ~20-fold and ~4-fold for CD8<sup>+</sup> and CD8<sup>+</sup> DC, respectively, compared with naive mice). Nevertheless, compared with DC<sub>naive</sub>, CFSE<sup>−</sup> DC from infected mice also had significantly elevated levels of expression of CD86. On CD8<sup>−</sup> DC, CD86 increased ~18-fold and expression of CD86 on CD8<sup>+</sup> DC increased by 4-fold (Fig. 1B). Herein, we refer to these matured but uninfected DC that we obtain from infected mice as “DC<sub>mat</sub>.” Although we cannot rule out the possibility that DC<sub>mat</sub> may have encountered trace amounts of soluble leishmanial Ag, only DC<sub>inf</sub> contained demonstrable mRNA for IL-12p40 (Fig. 1C) and indicator of maturation mediated by engagement of PRRs (12). Thus, DC<sub>mat</sub> represent a population of DC that have been partially matured in trans by “inflammatory” signals.

**DC matured by inflammation stimulate naive OT-I T cell proliferation**

To assess the capacity of DC<sub>mat</sub> to stimulate sorted naive CD8<sup>+</sup> T cells (supplemental Fig. S1),<sup>4</sup> we used OVA-specific CD8<sup>+</sup> OT-I cells. As expected, DC<sub>naive</sub> and DC<sub>mat</sub> induced a robust OT-I response in the presence of SIINFEKL, as measured by CFSE dilution analysis of viable (DAPI<sup>−</sup>) cells (Fig. 2A) and by quantifying the total viable T cell recovery after 5 days (Fig. 2B). DC<sub>mat</sub> (and to the same extent, DC<sub>inf</sub>) also stimulated OT-I proliferation in the absence of SIINFEKL, though the total recoverable cell yield was reduced by ~10-fold compared with that seen in the presence of this peptide. Examination of the CFSE content of DAPI<sup>−</sup> cells within these cultures (Fig. 2A) indicated that, similar to OT-I cells cultured in the absence of DC, most dead cells in the cultures stimulated with DC<sub>mat</sub> had undiluted CSFE content. This observation suggests that death by neglect rather than reduced survival after proliferation was responsible for the low cell recovery (Fig. 2B) compared with that seen in the presence of Ag. Collectively, therefore, these data indicate that only limited numbers of OT-I cells are activated by DC<sub>mat</sub> in the absence of their cognate peptide (31 ± 4% dividing at least once; supplemental Fig. S2). We next tested

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<sup>4</sup>The online version of this article contains supplemental material.
whether polyclonal naive CD8+ T cells from WT mice exhibited a similar response. Compared with DCnaive, DCmat caused significant proliferation of naive polyclonal CD8+ T cells (Fig. 3A), with ~10-fold greater cell recovery (Fig. 3B). Each of the three major DCmat subsets had similar potential to induce proliferation of polyclonal CD8+ T cells (Fig. 3C). In addition, the activity of DC was augmented in the presence of LPS, even when DCnaive were used as stimulators (Fig. 3D), suggesting that inflammation-induced activation and TLR-induced activation may have additive effects. Finally, to replicate inflammation-induced DC maturation in vivo but in the absence of an antigenic stimulus, we injected mice with LPS-coated fluorescent microspheres and purified DCmat that did not contain beads. DCmat in this system were again more efficient at inducing proliferation of naive polyclonal CD8+ T cells than naive DC (Fig. 3E).

Proliferation of OT-I cells is CD86-dependent

To dissect the mechanism by which DCmat are able to activate OT-I, we first tested the stimulatory capacity of fixed DCmat. SIINFEKL-independent proliferation of OT-I cells induced by DCmat was unaffected by fixation (Fig. 4A). Conversely, the ability of DCnaive to induce SIINFEKL-independent proliferation of OT-I was not markedly improved by addition of supernatant collected from DCmat. Not surprisingly, this supernatant also did not enhance the ability of fixed DCmat to induce OT-I proliferation (Fig. 4B). As CD86 expression was significantly increased on DCmat (Fig. 1) and this costimulatory pathway has been shown to be fixation-resistant (23), we blocked CD86-CD28 signaling. As shown in Fig. 4C, CD86 mAb significantly reduced OT-I proliferation induced by DCmat. The frequency of recoverable OT-I cells was also reduced by 58 ± 1% (p = 0.002; Fig. 4D).

To determine whether similar CD86-dependent proliferation also occurred in vivo, we transferred CFSE-labeled naive OT-I cells into mice and subsequently infected these mice with L. donovani. OT-I cells recovered from the spleen 5 days after infection had significantly diluted CFSE, indicating proliferation in vivo, and this response was significantly blocked by prior administration of CD86 mAb (Fig. 4, E and F). Similar results were noted 5 days post transfer of polyclonal CD8+ T cells into L. donovani infected mice (supplementary Fig. S3, A and B). In contrast, blockade of CD80, which we have previously shown is not up-regulated on DC from infected mice (20), had no effect on OTI-proliferation (Fig. 4, E and F). Furthermore, in vitro proliferation of OT-I cells by DCmat in the absence of SIINFEKL was blocked by addition of cyclosporine A (Fig. 4G) suggesting signals mediated via the TCR were also required.

IL-7 and IL-15 are both known to play a role in T cell proliferation and homeostasis and at least for IL-15, a membrane bound form has been observed on a variety of APC (24). To determine whether these cytokines played a role in the capacity of DCmat to induce proliferation of OT-I cells, we first measured mRNA accumulation for IL-7 and IL-15 in DCnaive and DCmat. Our data indicated an increase (~8-fold) in IL-15 mRNA accumulation in DCmat whereas IL-7 mRNA accumulation was unchanged (supplementary Fig. 4A). However, using soluble IL-15R, we found no evidence to support a role of IL-15 in the proliferation of OT-I cells in response to DCmat (supplementary Fig. 4B). We also did not find evidence of IL-2 production by DCmat during coculture with OT-I T cells (supplementary Fig. 4C).

Several reports have demonstrated that costimulation lowers the threshold of T cell activation (25, 26) and may allow recognition of weak TCR agonist peptides (27, 28). We therefore compared the capacity of DCnaive and DCmat to present variant OVA peptides to OT-I cells. As measured by OT-I recovery at 72 h, SIINFEKL induced a maximal response in OT-I cells at all concentrations tested (from 1 to 1000 pg/ml), irrespective of whether DCnaive or DCmat were used as APC (Fig. 5A). G4 was at least three orders of magnitude less effective than SIINFEKL at stimulating OT-I when presented by DCnaive. In contrast, stimulation by G4 was significantly enhanced by presentation on DCmat, with similar numbers of OT-I cells recovered as seen with SIINFEKL presented by DCnaive (Fig. 5B). E1 (27) was poorly presented by DCnaive, but near maximal numbers of OT-I cells were recovered when this peptide was presented by DCmat (Fig. 5C). Collectively, our data indicate that DCmat have a heightened capacity to induce proliferation of OT-I cells through enhanced CD86-dependent recognition of weak peptide agonists.
Characterization of proliferating CD8⁺ T cells

Next, we characterized OT-I cells that had been stimulated by DCmat. Compared with naive cells, OT-I cells activated by DCmat up-regulated CD44, and down-regulated CD62L and CCR7 (supplemental Fig. 5). Autocrine signals through the heterotrimeric IL-2R are known to promote naive T cell proliferation and survival. Therefore, we examined the requirements for IL-2R mediated signals to promote the proliferation of naive CD8⁺ T cells by DCmat. In the absence of Ag, OT-I cells stimulated by either DCmat or DCnaive produced little detectable IL-2, compared with cells stimulated for 24 h by these DC populations in the presence of SIINFEKL (Fig. 6A). Whereas addition of exogenous rIL-2 to OT-I cells expanded by DCmat in the presence of SIINFEKL enhanced OT-I proliferation, this response was more muted in OT-I cells expanded using DCmat in the absence of Ag (Fig. 6B). This reduced response correlated with low expression of CD25 on OT-I cells stimulated by DCmat in the absence compared with in the presence of SIINFEKL (MFI for CD25 expression of 15.5 ± 0.2 vs 455 ± 13, respectively, p = 0.005; Fig. 6C). To assess whether this relative lack of IL-2 production and CD25 expression affected the functional differentiation of OT-I cells, we cultured naive OT-I cells with DCmat for 3 days, followed by a further 2 days in the presence or absence of exogenous rIL-2. At day 5, surviving OT-I cells were restimulated with SIINFEKL and assessed for IFN-γ production. As shown in Fig. 6D, OT-I cells activated to proliferate in the absence of Ag required additional exogenous rIL-2 to develop into IFN-γ-secreting effector cells.

To determine whether proliferation induced by DCmat impacted on subsequent functional competence, we generated a population of OT-I cells that had been primed for 3 days with DCmat and then compared their response with that of naive OT-I cells to restimulation with SIINFEKL, G4 and E1. As shown in Fig. 7A, the frequency of IFN-γ secreting OT-I cells was comparable when SIINFEKL was presented by either DCmat or DCnaive, and irrespective of whether the OT-I cells had been primed using DCmat. Importantly, no IFN-γ was produced by cells primed and subsequently restimulated with DCmat alone. Priming, therefore, neither led to functional commitment in the absence of exogenous Ag nor to loss of function upon subsequent Ag exposure. Stimulation of naive OT-I cells using G4 mirrored that observed by proliferation (Fig. 5B), with DCmat inducing a greater response than DCnaive. In addition, a significant effect of priming OT-I cells was observed at
low Ag doses (Fig. 7B). With DCnaive, E1 failed to induce IFN-γ production and only a weak response was generated using DCmat. However, priming of OT-I using DCmat significantly enhanced the IFN-γ response to this otherwise ineffectual weak TCR agonist (Fig. 7C).

OT-I cells redistribute among lymphoid tissues

The results described above demonstrate that splenic DC activated by inflammation during L. donovani infection can stimulate OT-I cells to proliferate independently of exposure to their cognate Ag. To explore the generality of this response, we therefore examined the in vivo response of OT-I cells following infection with Streptococcus pneumoniae. Mice were infected with S. pneumoniae via the i.v. route (allowing comparison with responses generated with L. donovani) or via the intranasal route, to examine the response in the lung-draining LN (29). Forty-eight hours after i.v. infection with S. pneumoniae, splenic DC and, to a lesser extent, lung-draining LN DC had responded by an increase in expression of CD86. In contrast, following intranasal infection, DC maturation was restricted to the lung-draining LN (Fig. 8A). We next adoptively transferred naive OT-I cells into B6.CD45.1 mice before intranasal or i.v. administration of 10⁷ S. pneumoniae. Five days later, OT-I

FIGURE 5. Presentation of weak agonist peptides is enhanced by DCmat. OT-I cells were cultured with DCnaive (●) or DCmat ( ■) in the presence or absence of SIINFEKL (A), E1 (B), or G4 (C) and total number of OT-I cells was determined at day 5. The experiment was performed three times. Representative data set is shown. Data represent mean ± SD of triplicate cultures. p < 0.05; ** p < 0.01; *** p < 0.001.
cells were recovered and CFSE dilution assessed. OT-I cells were found in both spleen and lung-draining LN after both i.v. and intranasal infection (Fig. 8B). The increased numbers of recoverable cells in dLN from i.n. vs i.v. infected mice (21374 ± 5853 vs 549 ± 67, respectively) was likely a reflection of the LN enlargement that accompanies this route of infection (data not shown). More importantly, however, OT-I cells with identical profiles of CFSE dilution were isolated from both organs, irrespective of the route of administration and therefore independently of whether local DC maturation had occurred (Fig. 8C).

Discussion
We have shown that CD8\(^+\) T cells are stimulated to proliferate without functional commitment when exposed in vitro or in vivo to DC that have undergone maturation in the presence of inflammatory signals. This CD8\(^+\) T cell response is costimulation-dependent and requires signals through the TCR, suggesting that these CD8\(^+\) T cells are recognizing endogenous self- ligands that behave as weak peptide agonists when presented by matured DC. CD8\(^+\) T cells stimulated to proliferate in this manner were not retained at the site of priming and appeared to circulate to other lymphoid organs, suggesting that self recognition on DC\(_{\text{mat}}\) may increase the number of CD8\(^+\) T cells available for pathogen recognition at distal sites.

Earlier work by Reis e Sousa and colleagues working with CD4\(^+\) T cells (12) suggested that DC matured by inflammatory signals evoked by LPS injection were only capable of inducing T cell proliferation and not commitment to cytokine production. In contrast, DC that had been licensed for IL-12p40 production through MyD88-dependent signaling were able to present cognate Ag and induce functional commitment. In these studies, however, neither the ability of DC to induce proliferation of CD4\(^+\) T cells in the absence of Ag, nor their ability to stimulate CD8\(^+\) T cells was reported. In this study, we injected mice with CFSE-labeled \textit{L. donovani} parasites to generate an in vivo inflammatory microenvironment and then examined the functional ability of DC directly ex vivo. Using IL-12p40 expression as a means of distinguishing DC activated by cognate TLR signaling, we found that IL-12p40 was restricted to cells containing CFSE-labeled parasites or parasite fragments. We then showed that DC\(_{\text{mat}}\) could effectively induce proliferation in three independent target populations; OT-I and F5 TCR transgenic T cells and polyclonal CD8\(^+\) T cells. The capacity of DC\(_{\text{mat}}\) to induce proliferation in a seemingly Ag-independent manner was unlikely to merely reflect presentation of \textit{Leishmania} Ags, not detectable by CFSE labeling and that served as weak agonists for the OT-I and F5 TCRs. First, we used transgenic T cells on a RAG\(^{-/-}\) background, to rule out the possibility that recombination had generated new \textit{Leishmania}-specific TCRs. Second, if OT-I cells recognized \textit{Leishmania} Ags, it would have been expected that DC\(_{\text{mat}}\) would stimulate a greater proliferative response than DC\(_{\text{naive}}\); yet this was not observed. Third, proliferation in CD8\(^+\) T cells was also induced by “bead-negative” DC isolated from mice injected with LPS-coated fluorescent latex beads. Thus, inflammation alone appears sufficient to endow DC with the ability to induce naive CD8\(^+\) T cell proliferation. We did not set out to identify the factors responsible for inflammation-induced maturation of DC. However, these trans-acting inflammatory signals may include cytokines produced by DC themselves (20), cytokines produced by other infected cells such as macrophages and neutrophils (30), uric acid (8), or kinins and other products of the coagulation pathway (9, 10).

The role of CD28-dependent costimulation in regulating the activation threshold for Ag-specific T cell proliferation is well known (21). The data presented in this study indicate that CD86 but not CD80 are involved in the proliferation of CD8\(^+\) T cells induced by DC\(_{\text{mat}}\). This finding is in keeping with the minimal regulation of CD80 on DC following infection with visceralising species of \textit{Leishmania} observed by us and others (22). Lack of complete inhibition of the response using CD86 mAb likely reflects the additional participation of other pathways not investigated in this study. Costimulation-dependence, cyclosporine A-dependence and the ability to drive proliferation with DC that either do not contain or have not been exposed to exogenous Ag all collectively argue that the CD8\(^+\) T cell proliferation observed here is due to self-Ag recognition. We believe that the most likely explanation for the limited frequency of OT-I cells that respond to DC\(_{\text{mat}}\) in this manner is that although DC are known to express a variety of self Ags containing peptide analogues of SIINFEKL (31), such expression may not be uniform. In this regard, it would be of future interest to determine whether interference with negative costimulatory receptor-ligand interactions, such as that between PD-1 and PD-L1 (32), might further enhance the magnitude of this response. Our data are also consistent with the notion that
OT-I proliferation induced by DCmat is tempered by limiting availability of, and responsiveness to, IL-2, a feature of T cell activation also known to be dependent on strength of signal (33). Although we have not found a role for IL-15 and IL-7 in this response, and our data using fixed DCmat argue against an obligatory requirement for the release of soluble mediators, we do not rule out the possibility that other cytokines contribute to early OT-I proliferation, as suggested by others (34).

Our study bears some similarities yet has important differences to one recently reported by Bevan and colleagues. Zehn et al. (35) used the elegant approach of expressing different OVA peptide agonists as transgenes in *Listeria monocytogenes*, whereas we have used *Leishmania* with no detectable cross-reactivity with the OT-I TCR and have measured the response to OT-I agonist peptides selected from the endogenous self peptide repertoire. Nevertheless, both studies report expansion of OT-I cells by low affinity ligands, with similar observable division rates, low survival, and impaired CD25 expression. Our data, like that of Zehn et al., also indicates that CD8^+^ T cells
activated by weak agonists have differential migration patterns. Here, we showed that OT-I cells induced to proliferate by DCmat in the lung draining LN were found in the spleen 5 days after transfer, having presumably trafficked there via the efferent lymphatics and subsequently via the bloodstream. These kinetics are very similar to those obtained by Zehn et al. who showed 1) that OT-I cells stimulated with weak agonist peptides Q4 and V4 appeared in blood at day 4.5 post infection and 2) that the avidity of endogenous polyclonal CD8 + T cells in the blood was lower at day 4.5 than at day 7.5 post infection (35). Our data extends the scope of their conclusions, however, by including self peptide recognition as a means for expanding the pool of pathogen-specific T cell clones and broadening the avidity of the response. We further suggest that expansion of potentially pathogen-responsive CD8 + T cells, though self-peptide recognition promoted by augmented costimulation, provides a limited window of opportunity for enhancing peripheral responsiveness to microbial insult. Thus, pathogen-specific T cells primed in a foreign Ag-independent manner become more readily able to be functionally committed even by activation with weak agonists, and this priming therefore serves to effectively enhance the precursor frequency of pathogen-specific T cells available for recruitment into the systemic immune response. Our model also predicts that T cell responses would mature with time, partly as the relative advantage of bystander proliferation is lost due to poor survival and partly due to the numerical gain associated with activated and proliferation mediated by DC that acquire direct pathogen induced maturation signals.

In conclusion, we have shown that upon activation by inflammatory signals, mature DC can stimulate proliferation of naive CD8 + T cells in the absence of their cognate Ag. Although self recognition due to enhanced costimulation is likely to drive this proliferation, such activation remains below the threshold required for IFN-γ production. Nevertheless, proliferation induced in this way primes CD8 + T cells for more robust cytokine production on subsequent encounter with weak TCR agonist peptides. The continued requirement for exogenous Ag should help limit, though not necessarily exclude, any autoimmune consequence of such proliferation, but by reducing the threshold for activation by exogenous peptides, this pathway may provide for more effective surveillance against systemic pathogen spread.

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Disclosures

The authors have no financial conflict of interest.

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