IL-4 deficiency does not impair the ability of dendritic cells to initiate CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses <i>in vivo</i>

Joanna M. Roberts, Jianping Yang and Franca Ronchese

Malaghan Institute of Medical Research, Wellington, New Zealand

Keywords: antigen presentation, cytokines, cytotoxic T lymphocytes, tumor immunity

Abstract

Several reports have described a role of IL-4 in dendritic cell function. We have examined the number and phenotype of dendritic cells from C57Bl/6 wild-type and IL-4<sup>−/−</sup> mice, and compared their ability to induce T cell immune responses <i>in vivo</i> and <i>in vitro</i>. We observed that the number of dendritic cells in the spleens and lymph nodes of IL-4<sup>−/−</sup> mice is comparable to the number found in wild-type mice. In addition, the expression of maturation markers such as MHC II, CD40, CD80 and CD86, and of differentiation markers such as CD4, CD8 and CD11b, was also comparable in the two populations. Splenic wild-type and IL-4<sup>−/−</sup> dendritic cells were both able to present antigen to T cell receptor transgenic CD4<sup>+</sup> or CD8<sup>+</sup> T cells in culture. When pulsed with antigen <i>in vitro</i> and then injected subcutaneously into C57BL/6 host mice, both populations of dendritic cells were able to induce the division of T cell receptor transgenic CD4<sup>+</sup> or CD8<sup>+</sup> T cells <i>in vivo</i>. This was the case regardless of whether the antigen used in these experiments was a low or a high affinity T cell receptor ligand. Similarly, both populations of dendritic cells were able to activate antigen-specific cytotoxic T cell responses and initiate tumor-protective immune responses <i>in vivo</i>. We conclude that IL-4<sup>−/−</sup> and wild-type dendritic cells have a comparable ability to initiate T cell immune responses when in an IL-4-sufficient environment.

Introduction

The cytokine IL-4 has multiple effects on immune responses, of which the most notable are the ability to direct CD4<sup>+</sup> T cells development towards 'type 2' cells which secrete IL-4, IL-5 and IL-13, and the ability to induce the switching of B cells to IgE secretion (1,2). Thus, IL-4 has been principally regarded as a key factor in the initiation of type 2 immune responses.

In addition to the effects described, other effects of IL-4 have also been observed which extend beyond its recognized role in T helper 2 (Th2) immune responses. Somewhat paradoxically, IL-4 appears necessary for the development of cytotoxic T lymphocyte (CTL) responses and type 1 Th immune responses. For example, the presence of IL-4 promotes the development of Th1 and CTL responses to tumors (3,4) and can increase resistance to <i>Leishmania</i> infection if administered early during infection (5). Furthermore, IL-4 is also critical for the activation of alloreactive T cells <i>in vitro</i>, and for the rapid rejection of histoincompatible skin grafts <i>in vivo</i> (6). These effects of IL-4 on type 1 responses appear to be mediated through antigen-presenting cells and an increased secretion of IL-12. IL-4 has been demonstrated to directly increase IL-12 secretion by dendritic cells (DC) <i>in vitro</i> (7) and <i>in vivo</i> (5). Increased IL-12 in turn allows the differentiation of CD4<sup>+</sup> Tcells into IFN-γ-secreting Th1 cells (8), the activation of CD8<sup>+</sup> Tcells into potent effector cells (9) and prevents CD8<sup>+</sup> T cell anergy (10). In all these systems, IL-4 appears to be mainly derived from CD4<sup>+</sup> Tcells (4,11), but secretion of IL-4 from other cell types has also been observed (12). Altogether, these observations suggest a critical function of IL-4, not only in Th2 immune responses, but in most T cell mediated immune responses as well.

In contrast to some of the observations above, other authors have reported that IL-4 can have an inhibitory effect on CD8<sup>+</sup> Tcells and immune responses (13). Infection with a recombinant vaccinia virus induced stronger CD8<sup>+</sup> Tcell activation in IL-4<sup>−/−</sup> mice compared to WT mice (14). In addition, IL-4 has been shown to modulate expression of costimulatory molecules on DC (15), which correlated with a strongly decreased incidence of diabetes in transgenic mice where IL-4 secretion had been targeted to the pancreas using the rat insulin promoter.
IL-4-deficient dendritic cells

The antigen-presenting function of DC therefore appears to be regulated by IL-4 through its effects on expression of costimulatory molecules and production of IL-12. It is possible that defects in the activity of DC may also contribute to the multiple defects in immune responses described above, which affected both CD4+ and CD8+ T cell responses. However, no reports to date have directly addressed whether the presence of IL-4 during DC development affects the phenotype or function of DC. To address this point, we compared the ability of DC from WT and IL-4^{-/-} mice to activate T cell responses \textit{in vitro} and \textit{in vivo}, and find that this appears completely normal.

Methods

\textbf{Mice}

C57BL/6 mice were obtained from Jackson Laboratories and were maintained at the Biomedical Research Unit of the Wellington School of Medicine. IL-4^{-/-} mice were originally obtained from Dr Manfred Kopf (16). The ‘line 318’ mouse strain, transgenic for a TCR specific for H-2D\textsuperscript{b} + fragment 33–41 of the lymphocytic choriomeningitis virus (LCMV) glycoprotein (LCMV\textsubscript{33–41}) was kindly provided by Dr H. Pircher (Institute of Medical Microbiology, University of Freiburg, Germany). OT-II mice, transgenic for a TCR specific for I-A\textsuperscript{b} + OVA, were kindly provided by Dr Bill Heath (WEHI, Melbourne, Australia). All experimental protocols were approved by the Wellington School of Medicine Animal Ethics Committee and performed according to Institutional guidelines.

\textbf{In vitro culture media and reagents}

Unless otherwise stated, all cultures were maintained in complete medium comprising Iscove’s modified Dulbecco's medium with 2 mM glutamine, 1% penicillin–streptomycin, 5 mM 2-mercapto-ethanol and 5% fetal bovine serum (FBS) (all Invitrogen, Auckland, NZ). Chicken ovalbumin protein (OVA) was from Sigma (St Louis, MO, USA). The synthetic peptides OVA\textsubscript{323–339} (KISQAVHAAHAEINEAG), OVA\textsubscript{257–264} (SIINFEKL), LCMV-GP33–41 (KAVYNFATM) and A4Y (KAVANFATM) were from Chiron Mimotopes, Clayton, Australia.

\textbf{DC preparation}

Splenic lymph node cell suspensions were prepared by depleting sheep anti-mouse IgG Dynabeads (Dynal, Victoria, Australia). Fc receptors were blocked using 2.4G2, and CD11c\textsuperscript{+} cells were labeled with N418–biotin (affinity purified from culture supernatants) or HL-3–biotin (Pharmingen, San Diego, CA) and positively selected using MACS Strepavidin Microbeads (Miltenyi Biotec, Australia) and MACS positive selection columns. This typically yielded a product of greater than 60% N418\textsuperscript{high} or HL-3\textsuperscript{high} cells, other cells present were CD11c\textsuperscript{intermediate} cells or B cells. The number of cells used in each experiment was adjusted to contain the desired number of CD11c\textsuperscript{high} cells, as determined on the basis of FACS staining.

For purification by overnight adherence, low-density spleen cells were plated on 100 mm Petri dishes (Falcon, BD Biosciences, Bedford, MA) and incubated for 2 h at 37°C. Non-adherent cells were discarded and incubation continued overnight. The remaining non-adherent cells were collected by pipetting and depleted of B cells by magnetic adherence as described (17).

For antigen loading, DC were incubated at 2 \times 10^6/ml in 10 \mu M peptide or 100 \mu g/ml protein for 2 h at 37°C.

\textbf{T cell proliferation \textit{in vitro}}

Purified CD4+ T cells were prepared from lymph node cell suspensions by depleting CD8+ and B cells using 2.43–biotin and Dynabeads conjugated to streptavidin, and Dynabeads conjugated to rat anti-mouse IgG (Dynal, Victoria, Australia). Purified CD8+ T cells were prepared by depleting lymph node cell suspensions of CD4+ T cells and B cells using GK1.5–biotin and magnetic depletion with Dynabeads. Thymidine incorporation was measured by culturing 1–2 \times 10^5 CD4+ T cells from OT-II mice, or CD8+ T cells from line 318 mice, with various numbers of antigen-loaded DC in 96-well culture plates in complete medium. Cells were pulsed with 1 \mu Ci/well of [3H]thymidine for 8 min at room temperature. Unbound dye was quenched by the addition of an equal volume of PBS and then washed three times with ice-cold medium containing 5% FBS.

\textbf{Carboxy-fluorescein diacetate succinimidyl ester (CFSE) labeling}

Single cell suspensions were prepared from spleen and lymph nodes by teasing through nylon gauze, and erythrocytes were lysed in 0.14 M NH\textsubscript{4}Cl and 17 mM Tris–HCl. Cells were washed and resuspended at 2 \times 10^7 cells/ml in phosphate-buffered saline (PBS) at room temperature. An equal volume of 2.5 \mu M CFSE (Molecular Probes, Eugene, OR) in PBS was added to the cell suspension, immediately vortexed, and incubated for 8 min at room temperature. Unbound dye was quenched by the addition of an equal volume of FBS and then washed three times with ice-cold medium containing 5% FBS.

\textbf{T cell division \textit{in vivo}}

CFSE-labeled lymph node and spleen cell suspensions from OT-II mice or line 318 mice, containing a total of 0.7 \times 10^6 V\alpha2\textsuperscript{+}V\beta5.1\textsuperscript{+} transgenic T cells (OT-II) or 1 \times 10^6 V\alpha2\textsuperscript{+}V\beta8\textsuperscript{+} transgenic T cells (line 318), were injected intravenously (i.v.) into C57Bl/6 mice in a total volume of 0.3 ml. One day later, the mice were immunized with 1 \times 10^5 antigen-loaded DC (3H) into the anterior forelimb. Control mice received DC only. Cells from the draining axillary and brachial lymph nodes were harvested 72 h after immunization.

\textbf{FACS analysis}

Anti-Fc\gammaRII (2.4G2), anti-MHC Class II (3JP), anti-CD4 (GK1.5), anti-CD11c (N418) and anti-CD86 (GL-1) antibodies were affinity purified from culture supernatants of the relevant B cell hybridomas using protein G–Sepharose (Pharmacia Biotech, Uppsala, Sweden) and conjugated to biotin, FITC or...
The percentages of DC in the secondary lymphoid organs of WT and IL-4−/− mice were examined. Spleens and lymph nodes were harvested from WT and IL-4−/− mice, and 1.25% CD11chigh cells (mean ± SE). Measurements were terminated for each group when the first animal developed a tumor in excess of 200 mm².

Results

Numbers and phenotype of DC in IL-4−/− mice

The percentages of DC in the secondary lymphoid organs of WT and IL-4−/− mice were examined. Spleens and lymph nodes were harvested from WT and IL-4−/− mice, and 1.25% CD11chigh cells (mean ± SE). Measurements were terminated for each group when the first animal developed a tumor in excess of 200 mm².

CD11chigh cells, with 1.39 ± 0.12% CD11chigh cells in WT mice, and 1.25 ± 0.29% in IL-4−/− mice.

DC comprise a heterogeneous population of cells expressing different surface markers (18). The distribution of DC into different subpopulations was compared in WT and IL-4−/− mice. As shown in Fig. 1, the CD11chigh population from the spleens and lymph nodes of WT and IL-4−/− mice comprised comparable proportions of cells expressing the DC subpopulation markers CD11b, CD8 and CD4. The expression of other markers associated with DC function, MHC II, CD40, CD80 and CD54, were also compared using density-gradient enriched spleen DC populations. As shown in Fig. 2, each of the above markers was expressed at a comparable level on splenic DC from WT or IL-4−/− mice. Because the spleens and lymph nodes of WT and IL-4−/− mice comprised similar numbers of cells, we conclude that IL-4 deficiency did not cause a detectable defect in the development and differentiation of spleen or lymph node DC.

IL-4−/− DC can stimulate T cell responses in vitro and in vivo

To establish whether the ability of DC to initiate immune responses was compromised by development in an IL-4−/− deficient environment, we assessed the ability of WT and IL-4−/− splenic DC to initiate immune responses in vitro and in vivo. Splenic DC were isolated by positive selection using anti-CD11c antibodies and magnetic beads, incubated with the relevant antigens for 2 h, and then cultured for 2 days with specific CD4+ or CD8+ cells purified from the lymph nodes of TCR transgenic mice. The WT and IL-4−/− DC populations used in these studies were of comparable purity in each experiment, and comprised similar proportions of CD8+ and CD4+ cells (data not shown). Antigen-specific CD4+ T cells were obtained from OT-II mice, which carry a TCR specific for OVA323-339, while antigen-specific CD8+ T cells were from line 318, which carry a TCR specific for LCMV-GP33-41 + Dβ.

To induce the proliferation of CD4+ OT-II cells, DC were incubated in the presence of the minimal OVA epitope OVA323-339, or in the presence of whole OVA protein (OVAp), for 2 h before the assay. As shown in Fig. 3, at every cell concentration tested, DC from WT or IL-4−/− mice induced similar proliferation of OT-II T cells. This was the case regardless of whether OVAp or the minimal OVA epitope OVA323-339 were used as antigens. Figure 3 shows a similar experiment carried out to compare the proliferation of CD8+ T cells to antigen presented on WT or IL-4−/− DC. In this case, DC were incubated in the presence of the synthetic peptide LCMV-GP33-41, which is a high affinity agonist for the line 318 transgenic TCR, or the substituted peptide A4Y, which is a weak agonist. Although T cell proliferation to the weak agonist A4Y was much lower than to the agonist LCMV-GP33-41, undistinguishable thymidine incorporation was observed when using WT or IL-4−/− DC. Together these results indicate that the two populations of DC express comparable levels of MHC and costimulatory molecules, and have comparable abilities to process antigen (in the case of OVAp).

Induction of in vivo immune responses may have more stringent requirements than induction of in vitro proliferation, as both APC and T cells are required to migrate to the appropriate site where interaction can occur. To evaluate the
T cell response in vivo we monitored cell division of antigen-specific T cells labeled with CFSE, where CFSE fluorescence can be observed to halve at each consecutive cell division. TCR transgenic T cells were injected i.v. on day 0, and antigen-loaded DC were administered s.c. on the following day. T cell division was evaluated in the draining lymph nodes at 72 h after DC administration, as this time was found to be optimal in pilot experiments. As shown in Fig. 4, CD4+ OT-II cells underwent a comparable number of divisions regardless of whether antigen was presented in the context of a WT or an IL-4−/− DC. Similarly, CD8+ line 318 T cells divided a similar number of times after immunization with antigen presented on WT or IL-4−/− DC.

Again, we considered the possibility that the use of relatively high affinity TCR ligands, such as LCMV-GP33–41 for line 318 T cells, may fail to reveal smaller differences in the ability of IL-4−/− DC to induce T cell responses. We therefore used peptide A4Y which is a weak agonist for line 318 T cells. As shown in Fig. 4, using A4Y peptide resulted in a much reduced accumulation of divided T cells in vivo. However, no difference between WT and IL-4−/− DC became apparent in those experiments.

**Induction of effector CD8+ T cell responses by WT and IL-4−/− DC**

T cell division as evaluated by CFSE staining is a sensitive measure of early T cell responses to antigen recognition, but does not necessarily reflect the generation of effector cells (19). In order to establish whether early T cell division had been followed by generation of effector T cells, we evaluated the development of cytotoxic activity in C57Bl/6 mice that had been immunized s.c. with WT or IL-4−/− DC that had been coated with LCMV-GP33–41 or OVA257–264 peptide. Unlike the experiments in the previous paragraph, these experiments
were carried out in C57Bl/6 mice that had received no adoptive transfer of TCR transgenic T cells, thus reflecting the generation of CTL from a naive repertoire. To measure cytotoxic activity, 1 week after immunization, mice were injected i.v. with target spleen cells that had been labeled with CFSE and loaded with LCMV-GP 33–41 or OVA 257–264 peptide, or labeled with CMTMR and loaded with no peptide. The differential survival of these target spleen cells was evaluated 48 h after target cell transfer. Target cell recoveries were evaluated in immunized mice and compared to recoveries in naive C57Bl/6 mice to minimize confounding effects of the dye label on target survival. As shown in Fig. 5, cytotoxic activity was readily demonstrated in immunized mice. No difference was apparent in the LCMV-GP 33–41-specific CTL activity induced by WT or IL-4−/− DC immunization, even when limiting numbers of DC were used. In addition, IL-4−/− DC were also able to induce OVA 257–264-specific CTL, although the CTL activity was somewhat lower than in mice immunized with WT DC. Therefore, WT and IL-4−/− DC were both able to induce CTL differentiation in vivo.

As a further measure of the ability of DC to activate effector T cell responses, we also evaluated their capacity to induce tumor-protective immunity. We therefore immunized C57Bl/6 mice with LCMV-GP 33–41 peptide-loaded DC prepared from WT or IL-4−/− mice, and evaluated their ability to resist a challenge with LCMV-GP 33–41-expressing tumor cells.
administered 1 week later. As shown in Fig. 6(A), these freshly prepared DC induced only weak or undetectable anti-tumor immune responses. In the attempt to induce more powerful anti-tumor immune responses, we used DC that had been activated by overnight adherence to plastic. This method of purification also yielded similar numbers of WT and IL-4^{+/−} DC, which had similar surface phenotypes. As shown in Fig. 6(B), both DC populations could induce tumor protective immunity in recipient mice. When lower numbers of DC were used to immunize mice, no effect on tumor growth could be detected (data not shown). We conclude that both WT and IL-4^{+/−} DC are able to induce the activation of effector T cells with comparable tumor-protective activity.

Conclusions

Several reports have described a role for IL-4 in CD4^{+} T cell responses, CTL generation and DC function. IL-4 appears necessary for the early production of IL-12 by DC (7). Lack of IL-4 production causes altered resistance to infection with the protozoan parasite Leishmania major (5) and defective CTL generation after vaccination with a recombinant protein (12). T cells have been identified as the source of IL-4 in some of these experiments (4), but other cell types, including monocytic cells, have also been implicated (12). Although DC are critical components of the immune response, and effects of IL-4 on their function have been observed (7, 15), the properties of DC in IL-4^{+/−} mice have not been directly addressed.

In this report we compare the ability of WT and IL-4^{+/−} DC to induce the early activation of CD4^{+} and CD8^{+} T cells in vitro and in vivo, and initiate tumor-specific CD8^{+} effector T cells in vivo. In most experiments we used DC that were freshly isolated from murine spleen, and had not been activated by deliberate exposure to infectious stimuli or adherence to plastic. DC were loaded with synthetic peptide antigen and injected into host mice where their ability to induce specific immune responses

![Fig. 4.](https://example.com/fig4.png) WT and IL-4^{+/−} DC can prime T cell proliferation in vivo: C57Bl/6 mice received an adoptive transfer of CFSE-labeled TCR transgenic T cells on day 0, and 1 day later were immunized with 10^5 CD11c_{high} cells from either WT or IL-4^{+/−} mice, which had been prepared and loaded with antigen as indicated in the legend to Fig. 3. The results are representative of three independent experiments using populations that comprised 37–38% WT and 42% IL-4^{+/−} CD11c_{high} cells. T cell division was assessed in the draining lymph nodes 72 h after DC injection. Bars indicate the number of cells at each division ± SD.

![Fig. 5.](https://example.com/fig5.png) WT and IL-4^{+/−} DC can initiate cytotoxic T cell responses in vivo: C57Bl/6 mice were immunized with the indicated numbers of CD11c_{high} cells from either WT or IL-4^{+/−} mice, which had been prepared and pulsed with antigen as described in the legend to Fig. 2. For the response to LCMV-GP_{33-41}, the DC populations used comprised 53.5% CD11c_{high} cells for the WT DC population and 58% for the IL-4^{+/−} DC population. For the response to OVA_{257-264}, the percentages of CD11c_{high} cells were 48% and 40% for WT and IL-4^{+/−}, respectively. Seven days after immunization, the cytotoxic activity in recipient mice was evaluated by co-injecting CFSE-labeled and CMTMR-labeled spleen target cells that had been pulsed with the relevant peptide antigens or left untreated, respectively. The preferential elimination of peptide-loaded targets was assessed 24 h later by flow cytometry of lymph node cell suspension, and is expressed as percent killing ± SEM, according to the formula described in the Methods.
In contrast to the situation for CD8+ T cells, antigen transferred to host DC may contribute to the induction of CD4+ T cell responses by injected DC (24). Again, the observations that anti-tumor immune responses require CD4+ T cell help (21), and that CD4+ and CD8+ T cells must recognize antigen on the same APC (25) suggest that endogenous APC would have little role in most of the responses described in this paper.

Recent reports have indicated that IL-12 is required for the acquisition of effector function by CD8+ T cells in vitro, and suggested that IL-12 acts as a ‘signal 3’ for the activation of CD8+ T cells (9, 10). Secretion of IL-12 by DC requires exposure to IL-4 (7). Because effector CD8+ T cell responses were elicited regardless of whether WT or IL-4−/− DC were used in our experiments, we conclude that sufficient amounts of IL-12 were likely to be present to allow full CD8+ T cell activation. We therefore conclude that the presence of IL-4 during the final stages of DC maturation is sufficient for the full antigen presenting function of these cells.

**Acknowledgements**

We wish to thank all staff of the Biomedical Research Unit of the Wellington School of Medicine for breeding all mouse strains used in this study. We also thank Dr K. Shortman for helpful advice on the preparation of fresh dendritic cells. This work was supported by a Research grant from the Royal Society of New Zealand Marsden Fund, and an equipment grant from Lottery Health. FR was supported by a Fellowship from the Wellington Medical Research Foundation Malaghan Fund.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFSE</td>
<td>carboxy-fluorescein diacetate succinimidyl ester</td>
</tr>
<tr>
<td>CMTMR</td>
<td>chloromethyl-benzoylamino-tetramethylrhodamine</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>LCMV</td>
<td>lymphocytic choriomeningitis virus</td>
</tr>
<tr>
<td>OVA</td>
<td>chicken ovalbumin</td>
</tr>
<tr>
<td>OVAp</td>
<td>OVA protein</td>
</tr>
<tr>
<td>s.c.</td>
<td>sub-cutaneous</td>
</tr>
<tr>
<td>WT</td>
<td>wild type</td>
</tr>
</tbody>
</table>

**References**

7. Hochrein, H., O’Keeffe, M., Luft, T., Vandenberghe, S., Grumont, R. J., Maraskovsky, E. and Shortman, K. 2000. Interleukin (IL)-4 is protective immunity to tumor challenge is tumor responses required the use of DC that had been induced by the two types of DC were also similar, although anti-proliferation. In addition, CTL activity and anti-tumor activity T cells, as IFN-γ secretion and effector function. The injection of WT and IL-4−/− DC into WT mice could fail to reveal differences between the two types of DC, if antigen was transferred from the injected DC to host DC. However, we do not think this is a possible explanation for our results. Our in vivo experiments used DC coated with minimal MHC I-binding peptides, while cross-presentation requires the presence of protein in donor cells (22, 23). Indeed, peptide-loaded WT DC induced comparable division of line 318 TCR transgenic T cells that had been transferred into C57Bl/6 or bm13 recipients (D. Ritchie and F. Ronchese, unpublished data), indicating that the response was mostly or entirely due to antigen presented on donor DC. We therefore conclude that the CD8+ T cell responses assessed in this study reflect the antigen-presenting capacity of the injected WT or IL-4−/− DC. was evaluated. Our experiments revealed no defect in the ability of these DC to initiate either CD4+ or CD8+ T cell proliferation. In addition, CTL activity and anti-tumor activity induced by the two types of DC were also similar, although anti-tumor responses required the use of DC that had been activated by overnight culture. We have previously shown that in our model, protective immunity to tumor challenge is mediated in large part by secretion of IFN-γ by tumor-specific T cells, as IFN-γ−/− mice are unable to resist tumor challenge (20) but they are able to generate effective CTL responses after DC immunization (our unpublished observation). In addition, we have also shown that generation of effective anti-tumor activity requires CD4+ T cell help (21). Together, our data indicate that the presence of IL-4 in the environment was sufficient to provide the required set of stimuli for the activation of CD4+ T cells and the provision of T cell help, and for full CD8+ T cell activation to IFN-γ secretion and effector function.

The injection of WT and IL-4−/− DC into WT mice could fail to reveal differences between the two types of DC, if antigen was transferred from the injected DC to host DC. However, we do not think this is a possible explanation for our results. Our in vivo experiments used DC coated with minimal MHC I-binding peptides, while cross-presentation requires the presence of protein in donor cells (22, 23). Indeed, peptide-loaded WT DC induced comparable division of line 318 TCR transgenic T cells that had been transferred into C57Bl/6 or bm13 recipients (D. Ritchie and F. Ronchese, unpublished data), indicating that the response was mostly or entirely due to antigen presented on donor DC. We therefore conclude that the CD8+ T cell responses assessed in this study reflect the antigen-presenting capacity of the injected WT or IL-4−/− DC. was evaluated. Our experiments revealed no defect in the ability of these DC to initiate either CD4+ or CD8+ T cell proliferation. In addition, CTL activity and anti-tumor activity induced by the two types of DC were also similar, although anti-tumor responses required the use of DC that had been activated by overnight culture. We have previously shown that in our model, protective immunity to tumor challenge is mediated in large part by secretion of IFN-γ by tumor-specific T cells, as IFN-γ−/− mice are unable to resist tumor challenge (20) but they are able to generate effective CTL responses after DC immunization (our unpublished observation). In addition, we have also shown that generation of effective anti-tumor activity requires CD4+ T cell help (21). Together, our data indicate that the presence of IL-4 in the environment was sufficient to provide the required set of stimuli for the activation of CD4+ T cells and the provision of T cell help, and for full CD8+ T cell activation to IFN-γ secretion and effector function.

The injection of WT and IL-4−/− DC into WT mice could fail to reveal differences between the two types of DC, if antigen was transferred from the injected DC to host DC. However, we do not think this is a possible explanation for our results. Our in vivo experiments used DC coated with minimal MHC I-binding peptides, while cross-presentation requires the presence of protein in donor cells (22, 23). Indeed, peptide-loaded WT DC induced comparable division of line 318 TCR transgenic T cells that had been transferred into C57Bl/6 or bm13 recipients (D. Ritchie and F. Ronchese, unpublished data), indicating that the response was mostly or entirely due to antigen presented on donor DC. We therefore conclude that the CD8+ T cell responses assessed in this study reflect the antigen-presenting capacity of the injected WT or IL-4−/− DC. was evaluated. Our experiments revealed no defect in the ability of these DC to initiate either CD4+ or CD8+ T cell proliferation. In addition, CTL activity and anti-tumor activity induced by the two types of DC were also similar, although anti-tumor responses required the use of DC that had been activated by overnight culture. We have previously shown that in our model, protective immunity to tumor challenge is mediated in large part by secretion of IFN-γ by tumor-specific T cells, as IFN-γ−/− mice are unable to resist tumor challenge (20) but they are able to generate effective CTL responses after DC immunization (our unpublished observation). In addition, we have also shown that generation of effective anti-tumor activity requires CD4+ T cell help (21). Together, our data indicate that the presence of IL-4 in the environment was sufficient to provide the required set of stimuli for the activation of CD4+ T cells and the provision of T cell help, and for full CD8+ T cell activation to IFN-γ secretion and effector function.


