IL-4 blocks T_H1-polarizing/inflammatory cytokine gene expression during monocyte-derived dendritic cell differentiation through histone hypoacetylation

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Background: Whereas recent research has characterized the mechanism by which dendritic cells (DCs) induce T_H1/T_H17 responses, the functional specialization enabling DCs to polarize T_H2 responses remains undefined. Because IL-4 is essential during T_H2 responses not only by acting on $CD4^+$ T cells through the activation of GATA-3 but also by regulating IgE class-switching, epithelial cell permeability, and muscle contractility, we hypothesized that IL-4 could also have a role in the conditioning of DCs during T_H2 responses.

Objective: We sought to analyze whether IL-4 exerts an immunomodulatory function on DCs during their differentiation, leading to their functional specialization for the induction of T_H2 responses.

Methods: Monocyte-derived DCs (moDCs) conditioned by IL-4 during their differentiation (IL-4–conditioned moDCs [IL-4–moDCs]) were analyzed for T_H1-polarizing/inflammatory cytokine production in response to Toll-like receptor stimulation. The acetylation level of the promoters of the genes encoding these cytokines was analyzed by using chromatin immunoprecipitation. Gene expression profiling of IL-4–moDCs was defined by using mouse genome microarrays. IL-4–moDCs were tested for their capacity to induce house dust mite–mediated allergic reactions.

Results: Our data suggest that IL-4 inhibits T_H1-polarizing/inflammatory cytokine gene expression on IL-4–moDCs through the deacetylation of the promoters of these genes, leading to their transcriptional repression. Microarray analyses confirmed that IL-4 upregulated T_H2-related genes as eosinophil-associated ribonucleases, eosinophil/basophil chemokines, and M2 genes. IL-4 licensed moDCs for the induction of T_H2 responses, causing house dust mite–mediated allergic airway inflammation.

Conclusion: This study describes a new role for IL-4 by demonstrating that moDCs are conditioned by IL-4 for the induction of T_H2 responses by blocking T_H1-polarizing/inflammatory cytokine production through histone hypoacetylation and upregulating T_H2-related genes. (J Allergy Clin Immunol 2013;132:1409-19.)

Key words: *T_H2 responses, IL-4, dendritic cells, monocyte-derived dendritic cells, house dust mite–induced allergic reactions*

T_H2 responses are crucial for defense against infections by helminths and trigger allergic reactions that can lead to severe clinical disorders, such as asthma or anaphylaxis, and ultimately to death. The induction of T_H2 responses relies on specialized dendritic cells (DCs) that present peptides from pathogen- or allergen-derived antigens to unpolarized $CD4^+$ T cells, leading to the T_H2 polarization of these antigen-specific $CD4^+$ T cells. This process is dependent on the transcription factor GATA-3, controlling the production by T_H2-polarized $CD4^+$ T cells of T_H2 cytokines that are responsible for the initiation and regulation of T_H2 responses.[1]

Whereas recent research has allowed us to define the mechanism by which DCs induce T_H1/T_H17 responses, the mechanistic basis for the functional specialization enabling DCs to polarize T_H2 responses has remained elusive. In contrast to T_H1/T_H17 polarization, induction of T_H2 differentiation does not appear to depend on the production of T_H2-polarizing cytokines by DCs. Recent data support that promoting a T_H2 genetic program involves a specific antigen presentation process by T_H2-polarizing DCs (T_H2-DCs) that requires the presence of IL-4 to activate GATA-3, the expression of costimulatory molecules by T_H2-DCs for a productive $CD4^+$ T-cell activation, and the blockade of T_H1-polarizing cytokine production by T_H2-DCs.

The blockade of T_H1-polarizing cytokine production by T_H2-DCs appears essential for T_H2 response induction because recent evidence supports that helminth-derived compounds can activate Toll-like receptors (TLRs) on DCs and thus induce T_H1-polarizing cytokine production[2] and that T_H1-polarizing TLR ligands, such as LPS, can be present in allergenic microscopic arthropods or pollen grains.[3] However, the mechanism by which T_H1-polarizing cytokine production is blocked in T_H2-DCs during T_H2 responses is largely unknown.

Because IL-4 functions as a key mediator of T_H2 responses not only at the $CD4^+$ T-cell level but also by regulating IgE class-switching, epithelial cell permeability, and muscle cell contractility,[4] we hypothesized that IL-4 could also exert a role on the licensing of DCs for the induction of T_H2 responses. Our data demonstrate that the presence of IL-4 during monocyte-derived DC (moDC) differentiation blocked the potential of LPS-stimulated moDCs to produce T_H1-polarizing cytokines and inflammatory mediators while allowing a correct costimulatory molecule upregulation. Our results support that IL-4 blocks T_H1-polarizing/inflammatory cytokine gene expression

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Abbreviations used

AAMΦ: Alternatively activated macrophage
BMDC: Bone marrow–derived dendritic cell
BMMΦ: Bone marrow macrophage
cDC: Conventional dendritic cell
ChIP: Chromatin immunoprecipitation
cM-LN: Caudal mediastinal lymph node
C-moDC: Control moDC
C-moMΦ: Control moMΦ
CTL: Cytotoxic T lymphocyte
DC: Dendritic cell
Ear: Eosinophil-associated ribonuclease
HDAC: Histone deacetylase
HDM: House dust mite
IL-4–DC: IL-4–conditioned DC
IL-4–moDC: IL-4–conditioned moDC
IL-4–moMΦ: IL-4–conditioned moMΦ
iNOS: Inducible nitric oxide synthase
moDC: Monocyte-derived DC
moMΦ: Monocyte-derived macrophage
NO: Nitric oxide
PPARγ: Peroxisome proliferator-activated receptor γ
qPCR: Quantitative PCR
Stat: Signal transducer and activator of transcription
T_H2-DC: T_H2-polarizing DC
TLR: Toll-like receptor
TSA: Trichostatin A

by causing the deacetylation of the promoters of these genes, leading to their transcriptional repression. In addition, microarray analyses revealed that IL-4 promoted the upregulation on moDCs of genes related to T_H2 responses, such as eosinophil-associated ribonucleases (Ears), eosinophil/basophil attractants chemokines, and M2 genes. Finally, *in vivo* experiments revealed the potential of IL-4 to license DCs for the induction of T_H2-polarized immune responses *in vivo* during allergic airway inflammation reactions induced by house dust mite (HDM) allergens.

METHODS

Mice

C57BL/6 mice were purchased from Harlan (Bicester, United Kingdom). Bone marrow from LXR-deficient mice (*Nr1h3*$^{-/-}$, *Nr1h2*$^{-/-}$ mice generated by Dr Mangelsdorf) was provided by A. Castrillo (Universidad de Las Palmas, Gran Canaria, Spain), from LysM-Cre-PPARγ-deficient mice by L. Nagy (University of Debrecen, Hungary), and from signal transducer and activator of transcription (Stat) 6–deficient mice by P. Murray (St Jude Children's Research Hospital, Memphis, Tenn). All the experiments were approved by the Animal Care Committee of the Centro Nacional de Biotecnología (CNB/CSIC).

Monocyte isolation and *in vitro* moDC and monocyte-derived macrophage differentiation

Monocytes were isolated and differentiated into moDCs and monocyte-derived macrophages (moMΦs), as described in the Methods section in this article's Online Repository at www.jacionline.org.

Differentiation of alternatively activated macrophages

Bone marrow cells were cultured for 7 days in nontreated, cultured 60-mm Petri dishes in complete Dulbecco modified Eagle medium supplemented with 20% FCS and 20 ng/mL M-CSF (PeproTech, London, United Kingdom) at 37°C and 5% CO_2; these cultures contained more than 95% CD11b$^+$F4/80$^+$ bone marrow macrophages (BMMΦs). Alternatively activated macrophages (AAMΦs) were obtained after BMMΦ culture for 24 hours in the presence of 20 ng/mL IL-4 and subsequently stimulated with 1 μg/mL LPS for the indicated times. AAMΦs were analyzed by means of flow cytometry with a FACSCalibur flow cytometer (BD Biosciences, San José, Calif) after double staining with fluorescein-conjugated anti-CD11b and phycoerythrin-conjugated anti-F4/80.

Analysis of cytokine production

Cytokine production by moDCs, moMΦs, and AAMΦs was analyzed at the mRNA level, protein level, or both by using quantitative PCR (qPCR) and ELISA, respectively, as described in the Methods section and Table E1 in this article's Online Repository at www.jacionline.org.

Analysis of histone acetylation

Histone acetylation was analyzed by means of chromatin immunoprecipitation (ChIP), as described in the Methods section this article's Online Repository.

Gene expression profiling

The gene expression profiling of moDCs and IL-4–conditioned moDCs (IL-4–moDCs) was performed by using mouse whole-genome microarrays, as described in the Methods section in this article's Online Repository.

Induction of moDC-mediated allergic airway responses against HDM

Induction of moDC-mediated allergic airway inflammation was performed according to a protocol modified from that reported by Lambrecht's group.[5] Control moDCs (C-moDCs; 5×10^4) or IL-4–moDCs (5×10^4) incubated for 16 hours with 30 μg/mL HDM extracts (Greer Laboratories, Lenoir, NC) were transferred intraperitoneally into C57BL/6 mice. On days 7 and 11, mice were anesthetized with ketamine/xylazine and challenged intranasally with 10 μg of HDM in a total volume of 40 μL of PBS. On day 14, mice were killed, and the lungs and caudal mediastinal lymph nodes (cM-LNs) were collected. Bronchoalveolar lavage was performed with 3×1 mL EDTA-containing PBS, and eosinophil infiltration was analyzed by using fluorescence-activated cell sorting after staining with phycoerythrin-conjugated anti–Siglec-F (BD PharMingen, San Diego, Calif). cM-LN cells were restimulated with 15 μg/mL HDM for 96 hours in 24-well plates at 1×10^6 cells/well, and cytokine production was measured in the supernatants with BD OptEIA ELISA kits.

RESULTS

Blockade of LPS-triggered T_H1-polarizing cytokine production on moDCs differentiated in the presence of IL-4

To explore whether IL-4 has a role in blocking the production of T_H1-polarizing cytokines, we analyzed the responsiveness to LPS of moDCs differentiated in the presence of IL-4. Culture of bone marrow Ly-6C^{high} monocytes with GM-CSF for 24 hours led to their differentiation into CD11c$^+$/MHCII$^+$ C-moDCs, expressing intermediate levels of the costimulatory molecules CD86 and CD40. Monocyte differentiation with GM-CSF in the presence of IL-4 for 24 hours generated IL-4–moDCs, which expressed higher levels of CD86 and CD40 than C-moDCs (Fig 1, *A*). Compared with C-moDCs, IL-4–moDCs displayed similar forward scatter and side scatter values, lower levels of F4/80 and CD11b, and more complex dendritic-like cytoplasmic