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Endogenous Dendritic Cells Are Required for Amplification of T Cell Responses Induced by Dendritic Cell Vaccines In Vivo

Petra Kleindienst and Thomas Brocker

Dendritic cells (DCs) are specialized APCs of the immune system that, as immature cells, reside within non-lymphoid tissues and continuously collect foreign Ag. After receiving a maturation signal, DCs migrate to lymphoid tissues and are highly effective at presenting foreign Ag to naive T cells, thereby inducing Ag-specific immune responses. Because they are such potent mediators of immunity, DCs are considered prime candidates for an active cancer immunotherapy. In experimental animal models as well as in clinical trials, DCs have been cultured in vitro, loaded with tumor-derived Ag, and injected as vaccines. Despite the fact that DC vaccination has been shown to be a very promising strategy for antitumor vaccination, mixed success has been reported from clinical trials so far (2). Whereas DC vaccines showed high efficacy of eradication of animal tumors early after transplantation, several possible mechanisms for the partial failure of DC vaccines in the setting of well-established tumors have been discussed. It has been shown that, during continued growth, tumors might exert several direct immunosuppressive mechanisms that negatively influence the immunostimulatory capacities of endogenous DCs. For example, DCs isolated from metastases induced a state of Ag-specific anergy in T cells. This effect was caused by IL-10 production within the tumors (3). Also vascular endothelial growth factor-producing tumors increase the percentage of endogenous immature DCs and directly suppress immunostimulation by mature DCs (4–6). Furthermore, immature DCs have been found to reside preferentially within the tumor tissue, where they might suppress tumor-specific T cells (7). Recently, DCs from patients with myeloid leukemia were shown to display functional impairment (8).

These reports suggest that endogenously growing tumors in a clinical setting might negatively influence APC of the tumor patient. Previous reports described that Ag can be transferred between live DCs in vitro (9) and that DCs can take up Ag from apoptotic or necrotic material (10–12). We explored whether such a mechanism, in which Ag is transferred from DC vaccines to endogenous DCs, appears probable in vivo, and investigated its role for T cell priming.

Previous studies have shown that DCs injected as DC vaccines leave the site of injection and migrate to draining lymph nodes (13), where they can be found in very small numbers (<1%) (14). There, short-lived migratory DCs can act as a source of Ag (e.g., the MHC II IE polypeptide) to be processed by resident lymph node DCs (15). Recently, it has been reported that specifically CD8+ DCs are able to process and re-present material from dying tumor, infected, or allogenic cells in vitro and in vivo (16). However, it remains unclear whether such an Ag-recycling mechanism, which has so far been described for proteins expressed in DCs (15, 16), is also relevant for preprocessed peptides such as on peptide-pulsed DCs. It has further not been shown whether live functional peptide-pulsed DCs are needed for such a cross-presentation, or whether apoptotic/necrotic DCs are sufficient. Whether such a process could play an amplifying role during DC vaccination in vivo has not yet been investigated.

To explore whether and how endogenous DCs take part in induction or amplification of T cell immunity during DC vaccination, we developed a model system that allows us to study this question in vivo. Using mice with targeted expression of transgenic MHC molecules on DCs or B cells, we demonstrate that the participation of endogenous DCs, but not B cells, enhances Ag-specific T cell proliferation and effector function induced by DC vaccination.
vaccination in vivo severalfold. This observation argues that functional endogenous DCs are important for the efficacy of functional endogenous DCs vaccines.

Materials and Methods

Mice

T cells from AD-10 mice are transgenic for the TCR specific for moth cytochrome c (MCC) positions 88–103 (MCCss88-103) in the context of MHC class II IE and are positively selected by MHC class II IAβ (17). AD-10 transgenic mice were backcrossed from B10BR for >10 generations to the C57BL/6 background. DC-IEΔ b (17) mice were raised in the C57BL/6 background. DC-IEΔ b or the human CD19 promoter specifically in B cells (B-IEΔ). All mice were bred and maintained at Institute for Immunology, Ludwig-Maximilians-Universität, and were used between 6 and 10 wk of age.

Adoptive transfer

CD4+ T cells from lymph nodes and spleen were prepared as single-cell suspensions. Splenic RBC were removed using ACK buffer (0.15 M NH4Cl, 1 mM KHCO3, and 0.1% Na2EDTA (pH 7.4)) for 4 min at room temperature. The percentage of AD-10 TCR-transgenic T cells among CD4+ T cells was controlled via FACS analysis with anti-TCR Vβ3- and Vα11-specific mAbs. Before injection, cells were washed in PBS and filtered through nylon mesh (51-μm pore size; Reichelt Chemie Technik, Heidelberg, Germany). For adoptive T cell transfer, 5 × 106 AD-10 T cells were injected i.v. into the lateral tail vein of recipient mice. In all transfer experiments, donor and recipient mice were sex-matched.

Dendritic cells

DCs were generated from bone marrow progenitors using GM-CSF as described previously (21). Briefly, 5 × 106 total bone marrow cells were suspended in complete medium (IMDM supplemented with 5% FCS, 50 mM 2-ME, and 1% penicillin/streptomycin) and incubated at 37°C. Resuspended through nylon mesh (51-μm pore size; Reichelt Chemie Technik, Heidelberg, Germany). For adoptive T cell transfer, 5 × 106 AD-10 T cells were injected i.v. into the lateral tail vein of recipient mice. In all transfer experiments, donor and recipient mice were sex-matched.

3-Bromo-2'-deoxyuridine (BrdU) labeling studies

Mice received an injection of 100 mg of BrdU (Sigma-Aldrich, St. Louis, MO) 3 days before T cell transfer and were subsequently given 1 mg/ml BrdU in their drinking water until the day of sacrifice. BrdU incorporation was detected by intranuclear staining using the BrdU flow kit (BD Pharmingen, San Diego, CA). Briefly, cells were surface stained with anti-Vβ3 and anti-Vα11, and fixed for 20 min in Cytofix/Cytoperm (BD Pharmingen); then nuclei were opened by incubation with Cytoperm Plus (BD Pharmingen) for 30 min at 4°C. Fixed cells were reixed with Cytofix/Cytoperm solution. Fixed cells were incubated with DNase I solution (300 μg/ml) for 1 h at 37°C. Cells were then stained with FITC anti-BrdU (BD Pharmingen) in perm/wash buffer (BD Pharmingen) for 20 min at room temperature.

Immunostaining and flow cytometry

Draining popliteal lymph nodes were removed, and cell suspensions were prepared. The mAbs used were anti-Vβ3-PE (KJ25), anti-Vα11-FITC (RPA-T4), PE-conjugated anti-IL-2 (SPL-IL2), anti-BrdU-FTTC, and streptavidin-APC. To stimulate IL-2 production, cells were incubated for 6 h with 100 ng/ml PMA, 1 mM ionomycin, anti BrdU-FITC, and streptavidin-APC. To stimulate IL-2 production, cells were incubated for 6 h with 100 ng/ml PMA, 1 mM ionomycin, anti BrdU-FITC, and streptavidin-APC. To stimulate IL-2 production, cells were incubated for 6 h with 100 ng/ml PMA, 1 mM ionomycin, anti BrdU-FITC, and streptavidin-APC.
calculated with the Student’s t test. Cells showed down-regulation of CD62L as a sign of T cell activation, but no significant differences in IL-2 production (data not shown). Taken together, endogenous DCs that express the correct Ag-specific peptide (data not shown) are capable of enhancing the proliferation of AD-10 T cells. For this purpose, we took advantage of transgenic mice expressing MHC class II IE’ molecules under the control of the B cell-specific CD19 promoter selectively in B cells (20). When the draining lymph nodes of vaccinated animals were analyzed, in contrast to DC-IE’ recipients, the number of AD-10 T cells found in B-IE’ mice was not significantly different from that detected in IE’ animals (Fig. 3; Student’s t test, p = 0.09). Because in B-IE’ mice, in contrast to IE’ mice, T lymphocytes have been negatively selected against IE, these data argue against an alloreactive influence on the DC vaccine. We investigated this possibility further and injected CFSE-labeled IE’ DCs as a vaccine s.c. into DC-IE’ or IE’ recipients and subsequently analyzed the draining lymph node at different time points for the presence of labeled DCs. As reported by others (14, 22), we found that numbers of transferred DCs peaked after 2 days postinjection and decreased substantially. Identical numbers of DCs were detected in both hosts at all time points after injection, indicating that survival times of the DC vaccine was equivalent in both hosts (data not shown). This finding is in accordance with a previous report (15), which showed that no alloreactive effect could be observed when IE’ DCs were injected into IE’ mice. Therefore, we conclude that the amplification of the T cell response induced by a DC vaccine is a special property of endogenous DCs, while B cells are less able to process Ag from the vaccine to present it efficiently.

Re-presentation of Ag by endogenous DCs results in increased levels of activated, proliferating effector T cells

We next asked whether the Ag-specific T cells triggered by DC vaccines had different characteristics in the various recipients, and analyzed lymph node cells for the activation marker CD62L, in-nerve staining for IL-2 expression, or surface staining for Vα11 and Vβ3 (a, dot plot) followed by intranuclear staining for BrdU incorporation, intracellular staining for IL-2 expression, or surface staining for CD62L expression (a, histograms, solid lines). Endogenous CD4+ T cells from the same lymph nodes are shown as controls (a, histograms, dotted lines). Numbers of CD62L+ (b), BrdU+ (c), or IL-2-positive (d) AD-10 T cells were determined on day 5 postvaccination. Each group contained three mice (n = 3). Asterisks indicate significant differences between DC-IE’ and IE’ mice as calculated with Student’s t test (*, p < 0.05).

Endogenous DCs but not B cells enhance the effect of DC vaccines

We next wanted to investigate whether the re-presentation by endogenous cells following DC vaccination is a special property of DCs or whether B cells are also capable of enhancing the prolif-eration of AD-10 T cells. For this purpose, we took advantage of transgenic mice expressing MHC class II IE’ molecules under the control of the B cell-specific CD19 promoter selectively in B cells (20). When the draining lymph nodes of vaccinated animals were analyzed, in contrast to DC-IE’ recipients, the number of AD-10 T cells found in B-IE’ mice was not significantly different from that detected in IE’ animals (Fig. 3; Student’s t test, p = 0.09). Because in B-IE’ mice, in contrast to IE’ mice, T lymphocytes have been negatively selected against IE, these data argue against an alloreactive influence on the DC vaccine. We investigated this possibility further and injected CFSE-labeled IE’ DCs as a vaccine s.c. into DC-IE’ or IE’ recipients and subsequently analyzed the draining lymph node at different time points for the presence of labeled DCs. As reported by others (14, 22), we found that numbers of transferred DCs peaked after 2 days postinjection and decreased substantially. Identical numbers of DCs were detected in both recipients at all time points after injection, indicating that survival times of the DC vaccine was equivalent in both hosts (data not shown). This finding is in accordance with a previous report (15), which showed that no alloreactive effect could be observed when IE’ DCs were injected into IE’ mice. Therefore, we conclude that the amplification of the T cell response induced by a DC vaccine is a special property of endogenous DCs, while B cells are less able to process Ag from the vaccine to present it efficiently.
Transfer of Ag between DC in vitro requires cell-cell contact

Only a small fraction of DCs (<1%) from the injected DC vaccine seems to arrive in the draining lymphoid organs (data not shown and Ref. 14), suggesting that death of DCs occurs at the injection site. One consequence of DC death might be the release of antigenic material and its active uptake by or passive transfer to endogenous DCs. To control for such a possibility, we first analyzed Ag transfer independent of cell-cell contact using a Transwell assay. Viable, necrotic, or apoptotic peptide-pulsed DCs were cultured in the top chamber of a Transwell plate, while AD-10 T cells and peptide-free IE+ DCs were cultured in the lower compartment. Effective induction of apoptosis or necrosis of the Ag-pulsed DCs in the top chamber was controlled by flow cytometry using annexin V and propidium iodide (data not shown). As expected, T cells proliferated when soluble peptide was added to the upper compartment of the Transwell and could diffuse freely (Fig. 4a). In contrast, when viable peptide-pulsed DCs were cultured in the upper Transwell, only a background level of proliferation, not different from the medium control, was detected, indicating that the peptide did not reach the lower compartment. Therefore, it seems unlikely that the peptide is released from the live DC vaccines used in previous experiments. DCs that were rendered either apoptotic or necrotic were also unable to release antigenic peptide in a form capable of reaching the lower compartment and therefore did not stimulate T cells (Fig. 4a).

To address the possibility that Ag transfer requires direct cell-cell contact, we performed in vitro T cell stimulation assays with the same DC fractions used in the Transwell assay, but now without separation (Fig. 4b). Neither IE+ nor IE− DCs induced more than background proliferation when cultured together with AD-10 T cells in the absence of peptide (data not shown). When peptide-pulsed necrotic DCs were cultured in the presence of Ag-specific AD-10 T cells, they induced low, but specific proliferation (Fig. 4b). Addition of viable Ag-free DCs that did not express MHC class II IE further increased T cell proliferation. In contrast, addition of Ag-free IE+ DCs enhanced T cell proliferation ~2-fold as compared with necrotic DCs alone (Fig. 4b). Similar results have been obtained when Ag-pulsed apoptotic DCs were used (data not shown). These data indicate that Ag transfer from necrotic/apoptotic DCs to viable DCs is dependent on direct cell-cell interaction (Fig. 4, a and b) and can enhance T cell proliferation substantially. Further, expression of the correct MHC is needed for optimal cross-presentation.

Apoptotic or necrotic DCs cannot induce T cell proliferation in vivo

As mentioned before, only a small percentage of injected DCs reach the draining lymph nodes (14, 22). We wondered whether 1) these few surviving and migratory DCs from the DC vaccine actually reached the lymph nodes or 2) the majority of DCs dying at the injection site would be the source for Ag. To discriminate between these two possibilities, we injected either IE+, peptide-pulsed, necrotic or apoptotic DCs into DC-IE− recipients. Whereas the dead DCs were able to prime naive T cells to some extent in vitro (Fig. 4b), we could not detect statistically significant proliferation in the transferred T cell pool, when necrotic (Fig. 4c) or apoptotic (Fig. 4d) MCC peptide-loaded DC vaccines were injected. We concluded from these findings that, at the conditions we used, either transfer of Ag does not sufficiently take place at the site of injection or dead DCs cannot induce T cell proliferation in vivo.

Injection of viable DCs activate endogenous DCs before they die rapidly in the draining lymph node

To follow the fate of DCs after vaccination more closely, we vaccinated DC-IE− mice with a viable DC vaccine that was labeled with the life dye CFSE. At different time points after injection, draining lymph node cells were isolated and analyzed for viability of the DC vaccine. As shown in Fig. 5a, already at day 2 after injection, ~25% CFSE-positive DCs in the draining lymph nodes showed signs of secondary necrosis as judged from strong labeling with both annexin V and propidium iodide (Fig. 5a). In contrast, as control, only 2.5% necrotic, resident, CFSE− DCs could be detected in the same lymph node (Fig. 5a). Comparison of endogenous DCs in injected vs noninjected mice revealed activation of some endogenous DCs by the DC vaccine as detected by their up-regulation of CD86 (Fig. 5b). In a nonvaccinated mouse, ~34% of DCs expressed high levels of CD86, this percentage went up to ~50% in DC-vaccinated animals (Fig. 5b).

To visualize interactions between injected DCs from the vaccine and endogenous DCs, sections from the draining lymph nodes of these injected animals were stained with CD11c. As shown in Fig. 5c, injected and endogenous DCs can be localized in close contact in the T cell zone (data not shown) of draining lymph nodes. Many of those DCs from the vaccine are in intimate contact with endogenous DCs (Fig. 5c, arrows). Moreover, we could identify CFSE+ membrane patches, which had been transferred to otherwise CFSE− endogenous DCs (Fig. 5c, arrowheads). Taken together, these results suggest that Ag transfer becomes more than likely in the setting of a DC vaccination.

Injection of apoptotic or necrotic DC vaccines has no immunological effect on Ag-specific T cells

We then controlled to determine whether injection of apoptotic or necrotic DC would interfere with subsequent T cell activation by inducing T cell tolerance, as postulated before (15, 23, 24). To this end, we first immunized mice with Ag-pulsed necrotic or apoptotic DC vaccines and then challenged them with a viable DC vaccine (Fig. 6). Both groups of animals showed strong Ag-specific T cell expansion as compared with control mice (Fig. 6). These data argue against an induction of T cell tolerance by dead DC vaccines, but rather suggest that injection of apoptotic DCs fails to have an immunological effect in our experimental system.

FIGURE 3. Endogenous B cells do not enhance T cell proliferation induced by a DC vaccine. DC-IE−, B-IE−, or IE− mice were immunized with peptide-pulsed DCs s.c., and the total number of Ag-specific adoptively transferred AD-10 T cells in draining lymph nodes was determined on day 5 postimmunization by FACS analysis with TCR Vα and Vβ-specific mAbs. Each group contained three to five mice (n = 3–5). Asterisks indicate significant differences between DC-IE− and B-IE− mice as calculated with Student’s t test (**, p < 0.005), whereas differences between B-IE+ and IE− recipients were not significant.
These findings lead us to the hypothesis that few DCs from the DC vaccine, which migrate to the draining lymph node, are sufficient to prime naive T cells. As they die rather fast in situ, their antigenic peptide is taken up by endogenous DCs that further amplify the T cell response.

Discussion

Several studies have demonstrated transfer of Ag from living cells to DCs in vitro (9, 15) and in vivo (15). In addition, DCs are able to present phagocytosed material from necrotic or apoptotic cells (10–12). Although presentation of cellular material from dead cells resulted in efficient T cell stimulation in vitro (10–12), the relevance of such a mechanism in vivo remains hypothetical (24).

We have addressed this question using a novel in vivo system that allows us to focus on endogenous DCs or B cells (as control) and their participation in the immune response following DC vaccination. We found that endogenous DCs play an important role during DC vaccination and enhance Ag-specific activation, expansion/proliferation, and effector function of CD4+ T cells in vivo (Figs. 1 and 2). In contrast, participation of endogenous B cells had no impact on the efficiency of the DC vaccine (Fig. 3). Using this experimental system, we show that Ag transfer requires cell-cell contact (Fig. 4) and enables endogenous DCs to enhance T cell responses.

However, it remains unclear how Ag transfer takes place. Inaba et al. (15) proposed that transferred MHC class II molecules are endocytosed and the resulting antigenic peptide is subsequently re-presented in the context of endogenous MHC class II molecules. More recent work showed that entire membrane fragments containing MHC class II/peptide complexes may be transferred to endogenous DCs (9), allowing T cell recognition without further peptide processing. This could occur in the shape of exosomes or membrane vesicles. We found that even the presence of viable IE- DCs unable to express the restricting MHC class II element enhances T cell stimulation induced by necrotic or apoptotic MHC IE+ DCs in vitro (Fig. 4). This effect might well be explained as a bystander phenomenon; in this scenario, T cells might recognize MHC class II/peptide on necrotic/apoptotic IE- DCs, with live IE+ DCs simply providing an efficient costimulus. However, the addition of live DCs with the correct MHC class II enhanced T cell

![FIGURE 4. Dead peptide-pulsed DCs induce weak T cell proliferation in vitro but not in vivo. a, Proliferation assay where AD-10 T cells (1 x 10^5) and mature IE+ DCs (2.5 x 10^4) were cocultured in the lower chamber of a Transwell. This culture was separated by 0.2-μm pore filters from the indicated MCC88–103 peptide-pulsed (Ag+) DCs or controls in the upper chamber. T cell proliferation was measured, and SEM is shown for an average of three wells. b, Graded numbers of MCC88–103 peptide-loaded necrotic DCs were cultured in the same chamber with 1 x 10^5 AD-10 T cells in the presence of no additional DCs (Δ), 1 x 10^3 nonpulsed IE+ DCs (○), nonpulsed IE- DCs (□), or peptide-pulsed IE+ DCs (rhomb). T cell proliferation was measured in triplicate and error bars represent SEM. c and d, DC-IE mice were vaccinated with viable (c, □) or necrotic (c, △), or viable (d, □) or apoptotic (d, △) MCC88–103 peptide-pulsed IE+ DC vaccines. As controls, peptide-pulsed IE+ DCs (□) were used. Analysis of AD-10 T cells was performed as shown for Fig. 1. The group size was three to five (n = 3–5). Differences between injection of viable or necrotic DCs (c) and viable or apoptotic DCs (d) are both significant (*, p < 0.05; **, p < 0.005) as calculated with Student’s t test.](http://www.jimmunol.org/doi/abs/10.4049/jimmunol.2821250)
proliferation further. This led us to believe that DCs play an active role in the presentation of transferred Ag. This hypothesis is further supported by in vivo experiments, where T cell responses in hosts with endogenous IE\(^+\)/H\(11001\) DCs were very strong, as compared with the responses in IE\(^+\)/H\(11002\) hosts (Figs. 1 and 2). Taken together, our in vitro and in vivo experiments support both inactive (bystander phenomenon) and active roles for endogenous DCs in their enhancement of DC vaccinations, but the re-presentation of antigenic peptide on endogenous MHC class II seems to be the more efficient pathway. This interpretation is further supported by a report showing in vitro that a blockade of endocytic proteolysis inhibits re-presentation of transferred MHC class II molecules (15). Our findings show the in vivo consequence of this scenario and indicate that re-presentation via endocytosis accounts not only for peptides derived from the backbone of MHC molecules, as shown previously (15, 16), but also for (MCC\(_{88-103}\)) peptides which are already bound to the peptide-binding groove of the endocytosed MHC molecule. Therefore, we propose that such a pathway of re-presentation, which contains many potential degradation mechanisms for preprocessed small peptides with very short half-lives, is nevertheless very efficiently recycling them for re-presentation in the MHC context.

FIGURE 5. DCs from the vaccine die in the lymph node after interaction with and activation of endogenous DCs. CFSE-labeled DCs (1 \(\times\) 10\(^7\)) were injected s.c. into DC-IE mice. Two days after injection, draining lymph nodes were taken. a, Flow cytometry analysis of draining lymph nodes. The left dot plot shows representative annexin V/propidium iodide stainings either gated on CFSE\(^+\) DCs from the vaccine or gated on endogenous CFSE\(^+\) DCs. The bar graph on the right side shows a comparison of mean percentages of annexin V\(^+\) cells from either the CFSE\(^+\) or the CFSE\(^-\) DC cell populations. b, Flow cytometry analysis of CD86 up-regulation on endogenous DCs. The left histograms show representative analysis of CD86 on DCs in a mouse injected with a DC vaccine or in noninjected mice. Histograms are gated on CFSE\(^+\) CD11c\(^+\) cells. The bar graph on the right shows a comparison of the mean percentages of CD86 DCs in vaccinated vs non-vaccinated mice. c, Histological analysis of draining lymph nodes. CFSE\(^+\) DCs from the vaccine are shown in green, and endogenous CD11c-stained DCs are shown in red. Arrows indicate sites of interaction between injected and endogenous DCs, and arrowheads show CFSE\(^+\) membrane patches from injected DCs (green) on endogenous DCs (red).

FIGURE 6. Injection of necrotic or apoptotic DC vaccines does not lead to T cell unresponsiveness. DC-IE\(^+\) mice were injected s.c. on day 0 with either 10\(^7\) apoptotic (■), necrotic (□), or viable (△) peptide-pulsed DCs. Eight days later, the same mice were challenged with viable peptide-pulsed IE\(^+\) DCs (■ and □) to measure T cell responses or were not immunized (△) to determine background T cell expansion. On day 5 after the second injection, draining lymph nodes were prepared and cell suspensions were stained for the percentage of AD-10 T cells as described in Fig. 1. The group size was three to four (n = 3–4). Differences of T cell expansion in mice injected with apoptotic/viable DCs (■) vs necrotic/viable DCs (□) were insignificant (p = 0.24), whereas differences observed after treatments with necrotic/viable DCs (□) and viable/no DCs (△) were significant (p = 0.041) as calculated with Student’s t test.
injected DCs that die in situ at the site of injection might provide Ag to migratory DCs from peripheral tissues (e.g., skin). The latter would take up Ag, migrate to the draining lymph node, and present Ag. To discriminate between these two possibilities, we rendered the Ag-loaded DC vaccines either necrotic or apoptotic before injection. In neither case could we find a significant induction of T cell immunity (Fig. 4, c and d). These data suggest that efficient T cell stimulation requires the migration of viable Ag-loaded DCs to the draining lymph nodes, a conclusion that is in accordance with the current working hypothesis for Ag presentation by immature DCs in the steady state (24, 25); resident lymph node DCs present Ag derived from migratory DCs and induce in this case T cell tolerance. In contrast to this, we hypothesize that, in our in vivo model, the DC vaccine activates Ag-specific T cells first and then dies, and Ag is passed over to endogenous immature DCs. This is supported by data showing that DCs from the injected vaccine are in close contact with endogenous DCs in the lymph node (Fig. 5c) but die soon after their arrival there (Fig. 5a). Preactivated T cells can then receive Ag-specific immunostimulatory signals from immature endogenous DCs, a phenomenon described previously in vitro (26). This model might explain why resident lymph node DCs, which are most likely immature DCs, are able to enhance the T cell response. In addition, DC vaccination induces activation of a fraction of the endogenous DC as measured by CD86 up-regulation (Fig. 5b). The latter also could be immunostimulatory for naïve Ag-specific T cells.

DC vaccination leads to the activation of both CD4+ and CD8+ T cells. Our data show that endogenous DC significantly enhance the response of MHC class II-restricted CD4+ T cells following DC vaccination, but it is unclear whether a similar role for endogenous DCs exists for the stimulation of CD8+ T cells. In the latter case, transferred exogenous Ag would have to be endocytosed and re-presented (cross-presented) in the context of endogenous MHC class I.

Cross-presentation has recently been shown to exist in vivo (27–29), and it will be interesting to determine whether it has a role in the generation of CTL responses following DC vaccination. The findings reported in this study suggest that both DCs injected as vaccine and endogenous DCs from the recipient are necessary for the induction of an optimal CD4+ T cell response. Because CD4+ T cells play a central role in antitumor responses (for review, see Ref. 30), it may be critical to consider the immunological status of endogenous DCs before therapeutic DC vaccinations.

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