Skin dendritic cells induce follicular helper T cells and protective humoral immune responses

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Background: The contribution of individual subsets of dendritic cells (DCs) to generation of adaptive immunity is central to understanding immune homeostasis and protective immune responses.

Objective: We sought to define functions for steady-state skin DCs.

Methods: We present an approach in which we restrict antigen presentation to individual DC subsets in the skin and monitor the effects on endogenous antigen-specific $CD4^+$ T- and B-cell responses.

Results: Presentation of foreign antigen by Langerhans cells (LC) in the absence of exogenous adjuvant led to a large expansion of T follicular helper (T_{FH}) cells. This was accompanied by B-cell activation, germinal center formation, and protective antibody responses against influenza. The expansion of T_{FH} cells and antibody responses could be elicited by both systemic and topical skin immunization. T_{FH} cell induction was not restricted to LCs and occurred in response to antigen presentation by $CD103^+$ dermal DCs. $CD103^+$ DCs, despite inducing similar T_{FH} responses as LCs, were less efficient in induction of germinal center B cells and humoral immune responses. We also found that skin DCs are sufficient to expand $CXCR5^+$ T_{FH} cells through an IL-6– and IFN-α/β receptor–independent mechanism, but B cells were required for sustained $Bcl\text{-}6^+$ expression.

Conclusions: These data demonstrate that a major unappreciated function of skin DCs is their promotion of T_{FH} cells and humoral immune responses that potentially represent an efficient approach for vaccination. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Steady-state conditions, skin dendritic cells, T follicular helper cells, protective humoral immune responses*

The dendritic cell (DC) paradigm states that after a pathogen encounter, DCs become activated and migrate to the secondary lymphoid organs, where they initiate expansion and differentiation of pathogen-specific T-cell responses.[1] In the steady state DCs at barrier sites acquire self-antigens and benign environmental antigens. The presentation of these antigens by immature DCs results in T-cell tolerance through either activation-induced cell death or induction of anergic/regulatory T (Treg) cells.[2] This is believed to be the mechanism for the maintenance of peripheral tolerance and has been hypothesized to be a targetable pathway to induce immune tolerance for the treatment of autoimmune diseases.

In mouse skin there are 2 distinct populations of DCs that express the C-type lectin langerin: Langerhans cells (LCs), which reside in the epidermis, and $CD103^+$ DCs, which reside in the dermis.[1] We have previously demonstrated that LCs and $CD103^+$ DCs induced opposing T_H responses against *Candida albicans*. As such, LCs were necessary and sufficient for *in vivo* induction of T_H17 responses, whereas $CD103^+$ DCs were required for cross-presentation to CD8 T cells and T_H1 responses.[3] The role of $CD103^+$ DCs in cross-presentation has been supported by other studies using different models and also antigen targeting.[3-6] In the setting of contact hypersensitivity, the function of LCs and $CD103^+$ cells remains controversial.[7] 2,4-Dinitrocholrobenzene–induced tolerance was dependent on LC-induced Treg cell expansion.[8] In addition, LCs have been reported to promote deletion of antigen-specific $CD4^+$ T cells after complete Freund adjuvant peptide immunization[9] and expansion of Treg cells during *Leishmania* species infection.[10] LCs are also required for the induction of protective antibody responses after epicutaneous patch immunization.[11]

The function of langerin-expressing cells in the steady state can be examined by using intraperitoneal injection of low amounts of anti-langerin mAb/antigen conjugates. Because ligation of langerin does not activate LCs and $CD103^+$ DCs, this technique assays the effect of antigen presentation of langerin-positive DCs in the absence of exogenous adjuvants. Anti-mouse langerin/myelin oligodendrocyte glycoprotein (MOG) conjugates induced expansion of antigen-specific transgenic Treg cells and provided subsequent protection from experimental autoimmune encephalomyelitis (EAE).[12] This finding suggests that langerin-expressing DCs (LCs and $CD103^+$ DCs) promote tolerance through Treg cell expansion and is consistent with earlier studies using the DEC-205 mAb to target antigen to other DC subsets under homeostatic conditions.[13]

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Abbreviations used
CFA: Complete Freund adjuvant
CFSE: Carboxyfluorescein succinimidyl ester
DC: Dendritic cell
EAE: Experimental autoimmune encephalomyelitis
FluHA1: Influenza hemagglutinin A1
FoxP3: Forkhead box protein 3
GC: Germinal center
HRP: Horseradish peroxidase
IFNAR: IFN-α/β receptor
LC: Langerhans cells
LN: Lymph node
MHCII: MHC class II
MOG: Myelin oligodendrocyte glycoprotein
PD-1: Programmed cell death 1
PR8: Puerto Rico 8
RORγt: Retinoic acid–related orphan receptor γt
T-bet: T-box transcription factor
TCR: T-cell receptor
T_{FH}: T follicular helper
Treg: Regulatory T

The contribution of individual subsets of DCs to the generation of adaptive immunity is central to understanding immune homeostasis and protective immune responses. To date, DC function has been studied either *in vitro* or by using adoptive transfer of T-cell receptor (TCR) transgenic T cells. To determine the functional consequence of foreign antigen presentation without adjuvants exclusively by LCs or $CD103^+$ DCs, we developed an approach in which we restrict antigen presentation to these individual DC subsets and monitor the effects on endogenous antigen-specific $CD4^+$ T-cell responses by using MHC class II (MHCII) tetramers.[14] We also developed a novel system for concomitant analysis of endogenous B-cell responses. Using these techniques, we defined new functions for LCs and $CD103^+$ DCs in T follicular helper (T_{FH}) cell induction and humoral immune responses.

METHODS

Mice

huLangerin,[15] huLangerin-Cre-I-$A\beta^{fl}$,[16] and $Batf3^{-/-}$[17] mice have been previously described. CD90.1 congenic TEα *Rag1*$^{-/-}$ *Cd4* TCR transgenic mice to I-$E\alpha_{52\text{-}68}$ on the C57BL/6 background[18] were obtained from M. Jenkins (University of Minnesota), μMT and CD11c-Cre-MHCII mice were from K. Hogquist (University of Minnesota), and IFN-α/β receptor $(IFNAR)^{-/-}$ mice were from M. Mescher (University of Minnesota). $IL\text{-}6^{-/-}$ mice on the C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, Me). All experiments were performed with 6- to 12-week-old female mice. Mice were housed in microisolator cages and fed irradiated food and acidified water. The University of Minnesota Institutional Care and Use Committee approved all mouse protocols.

Antibodies and reagents

Fluorochrome-conjugated antibodies to CD4, CD11b, CD11c, CD40, B220, CD44, CD86, CD90.1, CD90.2, CD103, Gr-1, F4/80, and I-A/I-E were purchased from BioLegend (San Diego, Calif). Antibodies to forkhead box protein 3 (FoxP3), T-box transcription factor (T-bet), programmed cell death 1 (PD-1), CXCR5, Bcl-6, Gata-3, retinoic acid–related orphan receptor γt (RORγt), and Live/Dead dye were acquired from eBioscience (San Diego, Calif). Anti-langerin (929F3) was from Dendritics (Lyon, France). Anti-human/anti-mouse langerin mAb and conjugates (2G3-Eα, 2G3-2W1S, 4C7-2W1S, and 2G3–influenza hemagglutinin A1 [FluHA1]) were generated in house, as previously described.[3,19] Eα (I-$E\alpha_{52\text{-}68}$) is a well-characterized immunodominant T-cell epitope from the I-Eα MHCII molecule recognized by transgenic TEα cells in the context of I-Ab.[18] 2W1S is a variant of peptides 52 to 68 from the I-Eα chain.[14] 2W1S binds to the I-Ab MHCII molecule expressed in C57BL/6 (B6) mice and is immunogenic in this strain. FluHA-1 represents influenza hemagglutinin A1 (influenza A virus [A/Puerto Rico/8/34{H1N1}]) residues 18 to 331.[19]

Adoptive T-cell transfer

T cells were adoptively transferred, as previously described.[3] Briefly, skin-draining lymph nodes (LNs), spleens, and mesenteric LNs of TEα TCR transgenic mice were disrupted through a cell strainer, washed with sterile HBSS, and labeled with carboxyfluorescein succinimidyl ester (CFSE; Invitrogen, Carlsbad, Calif), according to the manufacturer's instructions. The cells were resuspended in sterile PBS at a concentration of 1×10^6 cells/mL, and 300 μL (3×10^5 cells) was injected intravenously into different mouse strains.

LC and $CD103^+$ DC targeting with anti-langerin antibody by means of intraperitoneal injection

Cohorts of mice were injected with either 10 μg of 2G3-AF647 or 10 μg of 4C7-AF647. Mice were killed 16 hours later, and the presence of the AF647 signal was analyzed by means of flow cytometry in cells isolated from the epidermis, LNs, and spleen, as previously described.[3] In separate experiments cohorts of mice were adoptively transferred with 3×10^5 TEα cells. Twenty-four hours later, mice were immunized by means of intraperitoneal injection of 1 μg of 2G3-Eα, and skin-draining LNs were harvested at day 4. Cohorts of mice were injected with either 1 μg of 2G3-2W1S or 1 μg of 4C7-2W1S to define endogenous T-cell responses. Six skin-draining LNs and spleens were harvested at the indicated time points and analyzed by using flow cytometry.

Topical application of antigen

Mouse back skin was wet-shaved under anesthesia by using razorblades. One hundred micrograms of 2W1S or $MOG_{35\text{-}55}$ (GenScript, Piscataway, NJ) diluted in 100 μL of endotoxin-free PBS was smeared onto the skin. A separate cohort of mice was painted with 1 μg of 2G3-2W1S.

Flow cytometry

Single-cell suspensions were obtained and stained, as previously described.[3] All the flow cytometric plots presented in this article were pregated on live (using Live/Dead stain) and singlet events. Intracellular transcription factor staining was performed with the BD Bioscience Cytofix/Cytoperm kit (BD Biosciences, San Jose, Calif), according to the manufacturer's instructions. Samples were analyzed on LSR II flow cytometers (BD Biosciences). Data were analyzed with FlowJo software (TreeStar, Ashland, Ore). Boolean gating was used to identify single transcription factor–expressing cells, and data are displayed as percentages of total values in a pie graph.

2W1S tetramer enrichment

Single-cell suspensions of LNs and spleens were stained for 1 hour at room temperature with 2W1S:$I\text{-}A^b$–streptavidin–APC tetramers (a generous gift of M. Jenkins). Samples were then enriched for bead-bound cells on magnetized columns, as previously described.[14]

Endogenous B-cell responses

Single-cell suspension of LNs from MD4, naive, and 2G3-immunized WT and huLangerin-positive mice were incubated *ex vivo* with AF647-conjugated 2G3 on ice for 30 minutes. After washing, the cells were stained for Live/Dead, CD11c, CD90.2, CD45.2, Gr-1, MHCII, B220, GL-7, and CD38 markers and analyzed on a flow cytometer.