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Cutting Edge: Polarized Th Cell Response Induction by Transferred Antigen-Pulsed Dendritic Cells Is Dependent on IL-4 or IL-12 Production by Recipient Cells[1]

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Cutting Edge: Polarized Th Cell Response Induction by Transferred Antigen-Pulsed Dendritic Cells Is Dependent on IL-4 or IL-12 Production by Recipient Cells[1]

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To assess the influence of dendritic cell (DC) production of polarizing cytokines on Th2 and Th1 development we transferred Ag-pulsed DC generated from wild-type, IL-4$^{-/-}$, or IL-12$^{-/-}$ mice into wild-type, IL-4$^{-/-}$, or IL-12$^{-/-}$ recipients. We found that DC IL-4 was not necessary for Th2 induction and that, surprisingly, DC IL-12 was not an absolute requirement for Th1 development. However, DC IL-12 production facilitated optimal Th1 response development. Critically, recipient ability to produce IL-4 or IL-12 was essential for either Th2 or Th1 development. These data help delineate the source and importance of IL-4 and IL-12 in the process of induction of polarized T cell responses by DC. *The Journal of Immunology*, 2002, 168: 3127–3130.

Key questions remain to be answered as to the relative contribution that specific cell types and cytokines make in shaping the phenotype of developing T cell responses. It is clear that dendritic cells (DC)[4] represent a highly specialized type of APC that possess the requisite abilities to potently activate T cells and initiate immune responses (1–4). Additionally, a strong body of evidence promotes the cytokines IL-4 and IL-12 as principal players in the process of T cell response polarization, with Th2 responses being promoted by IL-4 and Th1 by IL-12 (5, 6). However, exactly how and at what point IL-4 and IL-12 exert their influence remains an issue of some contention (7).

The type of response induced by DC appears to depend upon the nature of the activating signal (be it pathogen or model Ag) and may be associated with the activation status of the DC (8). The ability of DC to produce substantial amounts of IL-12, both directly in response to pathogen-inherent signals and on secondary stimulation of the DC during communication with T cells, is well documented and is generally thought to be contributory to Th1 response initiation and development (9–11). In contrast to this, there is a general paucity of data regarding DC induction of Th2 responses and whether DC might produce IL-4 during this process. Recently, it has been suggested that DC IL-4 may play an important role in Th2 development (12), although we have previously been unable to detect DC IL-4 production either at the protein or message level (13).

To determine whether the ability of DC to produce polarizing IL-4 or IL-12 was necessary for induction of polarized T cell responses, we generated murine bone marrow-derived DC from wild-type (WT), IL-4$^{-/-}$, or IL-12$^{-/-}$ mice. We then compared the ability of these DC, pulsed with Ag that inherently drive either strong Th1 or Th2 responses, to induce polarized T cell responses after transfer into recipient mice. Additionally, we assessed how important it is for the recipient to be able to produce IL-4 or IL-12 during the polarization process by transferring WT DC into IL-4$^{-/-}$ or IL-12$^{-/-}$ recipients. Using this approach we found that DC are not required to produce IL-4 for effective Th2 induction. More surprisingly, given the well-known ability of DC to produce IL-12, we also found that DC IL-12 production was not an absolute requirement for Th1 induction, although it did enhance the resultant Th1 response. Importantly, in the absence of recipient ability to produce IL-4 or IL-12, we were unable to detect either Th2 or Th1 response development, respectively. These data help define the source and importance of IL-4 and IL-12 during immune response development initiated by DC, and underscore the vital role of these cytokines in promoting response progression and expansion.

Materials and Methods

Animals and reagents

C57BL/6 mice were purchased from Taconic Farms (Germantown, NY), and IL-12(p40)-deficient mice were purchased from The Jackson Laboratory (Bar Harbor, ME). IL-4-deficient (14) mice were bred in-house. Six- to 12-wk-old age- and sex-matched mice were used as a source of bone marrow for DC generation or as recipients for transfer of DC. For a Th2 Ag we used *Schistosoma mansoni* egg Ag, which is a highly effective Th2 inducer (15, 16). Soluble egg Ag (SEA) was prepared from isolated schistosome eggs as previously described (13, 17, 18). For a Th1 stimulus we used heat-killed *Propionibacterium acnes* (Pa, a Gram-positive bacterium previously known as *Corynebacterium parvum*) (19), a stock of which was kindly provided by the Trudeau Institute (Saranac Lake, NY).

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[4] Abbreviations used in this paper: DC, dendritic cell; SEA, soluble egg Ag; WT, wild type; Pa, *Propionibacterium acnes*.

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DC generation

DC were generated from murine bone marrow by culture in GM-CSF (PeproTech, Rocky Hill, NJ) for 11 days as described in detail previously (13, 20). Cells generated by this method comprised 95% DC (class II^+CD11c^+), with the remainder of the cells being predominantly granulocytes. No contaminating B cells, macrophages, CD4 or CD8 T cells, or $CD8^+$ DC were generated under these conditions, as determined by FACS using mAbs specific for B220, F4/80, CD4, and CD8-α (data not shown). For activation of DC with polarizing Ag, cells were treated in the same way, but with the addition of the chosen Ag (50 μg/ml SEA or 5 μg/ml Pa) for the final 18 h of culture.

Determination of DC priming ability

For in vivo transfer experiments, mice were injected i.p. with 5×10^5 DC or DC that had been pulsed with SEA or Pa (as above). After 7 days, spleens were removed aseptically and splenocytes were incubated with medium, SEA (50 μg/ml), Pa (5 μg/ml), or mAb anti-CD3 (0.5 μg/well, plate bound; BD PharMingen, San Diego, CA). Splenocytes (2×10^6) were stimulated in 200 μl DMEM supplemented with 2 mM L-glutamine (Life Technologies, Gaithersburg, MD), 100 U/ml penicillin plus 100 μg/ml streptomycin (Life Technologies), and 50 μM 2-ME (Sigma-Aldrich, St. Louis, MO) and 3% normal mouse serum (Cedarlane Laboratories, Hornby, Ontario, Canada). Cytokine levels in supernatants harvested from 72-h cultures were measured by ELISA. Cytokine ELISAs were performed on culture supernatants using paired mAb purchased from BD PharMingen or purified from hybridoma supernatants in our laboratory. Using this system, we have previously shown that transfer of DC primed with SEA or Pa induces polarized Th2 or Th1 responses, respectively, in recipient mice via a mechanism that is dependent upon expression of MHC class II by the injected DC (13). Importantly, this class II dependence suggests that Ag is being presented by the transferred DC and not by resident cells that acquire Ag from these DC.

Statistical analysis

Student's *t* test was used to determine the statistical significance between groups. A *p* value <0.05 was considered to be a significant difference.

Results

DC IL-4 production is not required for Th2 induction

Previously, we have been unable to detect either IL-4 mRNA or protein by SEA (or Pa)-pulsed DC (13). However, this did not preclude the possibility that DC primed with SEA might produce IL-4 following interaction with T cells in vivo. Furthermore, given recent data indicating that DC IL-4 may play an important role in Th2 development (12), we wanted to more closely examine a role for DC-derived IL-4 in Th2 induction by SEA-pulsed DC. To this end, we generated DC from WT or $IL\text{-}4^{-/-}$ mice and then differentially activated them by overnight exposure to either SEA or Pa. DC generated from WT or $IL\text{-}4^{-/-}$ mice exhibited similar phenotypic characteristics both before and after exposure to Ag, with activation markers (class II, CD80, CD86, and CD40) not being significantly up-regulated after exposure to SEA, but in all cases being markedly elevated after exposure to Pa (data not shown). Mice that had been injected 1 wk previously with SEA-pulsed DC mounted a marked SEA-specific Th2 response irrespective of whether the DC were able to produce IL-4 (Fig. 1). Furthermore, the Pa-specific Th1 response did not differ between mice injected with Pa-pulsed WT or $IL\text{-}4^{-/-}$ DC.

DC IL-12 production is required for optimal Th1 induction

It is generally thought that the IL-12 made by DC at the initiation of T cell activation contributes to the development of a Th1 response. To test this hypothesis directly, and to see what impact removal of the ability of DC to produce IL-12 might have on Th2 response initiation, we generated $IL\text{-}12^{-/-}$ DC and examined their ability to induce Th1 and Th2 responses after in vivo transfer into WT mice. DC generated from WT or $IL\text{-}12(p40)^{-/-}$ mice did not differ phenotypically either before or after exposure to Ag, displaying similar activation states after exposure to SEA, and

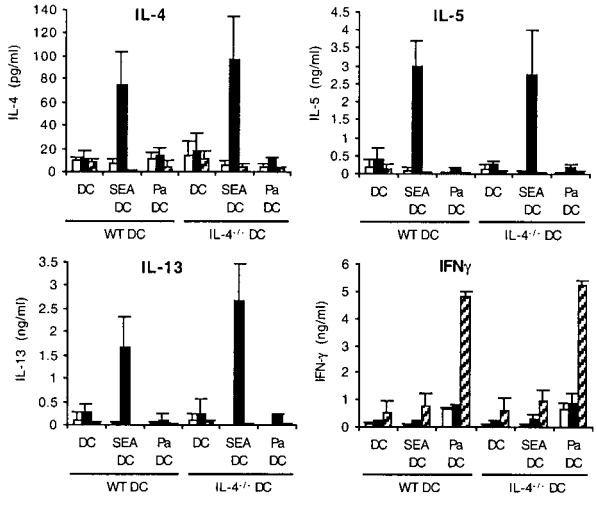

FIGURE 1. DC IL-4 is not required for Th2 induction in vivo. Cytokine production as measured by ELISA in culture supernatants from spleen cells taken from C57BL/6 mice that had been injected i.p. 7 days previously with DC generated from WT or $IL\text{-}4^{-/-}$ mice that were unstimulated, SEA pulsed, or Pa pulsed. Splenocytes were then stimulated in vitro with medium (open bars), SEA (filled bars), or Pa (hatched bars) for 72 h before supernatant harvest. Data shown are mean ± SD of triplicate wells from four to five mice per group that were individually assayed.

marked activation after exposure to Pa (data not shown). As expected, animals that received Pa-primed $IL\text{-}12^{-/-}$ DC mounted a reduced IFN-γ response in comparison to animals that had been injected with similarly activated WT DC ($p < 0.05$) (Fig. 2). However, although IFN-γ production was reduced in splenocyte cultures from these animals, the amount produced was still significant in comparison to mice injected with $IL\text{-}12^{-/-}$ DC alone ($p < 0.05$), indicating that DC IL-12 production is not an absolute requirement for initiation of a Th1 response to Pa. The SEA-specific

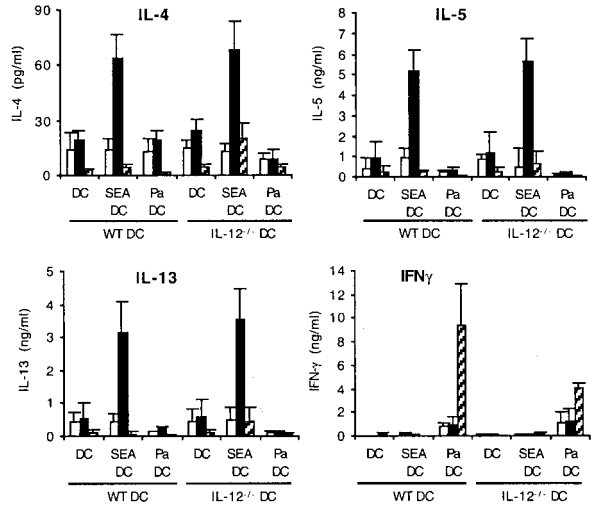

FIGURE 2. DC IL-12 is required for optimal Th1 induction in vivo. Cytokine production as measured by ELISA in culture supernatants from spleen cells taken from C57BL/6 mice that had been injected i.p. 7 days previously with DC generated from WT or $IL\text{-}12(p40)^{-/-}$ mice that were unstimulated, SEA pulsed, or Pa pulsed. Splenocytes were then stimulated in vitro with medium (open bars), SEA (filled bars), or Pa (hatched bars) for 72 h before supernatant harvest. Data shown are mean ± SD of triplicate wells from three to five mice per group that were individually assayed.

Th2 response did not differ between mice injected with SEA-pulsed WT or IL-12$^{-/-}$ DC.

Recipient IL-4 and IL-12 production is essential for Th2 and Th1 development induced by transferred Ag-pulsed DC

To assess the importance of the cytokine contribution made by resident cells in the induction of Th2 and Th1 responses by injected DC, we transferred SEA- or Pa-pulsed WT DC into WT, IL-4$^{-/-}$, or IL-12$^{-/-}$ recipient mice. Restimulation of splenocytes removed from these animals 1 wk later revealed that recipient IL-4 was absolutely required for Th2 induction, because spleen cells from IL-4$^{-/-}$ recipients of SEA-pulsed WT DC failed to produce IL-4, IL-5 (Fig. 3*A*), or IL-13 (data not shown) in response to restimulation with SEA. Splenocytes from IL-4$^{-/-}$ recipient animals stimulated with anti-CD3 also failed to produce Th2 cytokines (Fig. 3*B* and data not shown). Strikingly, spleen cells from IL-12$^{-/-}$ recipients of Pa-pulsed WT DC failed to produce IFN-γ on stimulation with Pa (Fig. 3*A*) and produced significantly less IFN-γ on stimulation with anti-CD3 (Fig. 3*B*) than WT recipients (2.6 $\pm$ 1.2 compared with 62.6 $\pm$ 17.7 ng/ml), indicating that recipient IL-12 production is necessary for Th1 induction by DC. In addition, IL-12$^{-/-}$ recipients of SEA-pulsed DC made significantly more Ag-specific IL-5 (Fig. 3*A*) and IL-13 (data not shown) than WT recipients. This was more evident following mitogenic stimulation, where IL-12$^{-/-}$ recipients of SEA-pulsed DC made significantly more IL-4, IL-5 (Fig. 3*B*), and IL-13 (data not shown) than WT recipients. Similarly, IL-4$^{-/-}$ recipients of Pa-pulsed WT DC made significantly more IFN-γ than WT recipients, which was evident on stimulation with either Pa (Fig. 3*A*) or anti-CD3 (Fig. 3*B*).

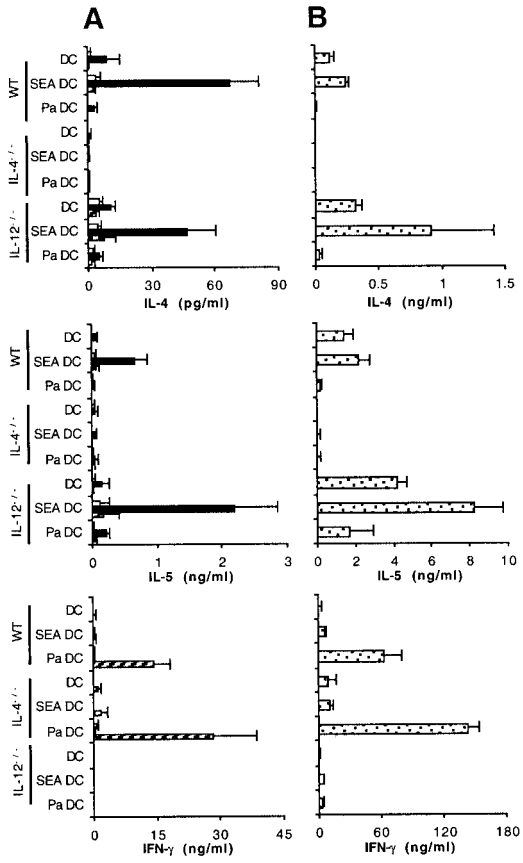

FIGURE 3. Recipient cytokine production is vital for DC induction of Th2 and Th1 responses. Cytokine production as measured by ELISA in culture supernatants from spleen cells taken from WT, IL-4$^{-/-}$, or IL-12(p40)$^{-/-}$ mice that had been injected i.p. 7 days previously with unstimulated, SEA-pulsed, or Pa-pulsed WT DC. Splenocytes were then stimulated in vitro with medium (open bars), SEA (filled bars), or Pa (hatched bars) (*A*) or with plate-bound anti-CD3 (*B*) for 72 h before supernatant harvest. Data shown are mean $\pm$ SD of triplicate wells from four to five mice per group that were individually assayed.

Taken together, these data suggest a scenario in which IL-4 from a source other than the transferred DC is required for Th2 response development. They also indicate that recipient IL-12 production is crucial for Th1 response development to Pa-pulsed DC. Additionally, because negation of recipient ability to produce IL-4 enhanced Th1 response development, and negation of recipient ability to produce IL-12 enhanced Th2 response development, these data support counterregulatory roles for IL-4 and IL-12 in this system. Last, the data indicate that Ag-pulsed DC initiate a sequence of events that leads to recipient IL-4 and IL-12 production.

Discussion

This work has yielded new information about the role of DC and recipient IL-4 and IL-12 in Th2 and Th1 development. Our data suggest that neither IL-4 nor IL-12 production by DC is required for Th2 or Th1 response induction, although DC IL-12 optimizes Th1 response development. These findings formally rule out a role for DC IL-4 in Th2 development in this system and are supportive of recent data suggesting that IL-4 is not necessary for Th2 induction by DC (13, 21). Surprisingly, and in contrast to previous work (11, 22), they also indicate that proinflammatory Th1 Ag can activate DC such that they are able to initiate Th1 responses even while unable to produce IL-12. Additional work is required to identify mediators that could compensate in the absence of DC IL-12 to induce Th1 response development, but IL-18, IFN-γ, and TNF-α are possible candidates.

Our data raise the interesting question of what role DC IL-12 might play if it is not essential for Th1 induction. Recent work addressing the kinetics of DC activation by proinflammatory Ag has shown that DC IL-12 production peaks and then is lost early after stimulation, and that DC ability to produce bioactive IL-12 on secondary stimulation and via CD40 is similarly transient (23, 24). We have shown that it is not necessary for DC to produce IL-12 to drive a potent Th1 response after activation with a proinflammatory Ag. Furthermore, we have previously shown that, following activation with a proinflammatory Ag, it is not necessary for DC to express CD40 to induce a Th1 response (25). Thus, these data suggest a role for DC IL-12 at the site of Ag encounter, rather than during DC interaction with naive T cells in the lymphatics. DC production of cytokines such as IL-12 in the periphery could affect the local response by promoting IFN-γ production by NK and memory T cells.

In contrast to our data indicating that a DC-derived IL-4 and IL-12 are not necessary for the induction of polarized responses, we have shown that recipient-derived IL-4 is vital for DC-induced Th2 development, and IL-12 for Th1 development. These results indicate that IL-4 and IL-12 function in this respect cannot be compensated by production of other cytokines such as IL-13 and IL-18. Surprisingly, particularly in the case of IL-12, these data also indicate that it is production of polarizing cytokines by cells other than the transferred DC that is vital for expansion/amplification of the T cell response initiated by DC. In relation to this, recent work has shown that the decision to become a Th1 cell can occur independently of IL-12, production of which then serves as an essential factor for prolonged Th1 survival (26). Similarly, it has been shown that Th2 development can occur in the absence of STAT-6 or IL-4 (27). Furthermore, expression of genes such as *Gata-3* and *T-bet* is alone sufficient to induce production of the key

polarizing cytokines IL-4 and IFN-γ (27, 28). Taken together, these data present the intriguing possibility that DC could initiate Th polarization by inducing the expression of genes that are important for Th polarization, but support the idea that selection, survival, and expansion of such cells then becomes reliant upon cytokines from other sources (29). In addition, they support the suggestion that continued IL-12 production is required for Th1 response maintenance (30, 31) and promote a similarly important role for IL-4 in Th2 response maintenance.

We do not yet know the source of the recipient-derived IL-12 or IL-4. It is possible that transferred Pa-pulsed DC may cross-prime resident cells (32) to make IL-12 via a mechanism that is not reliant upon DC IL-12 production. IL-4 production by resident cells could also be induced in a similar way. Such a mechanism could involve interaction of transferred DC either directly with resident DC or indirectly via priming of CD4 or CD8 T cells or other cell types. It is likely that CD4 T cells themselves provide the requisite source of IL-4 (33). We are currently addressing these possibilities.

In summary, we have shown that Th2 induction by DC occurs independently of DC IL-4 but is reliant upon recipient IL-4 production. Similarly, although DC IL-12 production can potentiate Th1 development, this process is ultimately dependent upon recipient IL-12.

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