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T_H1, T_H2, and T_H17 cells instruct monocytes to differentiate into specialized dendritic cell subsets

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Monocytes and T helper (T_H) cells rapidly infiltrate inflamed tissues where monocytes differentiate into inflammatory dendritic cells (DCs) through undefined mechanisms. Our studies indicate that T_H cells frequently interact with monocytes in inflamed skin and elicit the differentiation of specialized DC subsets characteristic of these lesions. In psoriasis lesions, T_H1 and T_H17 cells interact with

monocytes and instruct these cells to differentiate into T_H1- and T_H17-promoting DCs, respectively. Correspondingly, in acute atopic dermatitis, T_H2 cells interact with monocytes and elicit the formation of T_H2-promoting DCs. DC formation requires GM-CSF and cell contact, whereas T_H subset specific cytokines dictate DC function and the expression of DC subset specific surface molecules. Moreover, the pheno-

types of T cell-induced DC subsets are maintained after subsequent stimulation with a panel of TLR agonists, suggesting that T_H-derived signals outweigh downstream TLR signals in their influence on DC function. These findings indicate that T_H cells govern the formation and function of specialized DC subsets. (*Blood*. 2011;118(12):3311-3320)

Introduction

DCs are phenotypically and functionally divergent antigen presenting cells that originate from bone marrow precursors such as monocytes.¹⁻⁶ Encompassing a broad anatomical distribution, DCs continuously sample the microenvironment in search of danger signals recognized by a wide spectrum of pattern recognition receptors (PRRs).^{1,2,6} Depending on the cytokine milieu and the pathogen encountered, DCs differentially up-regulate costimulatory molecules and secrete a variety of cytokines that dictate the nature of the T cell response.⁷⁻¹⁰ DC exposure to particular intracellular pathogens or their products, including lipopolysaccharide (LPS) and double-stranded RNA, leads to DC-driven T_H1 differentiation primarily through IL-12 secretion.¹¹ Conversely, DCs mediate T_H2 differentiation in response to extracellular parasites, such as *S. mansoni*, which likely results from diminished IL-12 secretion and increased OX40L expression.^{7,8} Several reports have implicated DC-derived IL-1 β , IL-6, IL-23, and TGF- β in the polarization of T_H17 cells,^{9,12-17} which mediate defense against extracellular bacteria and various fungal infections.¹⁵

Psoriasis and atopic dermatitis are common inflammatory skin diseases characterized by the rapid accumulation of T_H cells and DCs.¹⁸⁻²² While both diseases share several pathologic features,^{18,22} the T_H cells implicated in the pathogenesis of each disease are distinct.^{19,20,23} Infiltration of T_H1 and T_H17 cells is commonly observed in psoriatic lesions,¹⁹ which is accompanied by elevated levels of the T_H1 cytokine, IFN- γ , and the T_H17 cytokines, IL-17A and IL-22.¹⁹ Conversely, acute atopic dermatitis lesions contain elevated levels of T_H2 cells and their associated effector cytokines, IL-4, IL-5, and IL-13.^{20,23} Not surprisingly, DCs that accumulate in

each of these diseases exhibit markedly different phenotypes.^{21,24,25} DCs present in psoriatic lesions express IL-12p40, IL-23, and TNF- α ²⁵⁻²⁷ while DCs present in atopic dermatitis lesions express low or undetectable levels of these cytokines.²⁵ DCs from each of these diseases also differentially express cell surface molecules such as DC-SIGN.²¹ However, the cell types and/or factors that influence the generation of these DC subsets remain elusive.

Given that memory T_H cells and monocytes rapidly infiltrate inflamed tissues² and that activated T_H cells secrete high levels of GM-CSF,²⁸ a cytokine that mediates DC differentiation from monocytes,^{29,30} we hypothesized that T_H cells instruct monocytes to differentiate into DCs. Our data demonstrate that not only do T_H cells direct monocytes to differentiate into DCs, but that each T_H cell subset drives the formation of phenotypically and functionally distinct DC subsets that resemble DCs previously identified in atopic dermatitis and psoriatic lesions.^{21,24,25} These data support the hypothesis that memory T_H cells influence the nature of the secondary immune response by regulating DC formation and function.

Methods

Cell isolation

Enriched CD4⁺ T cells and CD14⁺ monocytes were isolated from PBMCs obtained from healthy blood donors (Stanford Blood Center) by density gradient centrifugation using a custom RosetteSep cocktail (Stem Cell Technologies) containing mAbs against CD235a, CD8, CD19, CD20, CD56, CD66b and TCR $\gamma\delta$. CD14⁺ monocytes were isolated to > 97% purity using anti-CD14 conjugated microbeads (Miltenyi Biotec), and memory T_H cells were

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purified from the CD14⁺ fraction using a Memory CD4⁺ T cell Isolation Kit (Miltenyi Biotec). To sort unfractionated T_H cells, enriched memory T_H cells were stained with fluorescently labeled mAbs against CD14, CD16, CD19, CD25, CD36, CD56, CD123, CD235a, CD45RA, CD45RO (eBioscience), CD4 and CD8 (BD Bioscience) along with propidium iodide (PI; Invitrogen). T_H cells were defined as PI⁻CD4⁺CD45RA⁻CD45RO⁺CD25^{-lo}. To sort for each T_H subset, enriched memory T cells were stained with fluorescently labeled mAbs against CD45RA, CD4 (Invitrogen), CD25, CD127 (Biolegend), CD161, CCR4, CCR6 and CRTH2 (BD Biosciences) along with PI. T_H1 cells were defined as PI⁻CD4⁺CD45RA⁻CD25^{-lo}CRTH2⁻CD161⁻CCR4⁻CCR6⁻; T_H2 cells were defined as PI⁻CD4⁺CD45RA⁻CD25^{-lo}CRTH2⁺; T_H17 cells were defined as PI⁻CD4⁺CD45RA⁻CD25^{-lo}CRTH2⁻CD161⁺CCR4⁺CCR6⁺.

Monocyte/T-cell cocultures

Monocytes (1×10^6) were cultured with or without 1×10^5 autologous or allogeneic T_H, T_H1, T_H2 or T_H17 cells in 12-well plates (Corning) containing IMDM medium (Gibco) supplemented with 10% FCS, 2% human AB serum, 100 U/mL penicillin, 100 µg/mL streptomycin, 2mM L-glutamine, sodium pyruvate, nonessential amino acids, 50µM 2-ME and, where indicated, 20 ng/mL IL-2 (Peprotech). GM-CSF and IL-4 derived DC (DC_{GM}) were generated as described previously.³¹ For imaging studies, cells were evaluated at day 6 by Leica DMIRB microscopy with a 40×/0.55 Hoffman Modulation Contrast objective and images were acquired using a digital ORCA-ER camera and Openlab acquisition software. Otherwise, cells were processed on day 6 with 5mM EDTA and subsequently stained with DAPI (Invitrogen), fluorescently labeled isotype control mAbs, or mAbs against CD11c, CD40, CD123 (Biolegend), CD14 (eBioscience), CD3, CD80, CD83, CD86, CD205, CD209 (BD Bioscience) or CD303 (Miltenyi Biotec). Cells were also stimulated at day 6 with 2 µg/mL LPS, 5 µg/mL Pam3CSK4, 10 µg/mL CpG, 10 µg/mL zymosan, 25 µg/mL poly(I:C), 1 µg/mL flagellin, 5 µg/mL imiquimod, 1×10^8 cells/mL heat killed *Listeria monocytogenes* (HKLM), 1 µg/mL 3M-002 or 2 µg/mL N-glycol MDP for 36 hours and stained with the aforementioned mAbs against DC surface molecules or incubated with 100 µg/mL DQ-OVA (Invitrogen) on ice or at 37°C for an additional 30 minutes. Cells were analyzed on a BD LSRII flow cytometer, and FACS plots and MFIs were generated by FlowJo (Treestar). T cells were excluded according to their FSC:SSC profiles and CD3 expression. Cell-free supernatants were collected from LPS stimulated cultures for measurement of IL-1β, IL-6, IL-10, IL-12p70, IL-23p19, and TNF-α by ELISA. For neutralization studies, isotype control mAbs or mAbs against GM-CSF (Biolegend), CD119 (BD Bioscience), IL-17R, IL-21R (R&D Systems), IL-4R, IL-13, IFN-γ, TNF-α, or CD40L (eBioscience) were added at the initiation of culture at 10 µg/mL. Transwell experiments were performed using 24-well 0.4µM transwell inserts (Corning) with T_H cell and monocytes placed in the upper insert and monocytes below the insert or with T_H cells placed in the upper insert and monocytes in the lower insert.

Mixed leukocyte reaction (MLR)

Autologous T_H cells were magnetically depleted from LPS-stimulated monocytes, DC_{Th} and DC_{GM} using biotinylated anti-CD3 and anti-TCRα/β mAbs followed by anti-biotin microbeads (Miltenyi Biotec). Cells were irradiated (3000 rad) and cultured for 6 days at a 1:5 ratio with allogeneic naive CD4⁺CD45RA⁺CD45RO⁻CD25⁻ T cells isolated from PBMCs with a Naive CD4⁺ T Cell Isolation Kit II (Miltenyi Biotec). Cultures were pulsed with 1 µCi/well of ³H-thymidine for the last 20 hours and harvested by Harvester 400 (Tomtec), and radioactivity was measured by a 1450 MicroBeta counter (LKB Wallac). For DC_{Th} subsets, monocytes and T_H cells were cultured as described in "Monocyte/T-cell cocultures," and after 6 days, T cell-depleted DC_{Th} (> 95% by magnetic depletion; > 99% by FACS purification) were stimulated with 2 µg/mL LPS for 36 hours and subsequently cultured at a 1:1 ratio with allogeneic naive CD4⁺CD45RA⁺CD45RO⁻CD25⁻ T cells. After 6 days, supernatants were assayed for IFN-γ, IL-5, IL-13, and IL-17A by ELISA.

Immunohistochemistry

Sections were deparaffinized in 3 changes of xylene followed by graded alcohols. Antigen retrieval was performed using pH 6.0 Diva universal retrieval solution (Biocare Medical) inside a pressure cooker (Biocare Medical). Endogenous peroxidase activity was quenched using 3% H₂O₂ (Lab Vision). Sections were blocked with serum-free protein blocker (Dako). For immunohistochemical stains in which primary antibodies were raised in different species, sections were incubated with a cocktail of mouse and rabbit primary antibodies inside a moist chamber followed by detection using a MACH-2 detection kit (Biocare Medical). Color development was performed with freshly prepared 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (Vector Labs) and Ferangi Blue solution (Biocare Medical) before mounting with Faramount Mounting Medium (Dako).

For stains in which the primary antibodies were raised in the same species, the protocol was carried out sequentially. The first antibody was detected with either an alkaline phosphatase or HRP-conjugated polymer kit (Biocare Medical) and developed in fresh DAB solution. Before the addition of the next primary antibody, the first reaction complex was treated with denaturing solution (Biocare Medical).

The primary antibodies used were: mouse monoclonal anti-CD4 (1:10, Vector), rabbit polyclonal anti-CD14 (1:200; Atlas), mouse monoclonal anti-GATA-3 (1:100; Santa Cruz Biotechnology), rabbit monoclonal anti-T-bet (1:100; Santa Cruz Biotechnology) and rabbit polyclonal anti-RORγ (1:40; Novus Biologicals).

Sections were evaluated by an Olympus BX51 microscope with a 40×/0.95 objective and images were acquired using a CRI Nuance VIS Flex camera and CRI Nuance acquisition software. Images were subsequently imported into ImageJ (National Institutes of Health).

ELISA and statistical analysis

Antibody pairs for IL-1β, IL-4, IL-5, IL-10, IL-17A, IL-23p19 (eBioscience), IL-13, and GM-CSF (BD Bioscience) were used for ELISA analysis. BD OptEIA kits were used for IL-6, IL-12p70, and TNF-α measurements. Samples were run in duplicate, and data and statistics were analyzed with GraphPad Prism. A 2-tailed Wilcoxon rank-sum test was used to analyze all pairwise comparisons between conditions. Two-tailed Wilcoxon rank-sum tests were also used to analyze the flow cytometry data.

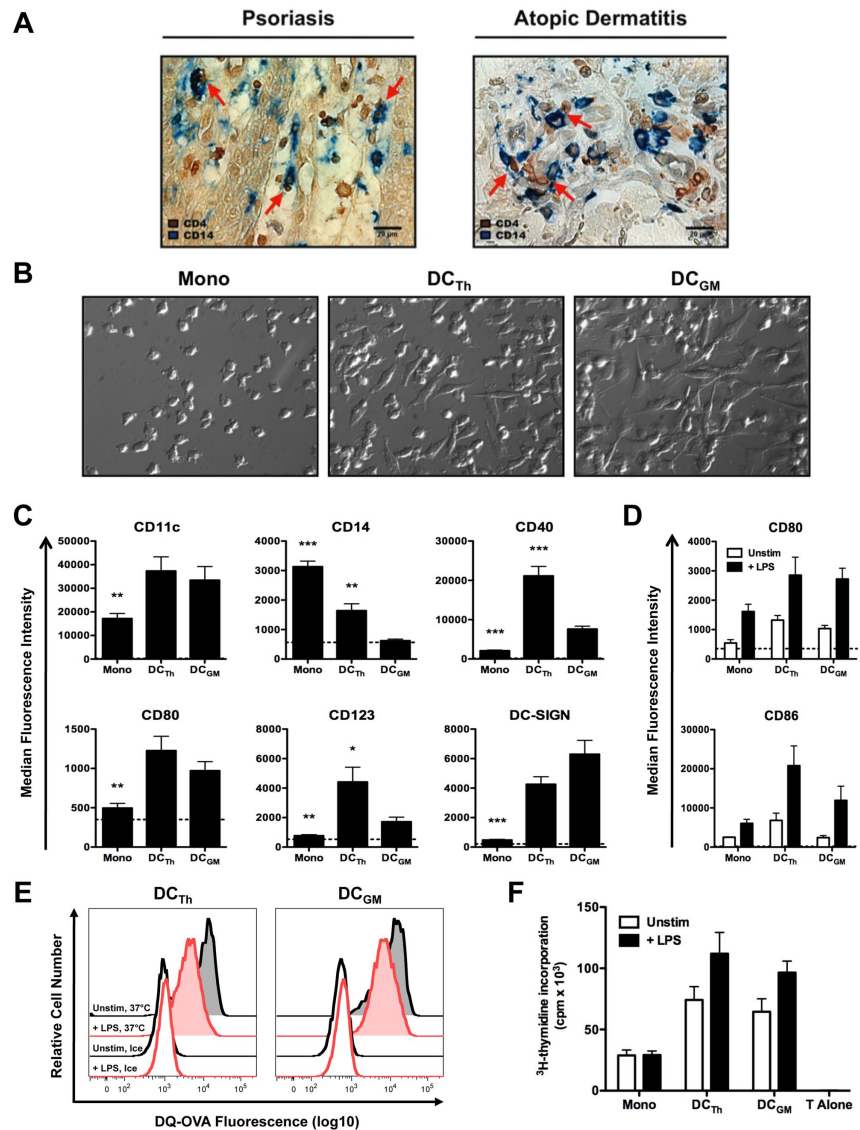
Results

T_H cells are juxtaposed with monocytes in inflamed skin and convert monocytes into DCs

Infiltration of tissues with T_H cells is a distinguishing feature of a variety of inflammatory diseases, including atopic dermatitis and psoriasis.^{19,20,22} To investigate the possible interplay between monocytes and T_H cells in situ, skin biopsies from patients with atopic dermatitis and psoriasis were stained by immunohistochemistry with anti-CD4 and anti-CD14 antibodies. In both diseases, CD4⁺ and CD14⁺ cells were found in close apposition, suggesting that T_H cells interact with monocytes in inflamed skin (Figure 1A).

After infiltrating inflamed tissues, monocytes can differentiate into DCs.³⁻⁵ To determine whether T_H cells trigger monocyte differentiation, FACS purified memory CD4⁺CD45RO⁺CD45RA⁻CD25^{-lo} T_H cells from healthy donors and autologous CD14⁺ monocytes were cocultured with IL-2 for 6 days. Monocytes cultured under these conditions (DC_{Th}) underwent morphologic changes similar to those of monocyte-derived DCs generated with GM-CSF and IL-4 (DC_{GM}; Figure 1B). Multicolor flow cytometry analysis indicated that DC_{Th} and DC_{GM} expressed similar levels of DC-associated molecules such as CD11c, CD80 and CD209 (DC-SIGN; Figure 1C). DC_{GM} expressed lower levels of CD14, CD40 and CD123 in comparison to DC_{Th} (Figure 1C). Both cell types expressed high levels of CD11c (Figure 1C) and low or undetectable levels of CD303 (BDCA-2; supplemental Figure 1A, available on the

Figure 1. T_H cells are juxtaposed with monocytes in inflamed skin and drive monocyte differentiation into DCs. (A) Representative skin lesions from psoriasis and atopic dermatitis patients were stained with antibodies against CD4 (brown) and CD14 (blue). Images are shown at 400 $\times$ magnification and were acquired using the equipment described in "Immunohistochemistry." Red arrows indicate potential monocyte/ T cell interactions. Scale bar equals 20 μ m. (B-F) CD14 $^{+}$ monocytes were cultured with IL-2 (Mono), autologous T_H cells and IL-2 (DC $_{Th}$), or GM-CSF and IL-4 (DC $_{GM}$) for 6 days. (B) Hoffman modulation contrast microscopy shown at 400 $\times$ magnification. Images were acquired using the equipment described in "Monocyte/ T -cell cocultures." Data are representative of > 10 donors. (C) Median fluorescence intensities (MFIs) of DC associated molecules are shown after excluding T_H cells. Dashed lines indicate the average MFIs of isotype control Abs used for staining from all culture conditions. Data are from 4 independent experiments and > 10 donors (mean and SEM); * P < .05, ** P < .01, *** P < .001 compared with DC $_{GM}$. (D-F) On day 6, Mono, DC $_{Th}$, and DC $_{GM}$ were cultured for 36 hours in the presence (+ LPS) or absence (Unstim) of LPS. (D) MFIs of CD80 and CD86 are shown after excluding T_H cells. Dashed lines indicate the average MFIs of isotype control Ab used for staining from all culture conditions. Data are from 2 independent experiments and 6 donors (mean and SEM). (E) Unstimulated (black histograms) and LPS-stimulated (red histograms) DC $_{Th}$ and DC $_{GM}$ were incubated with DQ-OVA for 30 minutes on ice (open histograms) or at 37 $^{\circ}$ C (shaded histograms). DQ-OVA expression is shown after excluding T_H cells. Data are representative of 2 independent experiments and 2 donors. (F) Allogeneic naive CD4 $^{+}$ T cells were cultured alone (T Alone) or with irradiated Mono, DC $_{Th}$, or DC $_{GM}$ with and without LPS stimulation at a 1:5 APC to T cell ratio in an MLR. After 6 days, ^{3} H-thymidine was added for an additional 20 hours and T cell proliferation was assessed. Data are from 2 independent experiments and 5 donors (mean and SEM).



Blood Web site; see the Supplemental Materials link at the top of the online article), which is consistent with the phenotype of myeloid DCs.³² Conversely, monocytes cultured in medium alone, IL-2 alone (Mono) or with T_H cells alone did not exhibit an altered morphology and uniformly expressed low levels of DC-associated molecules (Figure 1B-C and supplemental Figure 1A-B). Moreover, monocytes cocultured with T_H cells and nominal antigens, such as tetanus toxoid and influenza peptides, or monocytes cocultured with T_H cells and IL-15, differentiated into DCs with a phenotype similar to that described in Figure 1B and C, (supplemental Figure 1C,E and data not shown). Addition of IL-2, IL-15, or nominal antigens in this system was necessary for GM-CSF secretion by T_H cells (supplemental Figure 1D and data not shown). These data indicate that T_H cells instruct monocytes to form DCs under a variety of conditions.

DC $_{Th}$ are functionally similar to DC $_{GM}$

On activation, DCs increase the expression of costimulatory molecules, exhibit reduced endocytic activity and transform into potent antigen presenting cells.⁶ To assess the functional capacity of DC $_{Th}$, cells were cultured in the presence or absence of LPS for

36 hours. Under these conditions, DC $_{Th}$ up-regulated the expression of costimulatory molecules CD80 and CD86 to comparable or higher levels than DC $_{GM}$ (Figure 1D). After LPS stimulation, DC $_{Th}$ and DC $_{GM}$ exhibited reduced endocytic activity as measured by DQ-OVA fluorescence (Figure 1E). As expected, cells that were cultured on ice with DQ-OVA failed to internalize antigen (Figure 1E). To assess the capacity of DC $_{Th}$ to stimulate naive T cell proliferation, T cell-depleted DC $_{Th}$ were irradiated and subsequently cultured with allogeneic naive CD4 $^{+}$ CD45RA $^{+}$ CD45RO $^{-}$ CD25 $^{-}$ T cells in a mixed leukocyte reaction (MLR). Both LPS-stimulated and unstimulated DC $_{Th}$ induced naive T cell proliferation to a similar extent as DC $_{GM}$ (Figure 1F). Taken together, these data indicate that DC $_{Th}$ and DC $_{GM}$ are functionally similar.

Cell contact, GM-CSF, and TNF- α mediate DC $_{Th}$ formation

To investigate whether cell contact is required for DC $_{Th}$ differentiation, monocytes and autologous T_H cells were cocultured with IL-2 on opposite sides of a 0.4 μ m transwell membrane that allowed for the diffusion of soluble molecules, but not cells. Monocytes cultured under these conditions remained CD14 ^{hi} and did not up-regulate CD40 or CD123 (Figure 2A-B). DC-SIGN also

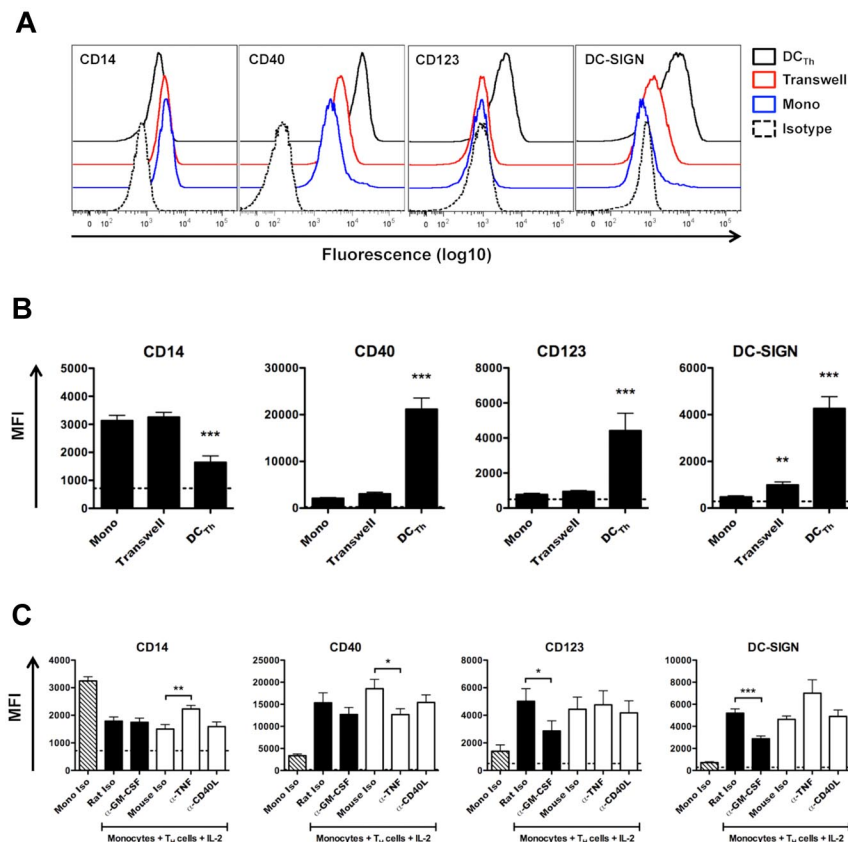


Figure 2. Cell contact, GM-CSF, and TNF- α mediate DC_{Th} differentiation. (A, B) CD14⁺ monocytes were cultured for 6 days with IL-2 (Mono), IL-2 and autologous T_H cells (DC_{Th}), or IL-2 and autologous T_H cells on opposite sides of a 0.4 μ m transwell membrane (Transwell) for 6 days. Cell surface expression of DC associated molecules is shown. (A) Representative FACS plots are shown from 4 independent experiments and > 10 donors. Isotype controls for staining (Isotype) are shown as dashed lines. (B) MFIs are shown. Dashed lines indicate the average MFIs of isotype control Ab used for staining from all culture conditions. Data are from 4 independent experiments and > 10 donors (mean and SEM), ** P < .01, *** P < .001 compared with Mono. (C) CD14⁺ monocytes were cultured for 6 days with IL-2 and isotype control Ab (Mono Iso) or autologous T_H cells, IL-2 and the indicated isotype control or neutralizing Ab. Filled columns indicate rat IgG1 Ab, while open columns indicate mouse IgG1 Ab. Mono Iso (hatched columns) represent combined values of rat IgG1 and mouse IgG1 isotype control Ab. MFIs are shown after excluding T_H cells. Dashed lines indicate the average MFIs of isotype control Ab used for staining from all culture conditions. Data are from 4 independent experiments and > 10 donors (mean and SEM), * P < .05, ** P < .01, *** P < .001.

remained low when monocytes were separated from T_H cells by a transwell membrane (Figure 2A-B). These data indicate that direct cell-cell contact is required for DC_{Th} differentiation.

To identify soluble or cell surface molecules capable of influencing DC_{Th} differentiation, monocytes and autologous T_H cells were cocultured with IL-2 and isotype control or neutralizing mAb against GM-CSF, TNF- α or CD154 (CD40L). Analysis of cocultures on day 6 demonstrated that GM-CSF neutralization inhibited DC_{Th} differentiation based on reduced acquisition of DC-SIGN and CD123 (Figure 2C). As expected, GM-CSF neutralization did not affect CD14 levels.³⁰ Conversely, TNF- α neutralization inhibited CD14 down-regulation and CD40 up-regulation (Figure 2C). Neutralization of GM-CSF or, to a lesser extent, TNF- α also prevented the acquisition of DC-like morphology (data not shown). These data suggest that GM-CSF and TNF- α play synergistic roles in DC_{Th} differentiation. Blocking CD40-CD40L interactions did not appear to alter DC differentiation (Figure 2C), which was surprising given the high levels of CD40 expression on DC_{Th} (Figure 1C).

Monocytes predominantly interact with T_H1 and T_H17 cells in psoriatic skin lesions and with T_H2 cells in atopic dermatitis

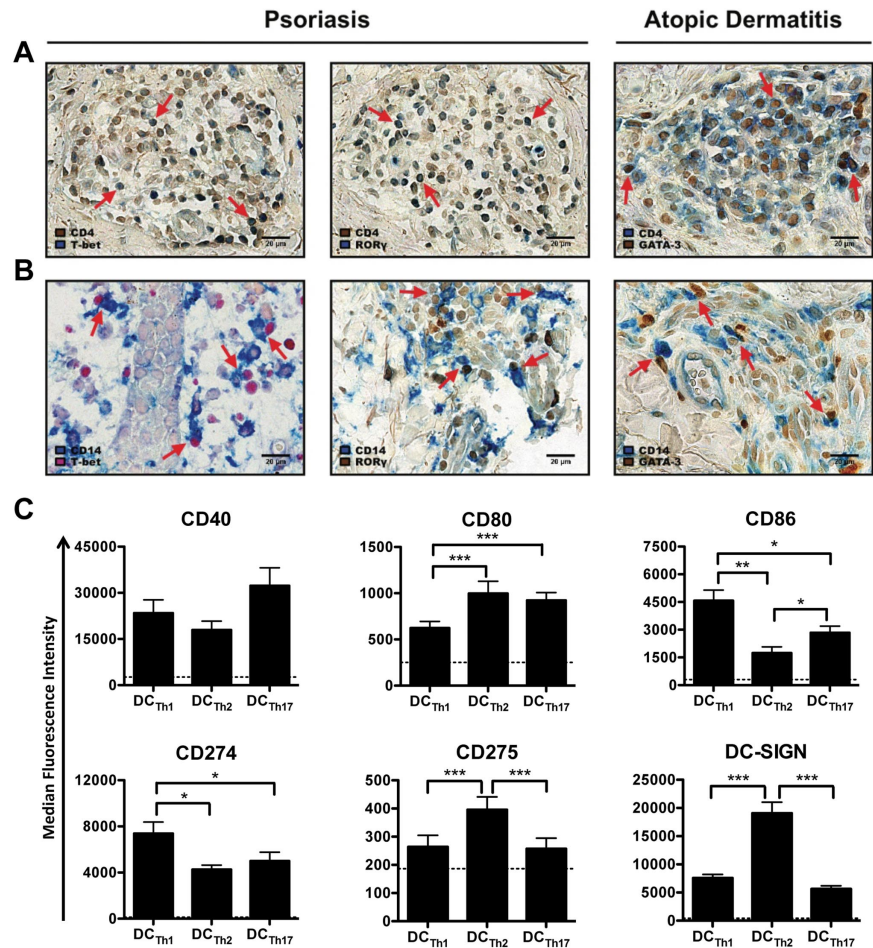
To investigate the potential interactions between T_H subsets and monocytes in atopic dermatitis and psoriatic lesions, T_H1, T_H2, and T_H17 cells were identified according to the expression of their master regulatory transcription factors T-bet, GATA-3 and ROR γ , respectively.³³⁻³⁵ In both diseases, the majority of T-bet⁺, GATA-3⁺ or ROR γ ⁺ cells were CD4⁺, which is consistent with their identity as T_H cells (Figure 3A). As expected, infiltrating GATA-3⁺ cells in atopic dermatitis lesions were present at higher levels than T-bet⁺ and ROR γ ⁺ cells (supplemental Figure 2A and data not shown).

Numerous CD14⁺ monocytes were also present in the infiltrate and were frequently juxtaposed to and/or in multipoint contact with GATA-3⁺ cells (Figure 3B). In contrast, most infiltrating CD4⁺ cells in psoriatic skin lesions were T-bet⁺ or ROR γ ⁺ (supplemental Figure 2A), and many of these cells were in close proximity with CD14⁺ cells (Figure 3B). Activated DCs, identified by CD208 (DC-LAMP) expression, were also found in close vicinity and/or multipoint contact with T_H cells in atopic dermatitis and psoriasis (supplemental Figure 2B). Given that DCs that arise in the presence of T_H1/T_H17 cells in psoriasis are phenotypically and functional divergent from DCs formed in the presence of T_H2 cells in atopic dermatitis,^{21,24,25} we investigated the impact of different T_H cell subsets on DC_{Th} differentiation.

Freshly isolated T_H1, T_H2, and T_H17 cells convert monocytes into specialized DC_{Th} subsets

To determine whether T_H cell subsets are sufficient to generate DC diversity, we FACS purified T_H1, T_H2, and T_H17 cells from healthy donors³⁶⁻³⁸ (supplemental Figure 2C-D) and subsequently cocultured each subset with allogeneic monocytes to simulate an inflammatory setting. Similar to monocytes cocultured with unfractionated T_H cells, monocytes cocultured with each T_H subset differentiated into DCs that resembled the DCs described in Figure 1C (supplemental Figure 2E). More importantly, each T_H subset elicited the formation of phenotypically distinct DC_{Th} subsets. DC_{Th}2 routinely expressed higher levels of DC-SIGN and CD275 (ICOSL; Figure 3C), which is consistent with reports indicating that T_H2-associated cytokines increase DC-SIGN and CD275 expression.^{39,41} Conversely, DC_{Th}1 expressed elevated levels of CD86 and the inhibitory receptor, CD274 (PDL-1; B7-H1), but reduced levels of CD80 (Figure 3C).

Figure 3. T_H subsets instruct monocytes to form specialized DC subsets. (A-B) Representative skin lesions from psoriasis and atopic dermatitis patients were stained with Abs against T-bet (left column), ROR γ (middle column) or GATA-3 (right column) and (A) CD4 or (B) CD14. Images are shown at 400 $\times$ magnification. Red arrows indicate examples of (A) double positive cells or (B) monocyte/ T_H cell interactions. Scale bar equals 20 μ m. (C) CD14 $^+$ monocytes were cultured with IL-2 and allogeneic T_H1 cells (DC $_{Th1}$), T_H2 cells (DC $_{Th2}$) or T_H17 cells (DC $_{Th17}$). After 6 days, DC $_{Th}$ subsets were analyzed for surface expression of DC-associated molecules. MFIs are shown after excluding T_H cells. Dashed lines indicate the average MFIs of isotype control Abs used for staining from all culture conditions. Data are from 5 independent experiments and > 10 donors (mean and SEM), * P < .05; ** P < .01; *** P < .001.



To examine the cytokine secretion profile of each DC $_{Th}$ subset, cell-free supernatants from LPS-stimulated cultures were analyzed by ELISA. Similar to previously purified DCs from psoriatic lesions,^{25,27} DC $_{Th1}$ and DC $_{Th17}$ secreted high levels of IL-1 β , IL-6, IL-23, and TNF- α (Figure 4A). DC $_{Th1}$ also secreted elevated levels of IL-12p70 (Figure 4A), a potent T_H1 polarizing cytokine that inhibits T_H2 and T_H17 differentiation.^{42,43} Intracellular analysis of IL-1 β , IL-12p40, and TNF- α confirmed these results (supplemental Figure 3A-B). Conversely, DC $_{Th2}$ more closely resembled DCs previously purified from atopic dermatitis lesions^{21,25} and produced little or no TNF- α , IL-12p70, and IL-23 (Figure 4A). DC $_{Th2}$ also produced high levels of IL-10 (Figure 4A). Similar cytokine secretion profiles were observed with DC $_{Th}$ generated with autologous T_H cells or by DC $_{Th}$ stimulated with LPS in the absence of T_H cells (supplemental Figure 3C-D). Overall, these data indicate that cytokines secreted by DC $_{Th1}$ would be expected to promote a T_H1 response,^{11,42} whereas the secretion of IL-1 β , IL-6 and IL-23, but not IL-12p70, by DC $_{Th17}$ would be expected to promote a T_H17 response.¹²⁻¹⁶ Similarly, the DC $_{Th2}$ cell surface and cytokine profile favors a T_H2 response.^{7,8,42,43}

DC $_{Th}$ subsets trigger distinct T-cell responses in an MLR

Given the marked differences in the cell surface and cytokine secretion profiles among the DC $_{Th}$ subsets, we investigated whether each subset could differentially impact naive CD4 $^+$ T-cell polarization. Magnetically purified DC $_{Th}$ subsets from autologous cocultures were stimulated with LPS for 36 hours

and cultured in the presence or absence of allogeneic naive CD4 $^+$ CD45RA $^+$ CD45RO $^-$ CD25 $^-$ T cells in an MLR. After 6 days, cell-free supernatants were assayed for the levels of IL-5, IL-13, IL-17A, and IFN- γ by ELISA. Notably, naive T cells cultured in the presence of DC $_{Th1}$ secreted elevated levels of the prototypic T_H1 cytokine, IFN- γ (Figure 4B). In contrast, DC $_{Th2}$ induced naive T cells to produce elevated levels of IL-5 and IL-13, indicative of a T_H2 response, while naive T cells cocultured with DC $_{Th17}$ secreted higher levels of IL-17A (Figure 4B). These data were confirmed by intracellular cytokine staining (supplemental Figure 4A). Because magnetic beads were used for DC $_{Th}$ isolation, it was possible that residual T_H cells from the original cocultures could confound the cytokine data. However, similar results were obtained with FACS purified DC $_{Th}$ subsets (> 99% purity; data not shown). Furthermore, the stated cytokines were largely undetectable when DC $_{Th}$ subsets were cultured in the absence of naive T cells (supplemental Figure 4B) indicating that the contribution of residual T_H cells was negligible. These data indicate that each DC $_{Th}$ subset promotes an immune response that mirrors the effector function of the T_H cell that initiated DC $_{Th}$ differentiation.

T_H cell specific cytokines and monocyte- T_H cell contact regulate the formation of specialized DC $_{Th}$ subsets

To dissect the mechanisms governing the formation of distinct DC $_{Th}$ subsets, monocytes and allogeneic T_H cells were cocultured in the presence of isotype control or T_H -specific neutralizing mAb.

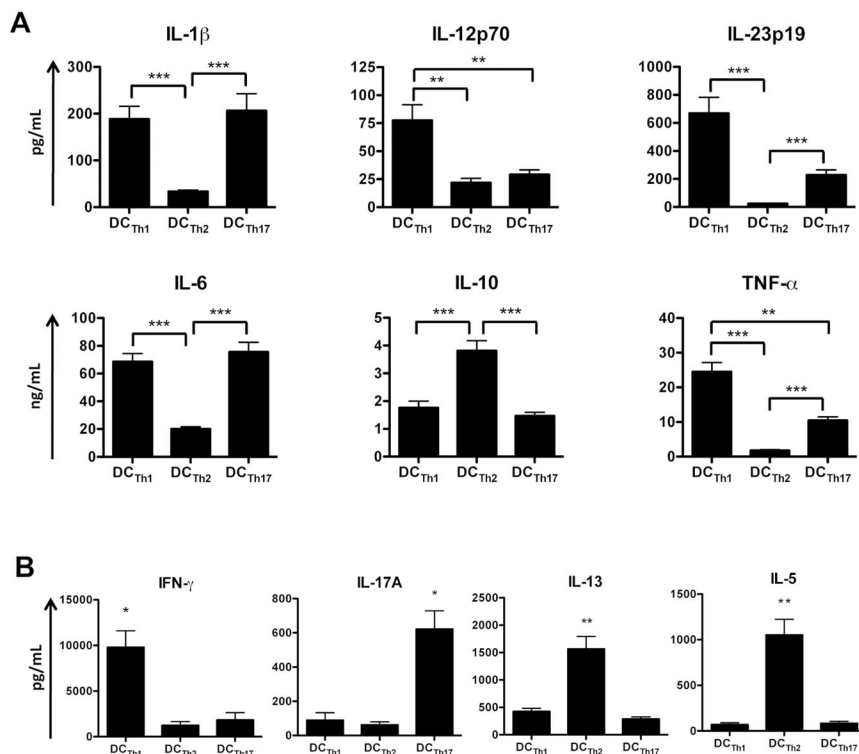


Figure 4. DC_{Th} subsets promote distinct T_H cell responses. CD14⁺ monocytes were cultured with IL-2 and allogeneic T_H1 cells (DC_{Th1}), T_H2 cells (DC_{Th2}), or T_H17 cells (DC_{Th17}). (A) On day 6 of culture, each DC_{Th} coculture was stimulated with LPS for 36 hours. Supernatants were subsequently collected for measurement of IL-1β, IL-6, IL-10, IL-12p70, IL-23p19, and TNF-α by ELISA. Data are from 5 independent experiments and > 10 donors (mean and SEM), **P* < .05; ***P* < .01; ****P* < .001. (B) T cell-depleted DC_{Th} subsets from autologous cocultures were stimulated with LPS at day 6 for 36 hours and subsequently cultured with allogeneic naive CD4⁺ T cells in an MLR. After 6 days, supernatants were collected for measurement of IL-5, IL-13, IL-17A, and IFN-γ by ELISA. Data are from 4 independent experiments and 4 donors (mean and SEM), **P* < .05, ***P* < .01 compared with all other subsets. See also supplemental Figure 4.

Analysis of cocultures on day 6 indicated that IFN-γ neutralization in DC_{Th1} cocultures reduced the acquisition of a DC_{Th1} phenotype as assessed by decreased CD86 and CD274 expression, but increased CD80 expression (Figure 5A). IFN-γ neutralization in DC_{Th1} cocultures and IL-4R/IL-13 neutralization in DC_{Th2} cocultures reduced the acquisition of a DC-like phenotype based on increased CD14 expression and decreased DC-SIGN expression (Figure 5A). Down-regulation of DC-SIGN after IL-4R/IL-13 neutralization is consistent with the high levels of DC-SIGN on DC_{Th2} in comparison to the other DC_{Th} subsets (Figure 3C). Neutralization of IL-17R/IL-21R or IL-22 did not alter the surface phenotype of DC_{Th17} according to any of the markers tested (Figure 5A and data not shown).

To determine which T_H-specific cytokines influence DC_{Th} cytokine secretion, DC_{Th} subsets were generated as in Figure 4 and stimulated with LPS in the presence of the appropriate isotype control or neutralizing mAb. After 36 hours, cell-free supernatants were analyzed by ELISA. The data indicate that IFN-γ neutralization reduced the acquisition of a DC_{Th1} phenotype in favor of a DC_{Th2} phenotype as assessed by increased IL-10 secretion and reduced secretion of IL-12p70, IL-23, and TNF-α (Figure 5B). Similarly, neutralization of IL-4R and IL-13 in DC_{Th2} cocultures shifted the acquisition of a DC_{Th2} phenotype toward a DC_{Th1}/DC_{Th17} phenotype as assessed by increased IL-1β secretion (Figure 5B). Interestingly, neutralization of IL-17R/IL-21R or IL-22 did not alter the cytokine secretion profile of DC_{Th17} (Figure 5B and data not shown) indicating that as of yet unidentified cytokines or cell surface molecules influence DC_{Th17} formation and function.

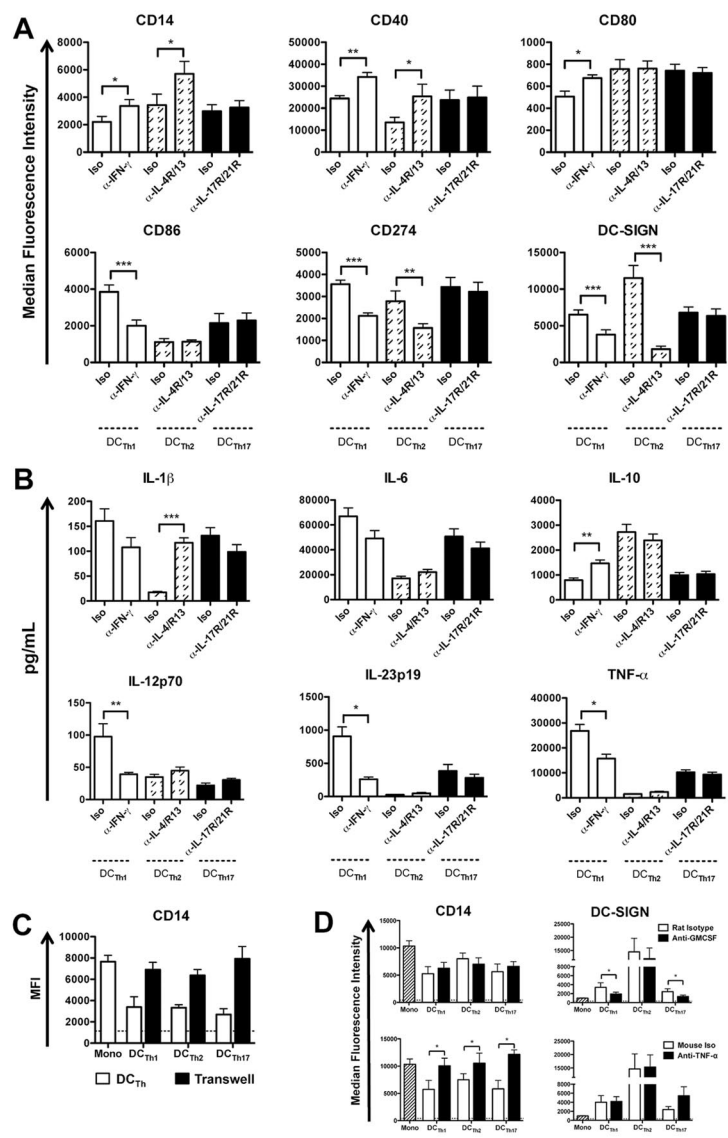
To assess whether direct contact between T_H cells and monocytes was required for the formation of DC_{Th} subsets, we separated monocytes from monocyte-T_H cell cocultures with a 0.4-μm transwell membrane. Analysis of the cells on day 6 indicated that cell contact was necessary for the formation of each DC_{Th} subset based on the failure of monocytes separated from monocyte-T_H cell cocultures to down-regulate CD14 (Figure 5C). We also assessed

the role of GM-CSF and TNF-α in the formation of DC_{Th} subsets by adding neutralizing mAb to monocyte-T_H cell cocultures. The data indicate that DC_{Th1} and DC_{Th17} formation required both GM-CSF and TNF-α, whereas DC_{Th2} formation was independent of GM-CSF and partly dependent on TNF-α (Figure 5D). Thus, T_H specific cytokines and direct T_H cell-monocyte contact are required for the full acquisition of a DC phenotype.

T_H cells, not PRR agonists, regulate DC_{Th} cytokine secretion profiles

As previously mentioned, DC responses are modulated according to the type of pathogen encountered.^{7,9,10} To assess whether memory T_H cells or PRR engagement dominate DC_{Th} responsiveness, DC_{Th} cocultures were stimulated with a range of PRR agonists (TLR1-9 and NOD2) for 36 hours. Surprisingly, analysis of cell-free supernatants indicated that differential PRR engagement modulated the total amount of cytokine secreted, but did not alter the overall cytokine secretion profile. For example, DC_{Th2} consistently secreted high amounts of IL-10 and relatively low amounts of IL-1β, TNF-α, and IL-23 regardless of PRR engagement (Figure 6). Instances where IL-10 secretion was higher in DC_{Th2} such as HKLM or zymosan stimulation were also associated with concomitant increases in IL-1β, IL-6, IL-23, and TNF-α (Figure 6). Similarly, DC_{Th1} and DC_{Th17} typically secreted higher levels of IL-1β, IL-6, IL-23, and TNF-α in comparison to DC_{Th2} (Figure 6). While some minor exceptions exist (ie DC_{Th1}, poly:IC), increases or decreases in the levels of IL-1β, IL-6, IL-23, and TNF-α were generally associated with concomitant increases or decreases in the other cytokines of this group (Figure 6). Taken together, these data indicate that not only do memory T_H cells elicit DC formation from monocytes, but that once formed, the DCs retain their functional phenotype regardless of subsequent TLR stimulation.

Figure 5. T_H cell specific cytokines and monocyte- T_H cell contact regulate the formation of specialized DC_{Th} subsets. (A,B) DC_{Th1} (open columns), DC_{Th2} (hatched columns) or DC_{Th17} (filled columns) were formed in the presence of isotype control (Iso) or the indicated neutralizing antibodies for 6 days. (A) MFIs of DC-associated molecules are shown after excluding T_H cells. Data are from 5 independent experiments and > 10 donors (mean and SEM), $*P < .05$; $**P < .01$; $***P < .001$. (B) On day 6 of culture, each DC_{Th} coculture was stimulated with LPS in the presence of the indicated isotype control (Iso) or neutralizing Ab for 36 hours. Supernatants were subsequently collected for measurement of IL-1 β , IL-6, IL-10, IL-12p70, IL-23p19, and TNF- α by ELISA. Data are from 5 independent experiments and > 10 donors (mean and SEM), $*P < .05$; $**P < .01$; $***P < .001$. (C) $CD14^+$ monocytes were cultured for 6 days with IL-2 (Mono), IL-2 and allogeneic T_H cells (DC_{Th}), or IL-2 and allogeneic T_H cells on top of a $0.4 \mu m$ transwell membrane and $CD14^+$ monocytes below the transwell insert (Transwell). MFIs of cells below the transwell insert are shown. Dashed lines indicate the average MFIs of isotype control Abs used for staining from all culture conditions. Data are from 1 experiment and 3 donors (mean and SEM). (D) $CD14^+$ monocytes were cultured for 6 days with IL-2 and isotype control Ab (Mono, hatched column) or T_H cells, IL-2 and the indicated isotype control (open columns) or neutralizing Ab (filled columns). MFIs are shown after excluding T_H cells. Dashed lines indicate the average MFIs of isotype control Ab used for staining from all culture conditions. Data are from 3 independent experiments and 6 donors (mean and SEM), $*P < .05$.



Discussion

Several studies have demonstrated that monocytes infiltrate inflamed tissues and differentiate into $CD11c^+$ DCs in vivo.³⁻⁵ However, the mechanisms responsible for DC formation have remained elusive. The present study extends these findings by elucidating the mechanism whereby human T_H cells drive DC differentiation from monocytes. Our results demonstrate that T_H cells are juxtaposed with monocytes in situ and convert monocytes into DCs in a cell contact, GM-CSF and TNF- α dependent manner. This T_H -mediated DC differentiation pathway proceeds upon IL-2 stimulation, which is consistent with our data demonstrating that IL-2 induces GM-CSF secretion from T_H cells. This study also indicates that T_H cells convert antigen-loaded (tetanus toxoid or influenza antigens) monocytes into DCs in the absence of exogenous stimulation. These data indicate that T_H cells may participate in the innate response by initiating a positive feedback loop with monocytes and DCs that govern an intensified and polarized immune response.

One of the striking findings of this study is the capacity of each T_H subset to elicit the formation of specialized DC subsets. The

data indicate that T_H1 cells instruct monocytes to form DCs that secrete IL-12 and express increased CD86 and CD274, whereas T_H17 cells elicit the formation of DCs that secrete IL-1 β , IL-6 and IL-23, but not IL-12. The cytokines secreted by DC_{Th1} and DC_{Th17} are among the key cytokines used in vitro to generate T_H1 and T_H17 cells, respectively.^{14,15} Interestingly, T_H2 cells drive the formation of T_H2 -biased DCs that express elevated levels of IL-10, CD275 and DC-SIGN. While CD275 is known to promote IL-4 responses, IL-10 and DC-SIGN have been implicated in the inhibition of T_H1 and T_H17 responses, respectively.^{17,44} Subsequent use of DC_{Th1} and DC_{Th17} as stimulators in an MLR induced responding $CD4^+$ T cells to produce IFN- γ and IL-17A, respectively. While the identification of DC-derived soluble factors that influence T_H2 differentiation remains elusive,¹⁰ DC_{Th2} nevertheless promoted IL-5 and IL-13 secretion from naive T cells. This implies that unidentified soluble factors or cell surface molecules such as OX40/OX40L, CD275 or DC-SIGN may be involved.^{7,8,41} Gene expression profiling of each DC_{Th} subset may prove useful for identifying additional molecules involved in the formation and functions of DC subsets.

Our data indicate that distinct sets of T_H -derived molecules were responsible for DC_{Th} formation and polarization. Whereas all

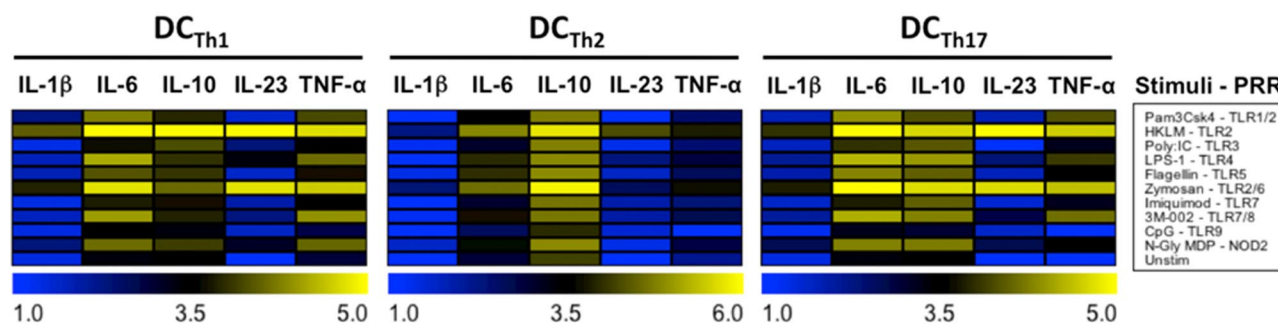


Figure 6. T_H cells, not PRR agonists, regulate DC_{Th} cytokine secretion profiles. DC_{Th1}, DC_{Th2}, and DC_{Th17} were generated as in Figure 3C. On day 6 of coculture, each DC_{Th} subset was stimulated with the indicated pattern recognition receptor (PRR) or left unstimulated (Unstim). After 36 hours, cell-free supernatants were collected for measurement of IL-1β, IL-6, IL-10, IL-23p19, and TNF-α by ELISA. IL-1β is displayed as log₂ values; IL-10 and IL-23 as log₅ values; and IL-6 and TNF-α as log₁₀ values. Data are from 2 independent experiments and 4 donors.

memory T_H subsets produced GM-CSF and TNF-α, the 2 cytokines required for generating DC morphology in our system, IFN-γ produced by T_{H1} cells was required for the induction of DC_{Th1} while IL-4 and IL-13 produced by T_{H2} cells played requisite roles in the induction of DC_{Th2}. Interestingly, T_{H17} cell-derived IL-17, IL-21, or IL-22 were not required for DC_{Th17} formation. Moreover, monocytes or DC_{GM} cultured with LPS in the presence of T_{H17}-associated cytokines, IL-17A and/or IL-21, did not secrete different cytokines compared with cells cultured with LPS alone (M.N.A., M.T.W., and E.G.E., unpublished data, August 2010). These data suggest that yet to be identified soluble and/or cell surface molecules may be involved in DC_{Th17} differentiation.

Taken together, our results indicate that T_H cells positively regulate the secondary immune response by instructing monocytes to differentiate into DCs, which in turn trigger naive CD4⁺ T cells to acquire similar effector functions as the T_H cell subset that initiated DC differentiation. Such an amplification loop would be beneficial during memory responses, but deleterious if uncontrolled during inflammatory diseases. Psoriasis and atopic dermatitis are 2 diseases that likely involve divergent feedback loops, as evidenced by the accumulation of markedly different T_H cells and DC subsets in inflamed skin.^{18–22} Psoriatic lesions contain primarily T_{H1} and T_{H17} cells,¹⁹ and possess CD11c⁺ myeloid DCs that strongly resemble the functional characteristics attributed to DC_{Th1} and DC_{Th17}.^{24–27} Most notably, CD11c⁺ DCs in psoriatic lesions express elevated levels of TNF-α, IL-12p40, and IL-23^{25,27} and drive the formation of T_{H1} and T_{H17} cells *ex vivo*.²⁴ Conversely, atopic dermatitis lesions contain large numbers of T_{H2} cells^{20,22,23} and CD11c⁺ DCs that share several characteristics with DC_{Th2}.^{21,25} Importantly, DCs from atopic dermatitis lesions express low or undetectable levels of TNF-α, IL-12p40, and IL-23^{21,25} and are believed to mediate T_{H2} differentiation during the acute phase of atopic dermatitis.²² DCs in atopic dermatitis also express higher levels of DC-SIGN compared with DCs from psoriatic lesions,²¹ which is in accordance with increased DC-SIGN expression observed on DC_{Th2}. These data indicate that CD11c⁺ DCs present in inflamed skin from classic T_H-associated diseases are phenotypically similar to those described in this report.

The numerical predominance of T_H cells in lesions from psoriasis and atopic dermatitis patients indicates that these cells may be principally responsible for shaping the local DC repertoire. However, this does not exclude the possibility that monocytes also receive signals from other cell types. Previous studies have shown that NK, CD8⁺ and NK T cells can also initiate DC differentiation.^{31,45,46} Similar to T_H cells, NK cells colocalize with monocytes in inflamed tissue and direct DC differentiation in a GM-CSF and cell contact dependent manner.⁴⁶ Comparison of the relative

contributions of each of these cell types to inflammatory responses merits further investigation.

The memory immune response is characterized by rapid responses to foreign agents encountered 2 or more times throughout the lifetime of the host. While this response is partially mediated by affinity maturation⁴⁷ and differential activation requirements,⁴⁸ our data suggest that memory T_H cells may also contribute to the speed and potency of the secondary response by eliciting the formation of specialized DC subsets. Interestingly, stimulation of these DC subsets with a panel of PRR agonists which were previously demonstrated to modulate DC cytokine secretion,^{7,9,10} affected total cytokine production, but failed to alter the relative ratios of individual cytokines produced by each DC_{Th} subset. These data indicate that, once formed, the phenotype of each DC_{Th} subset is stable and that signals from memory T_H cells outweigh subsequent signals from PRR engagement.

While our study elucidates a novel mechanism by which T_H cells can amplify the immune response, resolution of this response is essential to prevent ongoing inflammation and autoimmunity. Elimination of the initial immune stimulus, such as a pathogen or allergen, would presumably facilitate resolution. In addition, preliminary data from our laboratory indicate that regulatory T cells can instruct monocytes to differentiate into tolerogenic DCs, thereby providing another mechanistic brake on inflammation. It is also conceivable that T_H cells initiate a negative feedback loop after multiple rounds of activation.⁴⁹ This is supported by a recent report indicating that irradiated T_{H1} and T_{H2} clones prompt the formation of DCs that subsequently induce naive T cells to secrete IL-10 and IFN-γ, respectively.⁵⁰ Thus, whereas T_H cells may mediate an amplification pathway during the initiation of a secondary immune response, a variety of mechanisms likely exist to limit the intensity and duration of such responses.

This study identifies an important mechanism responsible for generating DC diversity that also provides a critical link between innate and adaptive immunity. The system we used for generating DC subsets with differential T_H polarizing capacity has facilitated the elucidation of cell surface and soluble factors governing the biology of T_H-promoting DC subsets. These and additional molecules expressed by DC subsets should prove useful for detecting and quantifying each subset *in situ* and enable the design of novel therapeutic strategies for T_H-associated inflammatory diseases.

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Authorship

Contribution: M.N.A., M.T.W., and A.L.Z. conceived and executed the studies; M.M.S. and M.G.D. assisted with experi-

mental design and provided technical guidance; D.W. and J.G. performed the IHC studies; T.H.K., D.M.G., and L.T. assisted in the execution of select studies; K.C.D. and J.K. provided psoriasis and atopic dermatitis tissues; P.J.U. supervised select studies. K.S. and E.G.E. conceived and supervised the studies; M.N.A., M.T.W., A.L.Z., M.M.S., D.W., M.G.D., P.J.U., K.S., and E.G.E. analyzed and/or interpreted the data; and M.N.A., M.T.W., and E.G.E. wrote the manuscript.

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Figure S1, related to Figure 1. T_H cells convert monocytes into DCs in the presence of IL-2, IL-15 or tetanus toxoid antigen. CD14⁺ monocytes were cultured as indicated. After 6 days, cells were analyzed for surface expression of DC-associated molecules after excluding T_H cells. Isotype controls are shown as dashed lines. **(A)** CD14⁺ monocytes were cultured alone (blue), with autologous T_H cells (orange), IL-2 (black), autologous T_H cells and IL-2 (red), or GM-CSF and IL-4 (purple). Data are representative of 4 independent experiments and >10 donors. **(B)** CD14⁺ monocytes were cultured alone (Mono), with autologous T_H cells (Mono + T_H), IL-2 (Mono + IL-2), or autologous T_H cells and IL-2 (DC_{Th}). Median fluorescence intensities (MFIs) of DC-associated molecules are shown. Data are from 4 independent experiments and >10 donors (mean and SEM); *p<0.05, **p<0.01, ***p<0.001 as compared to all other subsets. **(C)** CD14⁺ monocytes were cultured with IL-2 alone (black, open), tetanus toxoid alone (Tet, black, filled), autologous T_H cells and IL-2 (DC_{Th}, red, open), or autologous T_H cells and tetanus (Tet, red, filled). Data are representative of 3 independent experiments with 5 donors. **(D)** FACS sorted T_H cells were cultured in medium alone (Unstim) or IL-2. After 5 days, supernatants were collected for measurement of GM-CSF by ELISA. Data are from 2 independent experiments and 2 donors (mean and SEM). **(E)** CD14⁺ monocytes were cultured in the presence (filled columns) or absence (open columns) of autologous T_H cells and either 20ng/mL IL-2, 20ng/mL IL-7, 20ng/mL IL-15 (Peprotech) or left unstimulated (Unstim). MFIs of DC-associated molecules are shown. Data are from 2 independent experiments with 6 donors (mean and SEM); *p<0.05 as compared to Mono Alone in its respective condition.

Figure S2, related to Figure 3. T_H subsets interact with monocytes and DCs in inflamed tissues, and T_H subsets isolated from peripheral blood elicit DC formation. **(A, B)** Representative skin lesions from psoriasis and acute atopic dermatitis patients were stained as indicated. Images are shown at 200X magnification. Scale bar equals 40 μ m. **(A)** Skin lesions were stained with CD4 or T-bet, ROR γ , GATA-3 to identify T_H1, T_H17 or T_H2 cells, respectively. **(B)** Skin lesions were stained with DC-LAMP (1:100 dilution, Dendritics, clone 104G4) to identify DCs and T-bet (left column), ROR γ (middle column) or GATA-3 (right column) to identify T_H1, T_H17 and T_H2 cells, respectively. Red arrows indicate potential DC/T_H interactions. **(C)** Memory CD4⁺CD45RO⁺CD45RA⁻ T cells were isolated by magnetic cell sorting from PBMCs derived from healthy donors and subsequently labeled with surface markers specific for T_H subsets. After gating out CD4⁺CD45RA⁻CD25^{hi}CD127⁻ cells to deplete Tregs, T_H2 cells were defined as PI⁻CD4⁺CD45RA⁻CD25^{-lo}CRTH2⁺. T_H1 cells were defined as PI⁻CD4⁺CD45RA⁻CD25^{-lo}CRTH2⁻CD161⁺CCR4⁺CCR6⁻, while T_H17 cells were defined as PI⁻CD4⁺CD45RA⁻CD25^{-lo}CRTH2⁻CD161⁺CCR4⁺CCR6⁺. Shaded boxes indicate sorted populations. **(D)** Bulk memory CD4⁺CD45RA⁻ T cells (Bulk) and T_H subsets were stimulated with 25 ng/mL PMA and 750 ng/mL ionomycin for 12 hours, and supernatants were collected for measurement of IL-4, IL-5, IL-13, IL-17A, IFN- γ , TNF- α and GM-CSF by ELISA. Data are from 4 independent experiments and 4 donors (mean and SEM), *p<0.05 as compared to the other T_H subsets for the same cytokine. **(E)** CD14⁺ monocytes were cultured with IL-2 alone (Mono); with IL-2 and allogeneic (filled columns) or autologous (open columns) T_H1 cells (DC_{Th1}), T_H2 cells (DC_{Th2}) or T_H17 cells (DC_{Th17}). After 6 days, each culture was analyzed for surface expression of DC-associated molecules after excluding T_H cells. Isotype controls are shown as dashed lines. Data are from 3 donors (mean and SEM).

Figure S3, related to Figure 4. Cytokine secretion profile of different DC_{Th} subsets. Monocytes (Mono) or each DC_{Th} co-culture was stimulated with 2 μ g/mL LPS at day 6. (A, B) Brefeldin A (BFA, eBioscience) was added after (A) 12 or (B) 4 hours to Mono and allogeneic

DC_{Th} co-cultures. Cells were analyzed 6 hours later for intracellular expression of IL-1 β and IL-12p40 (eBioscience) or TNF- α (BD Pharmingen) after excluding T_H cells. Data are representative of 3 independent experiments and 7 donors. (C) Supernatants from autologous co-cultures were collected for measurement of IL-1 β , IL-6, IL-10, IL-12p70, IL-23p19 and TNF- α by ELISA 36 hours after LPS stimulation. Data are from 3 independent experiments and 5 donors (mean and SEM), *p<0.05 as compared to all other subsets; †p<0.05 as compared to Mono and DC_{Th2}. (D) Prior to LPS stimulation, T cells were magnetically depleted from DC_{Th} co-cultures. Mono and DC_{Th} co-cultures were subsequently stimulated with 2 μ g/mL LPS. After 36 hours, cell-free supernatants were collected for measurement of IL-1 β , IL-6, IL-10 and TNF- α by ELISA. Data are from 3 independent experiments and 5 donors (mean and SEM), *p<0.05 as compared to Mono and DC_{Th2}, †p<as compared to DC_{Th1} and DC_{Th17}.

Figure S4. DC_{Th} subsets promote distinct T_H-cell responses. T-cell depleted DC_{Th} subsets from autologous co-cultures were stimulated with LPS for 36 hours. (A) DC_{Th} subsets were subsequently cultured with allogeneic naïve CD4⁺ T cells for six days and stimulated with 25 ng/mL PMA and 750 ng/mL ionomycin for 4 hours prior to the addition of BFA and subsequent intracellular cytokine analysis of IFN- γ , IL-13 and IL-17. Percent cytokine positive cells (% Cytokine Pos.) are shown after gating on CFSE⁺CD14⁻ T cells. Data are from 4 independent experiments and 4 donors (mean and SEM). (B) DC_{Th} subsets were subsequently cultured alone (open columns) or with allogeneic naïve CD4⁺ T cells (filled columns) for six days. Cell free supernatants were collected for measurement of IL-5, IL-13, IL-17A and IFN- γ by ELISA. Instances where the level of cytokine present was below the level of detection are indicated as not detected (N.D). Data are from 2 independent experiments and 2 donors (mean and SEM).

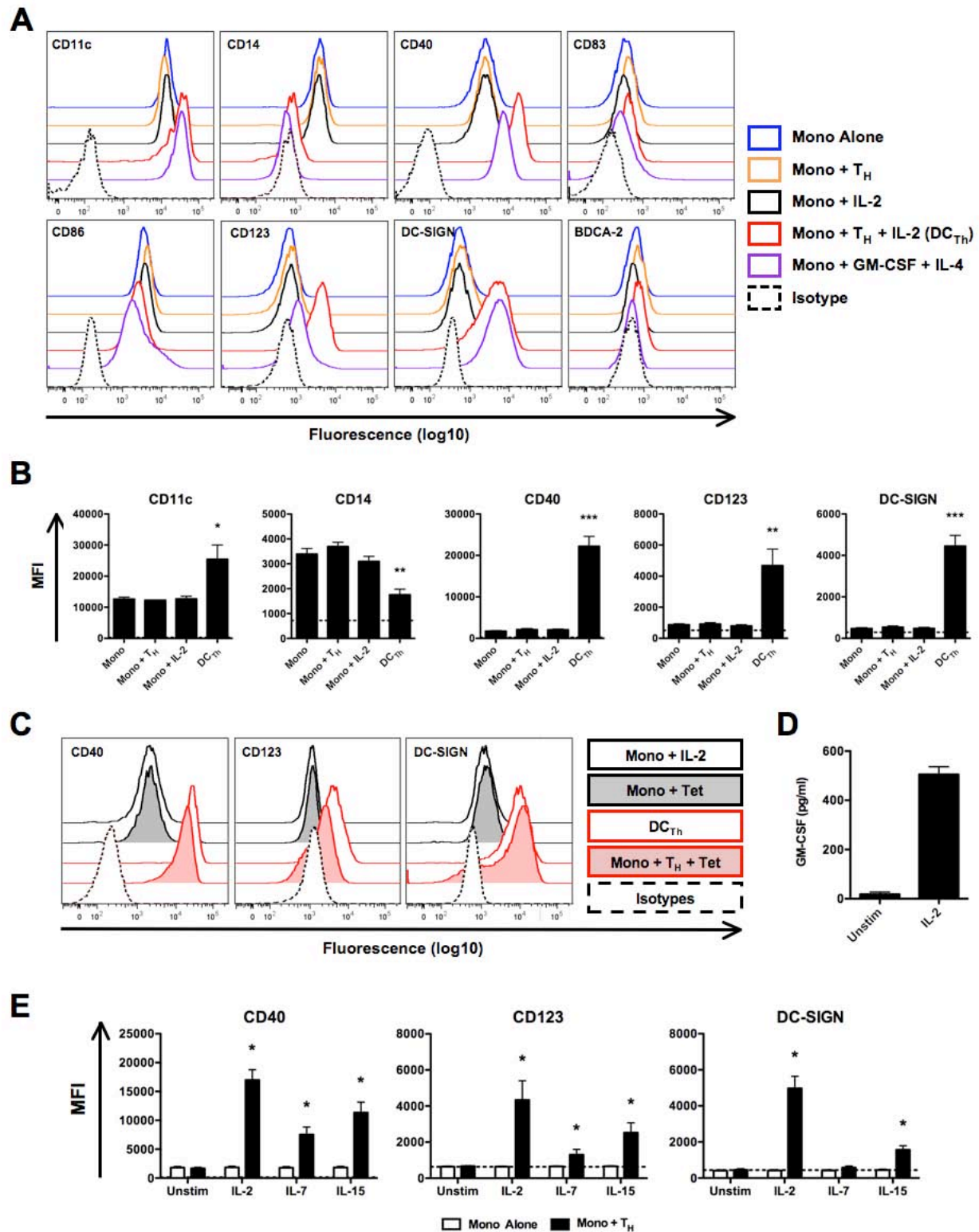


Figure S1, related to Figure1. T_H cells convert monocytes into DCs in the presence of IL-2, IL-15 or tetanus toxoid antigen

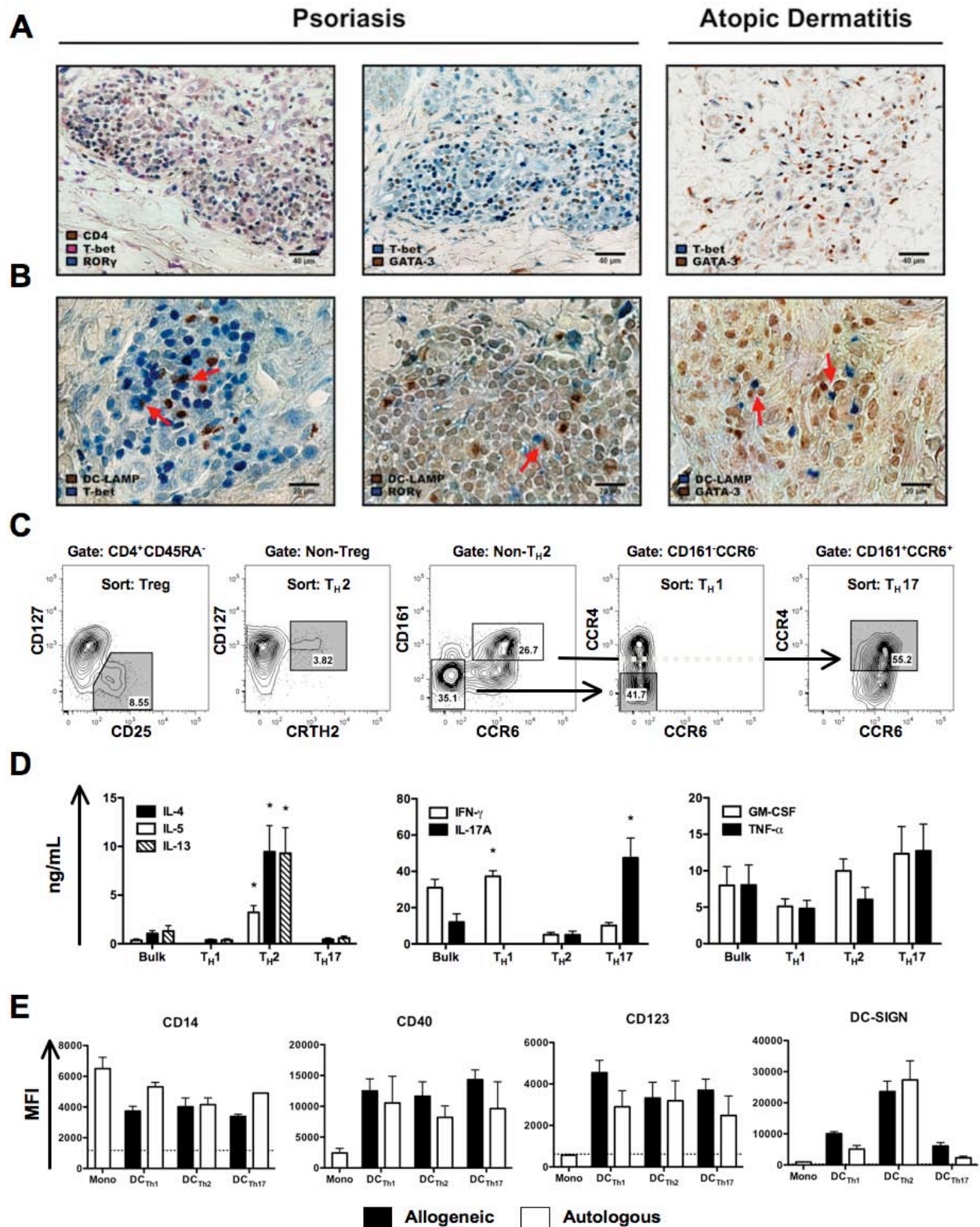


Figure S2, related to Figure 3. T_H subsets interact with monocytes and DCs in inflamed tissues, T_H subset isolation from peripheral blood and allogeneic T_H cells elicit DC formation

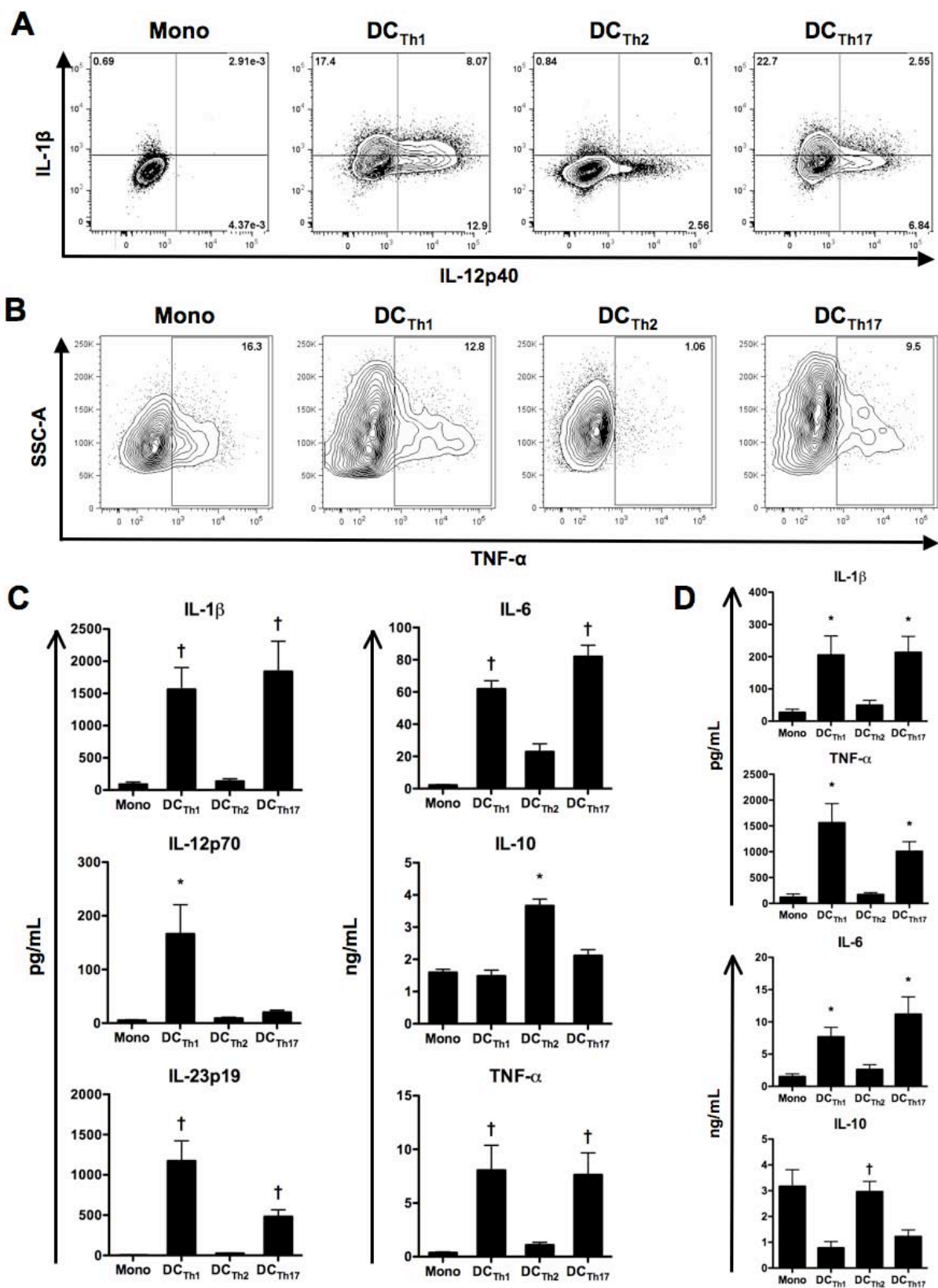
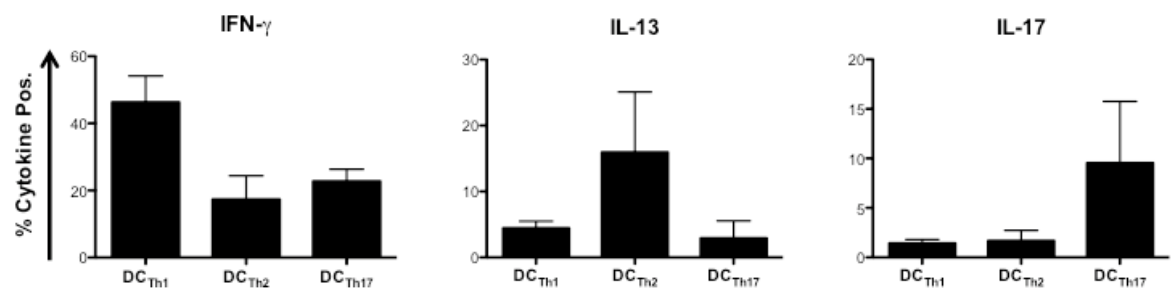


Figure S3, related to Figure 4. Cytokine secretion profile of different DC_{Th} cultures

A



B

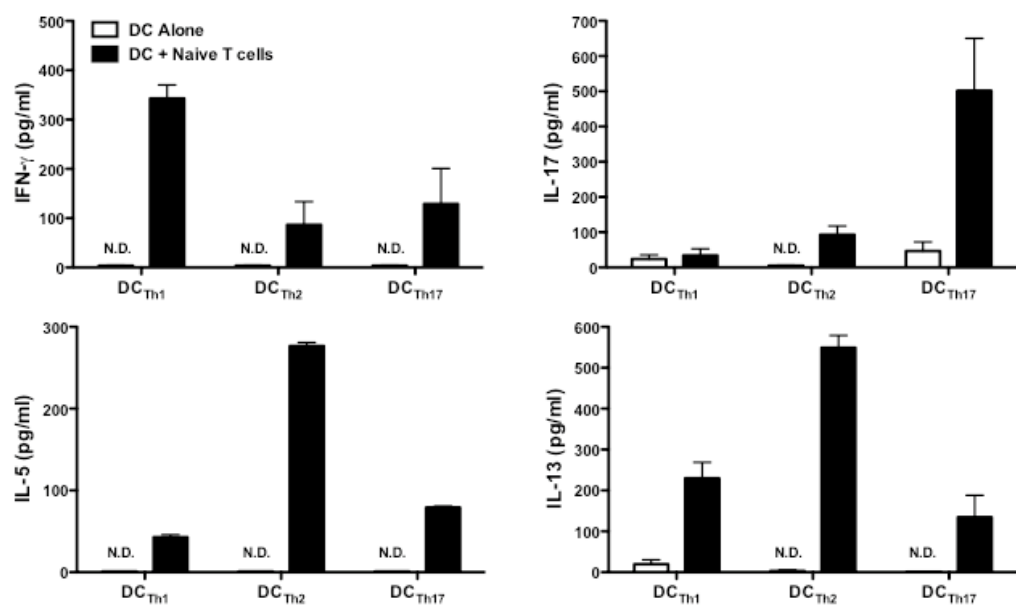


Figure S4. DC_{Th} subsets promote distinct T_H-cell responses