Control of dendritic cell maturation and function by triiodothyronine

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ABSTRACT **Accumulating evidence indicates a functional crosstalk between immune and endocrine mechanisms in the modulation of innate and adaptive immunity. However, the impact of thyroid hormones (THs) in the initiation of adaptive immune responses has not yet been examined. Here we investigated the presence of thyroid hormone receptors (TRs) and the impact of THs in the physiology of mouse dendritic cells (DCs), specialized antigen-presenting cells with the unique capacity to fully activate naive T cells and orchestrate adaptive immunity. Both immature and lipopolysaccharide-matured bone marrow-derived DCs expressed TRs at mRNA and protein levels, showing a preferential cytoplasmic localization. Remarkably, physiological levels of triiodothyronine (T3) stimulated the expression of DC maturation markers (major histocompatability complex II, CD80, CD86, and CD40), markedly increased the secretion of interleukin-12, and stimulated the ability of DCs to induce naive T cell proliferation and IFN-γ production in allogeneic T cell cultures. Analysis of the mechanisms involved in these effects revealed the ability of T3 to influence the cytoplasmic-nuclear shuttling of nuclear factor-κB on primed DCs. Our study provides the first evidence for the presence of TRs on bone marrow-derived DCs and the ability of THs to regulate DC maturation and function. These results have profound implications in immunopathology, including cancer and autoimmune manifestations of the thyroid gland at the crossroads of the immune and endocrine systems.—Mascanfroni, I., Montesinos, M., Susperreguy, S., Cervi, L., Ilarregui, J. M., Ramseyer, V. D., Masini-Repiso, A. M., Targovnik, H. M., Rabinovich, G. A., Pellizas, C. G. Control of dendritic cell maturation and function by triiodothyronine. *FASEB J.* 22, 1032–1042 (2008)**

Key Words: adaptive immunity · thyroid hormones · antigen presentation

THE ENDOCRINE AND IMMUNE SYSTEMS are interrelated *via* a bidirectional network in which hormones affect immune function, and, in turn, immune responses are reflected in neuroendocrine changes. This bidirectional communication is possible because both systems share common ligands (hormones and cytokines) and their specific receptors (1).

Thyroid hormones (THs) play critical roles in differentiation, growth, and metabolism (2). Despite the role assigned for THs in maintaining immune system homeostasis (3–5), the study of TH effects on cells of the immune system received relatively less attention than the study of effects exerted by other hormones of the hypothalamus-pituitary-adrenal (HPA) axis (6, 7). Interactions between hormones from the pituitary and thyroid glands and the immune system were revealed mainly by the presence of specific receptors for thyrotropic and thyroid hormones on lymphocytes or by the frequent immune alterations associated with physiological or pathological fluctuations of THs (1, 8). Evidence arising from analysis of lymphocyte development and function in mice with genetic defects in the expression of THs or thyroid hormone receptors (TRs) suggested that THs may play an essential role in maintaining immune system homeostasis in response to environmental changes or stress-mediated immunosuppression (4, 6, 8). Whereas studies on the effects of THs in the control of immune responses were mainly conducted on effector B and T lymphocytes, the role of THs in the initiation of adaptive immune responses still remains uncertain. Dendritic cells (DCs) are highly specialized antigen-presenting cells (APCs) that recognize, process, and present antigens to naive T cells for the induction of antigen-specific immune responses (9). Because DCs are pleiotropic modulators of T cell activity capable of orchestrating adaptive immunity and

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doi: 10.1096/fj.07-8652com

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are endowed with exquisite plasticity, manipulation of their function to favor the induction of DCs with immunogenic or tolerogenic properties could be exploited to positively or negatively regulate adaptive immune responses (10). Several factors may influence the decision of DCs to become immunogenic or tolerogenic, including the maturational and activation status, and the cytokine milieu (including growth factors, neuropeptides, and hormones) at sites of T cell activation and inflammation (11, 12).

After *in vitro* or *in vivo* exposure to lipopolysaccharides (LPSs) or other bacterial products, DCs undergo activation and maturation through different signaling pathways including mitogen-activated protein kinase kinase 1/extracellular signal-regulated kinase, which favors DC survival, and the NF-κB pathway, which allows for DC maturation (13). Signaling through NF-κB also determines the increased expression of major histocompatability complex (MHC) II and costimulatory molecules, release of proinflammatory cytokines and chemokines, and DC migration and recruitment. This coordinated process leads to sustained T cell stimulatory capacity and interleukin (IL)-12 release, which result in the induction of protective immunity (13).

The classic genomic actions of THs are mediated by nuclear TRs that act as hormone-inducible transcription factors. Several TRα and TRβ isoforms are encoded by the *TRA* and *TRB* genes, respectively. The $TR\alpha_1$, $TR\alpha_2$, $TR\beta_1$, and $TR\beta_3$ isoforms are widely expressed, whereas $TR\beta_2$ is predominantly restricted to the hypothalamus-pituitary (HP) axis (14, 15). However, nongenomic actions of THs have also been described at the level of the plasma membrane, cytoskeleton, cytoplasm, and distinct organelles of mammalian cells (8, 16).

Despite major advances in understanding of the interplay between distinct hormones and the immune cell network (11, 12, 17–19), the role of THs in the initiation of adaptive immunity still remains uncertain (20). In the present study we provide the first evidence of the expression of TRs on bone marrow-derived DCs and their striking localization in the cytoplasmic compartment of immature and mature DCs. Furthermore, we demonstrate the effects of THs on DC maturation and IL-12 secretion and the capacity of these cells to stimulate T cell responses.

MATERIALS AND METHODS

Mice

Female C57BL/6 (B6; H-2b) and BALB/c (H-2d) mice were obtained from Ezeiza Atomic Center (Buenos Aires, Argentina). Mice were maintained under specific pathogen-free conditions and used at 6–10 wk of age. Animal protocols complied with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health and local institutional animal care committee guidelines.

DC preparation and culture

DCs were obtained as described by Inaba *et al.* (21). Briefly, bone marrow progenitors were collected from the femurs of 4- to 6-wk-old female C57BL/6 mice and cultured in RPMI 1640 10% fetal calf serum (FCS) depleted of THs by treatment with resin AG-1-X8 (Bio-Rad Laboratories, Hercules, CA, USA) in the presence of granulocyte-macrophage colony-stimulating factor from supernatant of the J558 cell line and fed every 2 days. At day 10 of cell culture, >85% of the harvested cells expressed MHC class II, CD40, CD80, and CD11c, but not Gr-1. Immature DCs (iDCs) were stimulated with LPS (100 ng/ml; *Escherichia coli* strain 0111:B4; Sigma-Aldrich, St. Louis, MO, USA) for 18 h to obtain mature DCs (mDCs). Alternatively, iDCs were incubated with 3,3′,5-triiodo-L-thyronine (T3) (0.05–500 nM) or T3 (5 nM) plus LPS (100 ng/ml) for 18 h. Afterward, DCs were collected and washed. T3 was purchased from Sigma-Aldrich and prepared according to the manufacturer's recommended protocol. To rule out endotoxin contamination of the T3 preparation, the same set of experiments were performed in the presence of polymyxin B (10 μg/ml; Sigma-Aldrich). In addition, we checked the endotoxin content of the T3 preparation after reconstitution, which raised levels <0.03 endotoxin unit/ml (limit of detection), by using the Limulus amebocyte lysate assay (Sigma-Aldrich).

Flow cytometric analysis of DC phenotype

DCs were washed twice with PBS supplemented with 2% FCS and resuspended in 10% FCS-PBS. Cells were then incubated with the following fluorochrome-conjugated monoclonal antibodies (mAbs) for 30 min at 4°C: fluorescein isothiocyanate (FITC)-anti-CD11c, phycoerythrin (PE)-anti-IA/IE (MHC II), PE-anti-CD40, PE-anti-CD80, and PE-anti-CD86 (all from BD PharMingen, San Diego, CA, USA). Cells were then processed and analyzed in an Ortho Cytoron Absolute flow cytometer (Ortho Diagnostic Systems, Raritan, NJ, USA) using FlowJo software (Tree Star, Ashland, OR, USA).

Transfection of COS-7 cells with $TR\beta_1$ expression vector

COS-7 cells do not express TRs and were used as a negative control for Western blot analysis. As a positive control, COS-7 cells were transiently transfected with $TR\beta_1$ expression vector as described previously (22). Briefly, cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen/Life Technologies Corporation, Carlsbad, CA, USA) supplemented with 10% FCS, antibiotics, and glutamine at 37°C in a humidified atmosphere (5% CO_2). Cells (3×10^5) were seeded in a 60-mm dish 24 h before transfection. Cells were transfected with 5 μg of pCDM8-$TR\beta_1$ expression vector by calcium phosphate coprecipitation as described previously (23). After 2 h of incubation with the precipitate, cells were shocked with 15% glycerol in PBS. Twenty-four hours after transfection, cells were harvested and lysed as described below to obtain cell lysates for Western blot analysis.

Reverse transcription (RT) and polymerase chain reaction (PCR)

Cells were homogenized with TRIzol, and RNA extraction was performed according to the manufacturer's recommended protocol based on the Chomczynski and Sacchi method (24). mRNA was reverse transcribed and amplified by PCR essentially as described (22) with minor modifications. Briefly, 1 μg of total RNA was incubated with 0.1 μM degenerated oligo dT12VG primers at 65°C. After 3 min on ice, the following

reagents were added: 20 U of RNase inhibitor (RNaseOUT; Promega, Madison, WI, USA), 4 μl of 5× RT buffer [250 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$ and 10 mM dithiothreitol (DTT)], 0.5 mM concentrations of each dNTP, and 200 U of Moloney murine leukemia virus reverse transcriptase (Promega). After 1 h at 37°C, remnant reverse transcriptase was inactivated at 95°C for 5 min. Expressions of $TR\alpha_1$ and $TR\beta_1$ mRNAs were normalized using the housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Primers (Sigma-Aldrich, Buenos Aires, Argentina) were designed to distinguish cDNA and genomic DNA/pseudogenes (25) to amplify a 505-bp band for GAPDH, a 371-bp band for $TR\alpha_1$, and a 590-bp band for $TR\beta_1$ mRNAs as follows: 5′-CAGTGCCAGGAATGTCGCTTTAAG-3′ ($TR\beta_1$ forward), 5′-ACTCTGGTAATTGCTGGTGTGATGAT-3′ ($TR\beta_1$ reverse), 5′-TTCAGCGAGTTTACCAAGATCATCAC-3′ ($TR\alpha_1$ forward), 5′-TTAGACTTCCTGATCCTCAAAGACCTC-3′ ($TR\alpha_1$ reverse), 5′-GAAGGTGAAGGTCGGAGTCAACG-3′ (GAPDH forward), and 5′-GATACCAAGTTGTCATGGATGACCTT-3′ (GAPDH reverse).

PCR was carried out in a 20 μl final volume using 1.5 mM $MgCl_2$, 4 μl of 5× PCR buffer, 1 U of *Taq* polymerase (Promega), 0.25 mM concentrations of each dNTP (Promega), and 2 μl of RT product. A negative control (sterile water instead of RT product) was included in each PCR run. The PCR amplification was performed on a ICycler PCR System (Bio-Rad). The thermal profile was 94°C for 5 min (34 cycles for TRs and 26 cycles for GAPDH); 94°C for 1 min, 56°C for 1 min, 72°C for 2 min, and 72°C for 10 min. The mass of total RNA for RT, the number of cycles for PCR, and $MgCl_2$, primer, and dNTP concentrations were selected experimentally (data not shown). RT-PCR products were resolved by electrophoresis in 2% agarose gels followed by ethidium bromide staining.

Preparation of total, nuclear, and cytoplasmic extracts

To obtain total cell lysates of COS-7 and DCs, 3×10^6 cells were resuspended in 50–200 μl of RIPA buffer, disrupted by passages through a 25-G needle, and incubated on ice for 30 min, followed by removal of DNA and cell debris by centrifugation at 10,000 *g* for 20 min at 4°C. Nuclear and cytoplasmic DC extracts were obtained by subcellular fractionation essentially as described by Schreiber *et al.* (26). The supernatant containing cytoplasm was collected and frozen at −70°C or used immediately. The nuclear pellet was resuspended in 50 μl of ice-cold buffer (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM PMSF), and the tube was vigorously rocked at 4°C for 15 min on a shaking platform. The nuclear extract was centrifuged for 5 min in a Microfuge at 4°C, and the supernatant was frozen in aliquots at −70°C or used immediately. Rat liver nuclear extracts used as positive controls were obtained as described previously (22).

Western blot analysis

Total cell lysates and nuclear and cytoplasm extracts of DCs (40 μg), COS-7 cells (40 μg; negative control), COS-7 cells overexpressing $TR\beta_1$, and rat liver nuclear extract (40 μg) (positive controls) were used for immunodetection of $TR\alpha_1$ (47 kDa) and $TR\beta_1$ (55 kDa). The rabbit anti-TR polyclonal antibody (Ab) (FL-408, sc-772; Santa Cruz Biotechnology, Santa Cruz, CA, USA), which cross-reacts with chicken, mouse, rat, and human $TR\alpha_1$ and $TR\beta_1$, was used at a 1:2000 dilution. NF-κB (p65) expression was evaluated with an anti-p65 Ab (sc-8008; Santa Cruz Biotechnology). Anti-α-tubulin (Clone B-5-1-1) and antihistone deacetylase 1 (HDAC1) Abs (Sigma-Aldrich) were used to control the purity of the subcellular fractions. Equal protein loading was checked using an anti-β-actin mAb (sc-1616; Santa Cruz Biotechnology). Western blot analysis was performed as described (22) and revealed using the enhanced chemiluminescence protocol (NEL-100; DuPont NEN Research Products, Wilmington, DE, USA).

Immunofluorescence microscopy

Bone marrow-derived DCs were generated as described above and cultured on coverslips for 3 days. After treatments, cells were fixed in 4% paraformaldehyde, permeabilized in 0.25% Triton X-100 in PBS, blocked for 1 h in PBS (pH 7.4) plus 3% BSA fraction V (Fisher), incubated with a primary Ab (mouse anti-$TR\beta_1$ sc-738; Santa Cruz Biotechnology) at a dilution of 1:100 for 1 h, washed, and further incubated with an Alexa conjugated goat anti-mouse secondary Ab (Molecular Probes, Inc., Eugene, OR, USA) for 1 h at a dilution of 1:1000. Nuclei were stained with 4,6-diamidino-2-phenylindole for 5 min, and samples were washed in PBS and mounted on glass slides using Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL, USA) for examination using a Leica DM IRBE inverted microscope (Hamamatsu Corporation, Bridgewater, NJ, USA). Images were captured using Openlab 3.1 software (Improvision, Lexington, MA, USA) at a magnification of ×1000.

Allogeneic T cell cultures

Allogeneic T cell cultures were performed to assess the ability of DCs to stimulate allogenic splenocytes *in vitro* as described (27). Briefly, allogenic splenocytes (1×10^5 cells/well, responder cells) were incubated for 3 days with irradiated DCs (30 Gy, stimulator cells) at a ratio of 1:10 to 1:40 DCs/splenocyte) in 96-well round-bottom plates. On day 2, 0.5 μCi (0.0185 MBq)/well of [^{3}H]thymidine (Amersham Life Sciences, Buckinghamshire, UK) was incorporated into each well for 18 h. Proliferation was determined as counts per minute of triplicate determinations.

Cytokine determination

IL-12p70, IL-10, and IFN-γ detection was performed in cell culture supernatants using standard capture ELISAs. Coating Abs included a rat anti-mouse IL-12p70 mAb (clone C15.6; PharMingen), rat anti-mouse IL-10 mAb (clone JES5–2A5; PharMingen), and rat anti-mouse IFN-γ mAb (clone R4–6A2; PharMingen). Detection Abs included biotinylated rat anti-mouse IL-12p70 mAb (clone C17.8; PharMingen), biotinylated rat anti-mouse IL-10 mAb (clone SXC-1; PharMingen), and biotinylated rat anti-mouse IFN-γ mAb (clone XMG1.2; PharMingen). Streptavidin-horseradish peroxidase and 3-ethylbenzthiazoline-6-sulfonic acid (Sigma-Aldrich) were used as enzyme and substrate, respectively. Intracellular cytokine was detected by flow cytometry as described previously (28) using PE-conjugated anti-IL-12, PE-conjugated anti-IL-5, and FITC-conjugated anti-IFN-γ mAbs (all from BD PharMingen). Briefly, cells were exposed to brefeldin A (10 μg/ml; Sigma) for the last 6 h of cell culture. Allogeneically activated splenocytes were labeled with PE- or FITC-conjugated anti-CD4 mAbs (BD Biosciences, San Jose, CA, USA) for 30 min. Cells were then fixed with 1% paraformaldehyde, treated with fluorescence-activated cell sorter permeabilizing solution (BD Biosciences) and stained with an optimal concentration of anticytokine mAb or an appropriate isotype control mAb (all from BD Biosciences). Cells (at least 10,000 viable cells) were then analyzed in an Ortho Cytoron Absolute flow cytometer using FlowJo software.

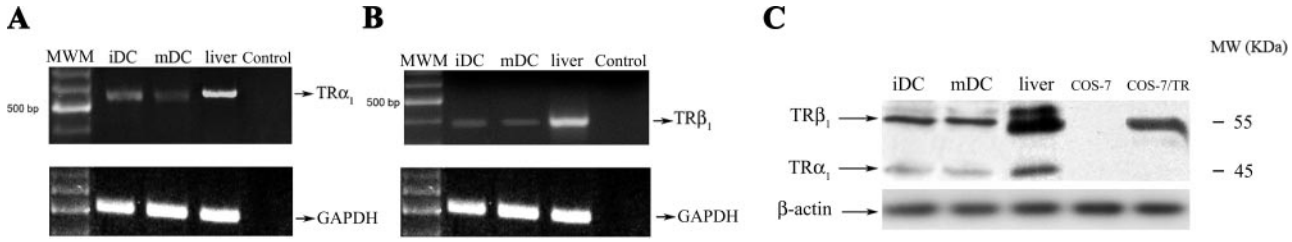

Figure 1. Expression of TRs in iDCs and mDCs. *A, B*) Detection of mRNA for $TR\alpha_1$ (*A*) and $TR\beta_1$ (*B*) by RT-PCR analysis of total RNA of iDCs and LPS-matured DCs. Total RNA from rat liver was run in parallel as a positive control. The expression of $TR\alpha_1$ and $TR\beta_1$ mRNAs was normalized with the housekeeping GAPDH mRNA. RT-PCR products were resolved by electrophoresis in 2% agarose gels followed by ethidium bromide staining. *C*) Western blot analysis of total cell lysates of iDCs, mDCs, COS-7 cells (negative control), COS-7/TR transfected cells (positive $TR\beta_1$ control), and nuclear extracts from rat liver (positive $TR\beta_1$ and $TR\alpha_1$ control). Samples were separated by 10% SDS-PAGE, transferred onto nitrocellulose membranes, and blotted with anti-TR Abs. Equal loading was checked using an anti-β-actin mAb. Representative of three independent experiments with similar results are shown.

Statistical analysis

Statistical analysis was performed using Student's paired *t* test. A Wilcoxon nonparametric test for paired data was used to determine the significance of the time-response curves. Values of $P < 0.05$ were considered statistically significant. To adjust the significance level for multiple comparisons, a Bonferroni correction was applied using a corrected significance level of 0.017. All experiments were performed at least in triplicate.

RESULTS

TRs are highly represented in iDCs and mDCs and are confined mainly to the cytosolic compartment of these cells

To investigate the role of THs in the physiology of DCs and their influence in the initiation of adaptive immune responses, we first examined the expression and subcellular localization of TRs in bone marrow-derived DCs. Both iDCs and LPS-matured DCs (mDCs) expressed $TR\alpha_1$ and $TR\beta_1$ mRNA (**Fig. 1*A, B***), although at a lesser extent than rat liver tissue, which was used as a positive control. Moreover, expression of TRs was confirmed at the protein level by Western blot analysis of iDCs and mDCs (Fig. 1*C*). We could not find significant differences in TR expression between iDCs and mDCs (Fig. 1*A–C*). However, both DC subsets showed higher expression of $TR\beta_1$ than $TR\alpha_1$ (Fig. 1*C*), similar to B and T lymphocytes (29, 30).

To examine the subcellular compartmentalization of TRs on iDCs and mDCs, we performed Western blot analysis of subcellular DC fractions. Strikingly, the expression of cytoplasmic $TR\beta_1$ was markedly increased compared with that of nuclear $TR\beta_1$ in both iDCs and mDCs (**Fig. 2**). The purity of subcellular fractions was checked by using α-tubulin and HDAC1 as specific markers of cytoplasmic and nuclear fractions, respectively. In addition, immunofluorescence staining showed bright cytoplasmic labeling of iDCs and mDCs, whereas nuclear staining was scarce and diffuse, in broad agreement with Western blot analysis (**Fig. 3**). Of importance, no substantial differences were observed in $TR\beta_1$ staining between both DC subsets.

T3 induces the maturation of bone marrow-derived iDCs

To determine the physiological relevance of TR expression in DCs, we cultured iDCs in the presence of LPS or T3 and evaluated their cell surface pheno-

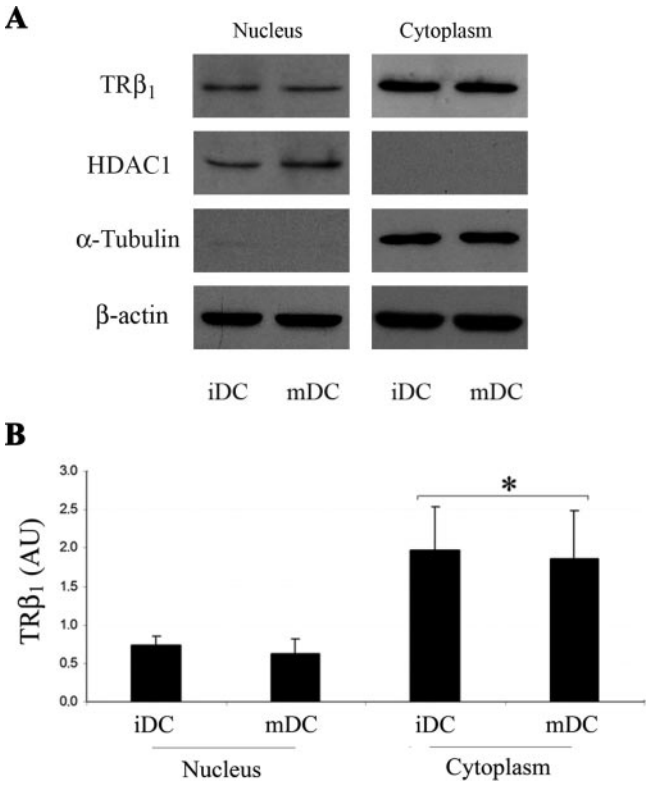

Figure 2. Subcellular compartmentalization of TRs in the nuclear and cytoplasmic fractions of iDCs and mDCs. *A*) Western blot analysis of nuclear and cytoplasmic fractions of iDCs and mDCs for detection of $TR\beta_1$. Anti-α-tubulin and HDAC1 Abs were used to check the purity of cytoplasmic and nuclear fractions, respectively. β-Actin was used as a control of equal protein loading (lower panel). *B*) Densitometric analysis of immunoreactive protein bands. Results are expressed as arbitrary units (AU) calculated from the densitometric analysis of $TR\beta_1$ signal normalized to β-actin. Data are presented as mean ± SD. $^*P < 0.01$ *vs.* nucleus. Blots are representative of six independent experiments.

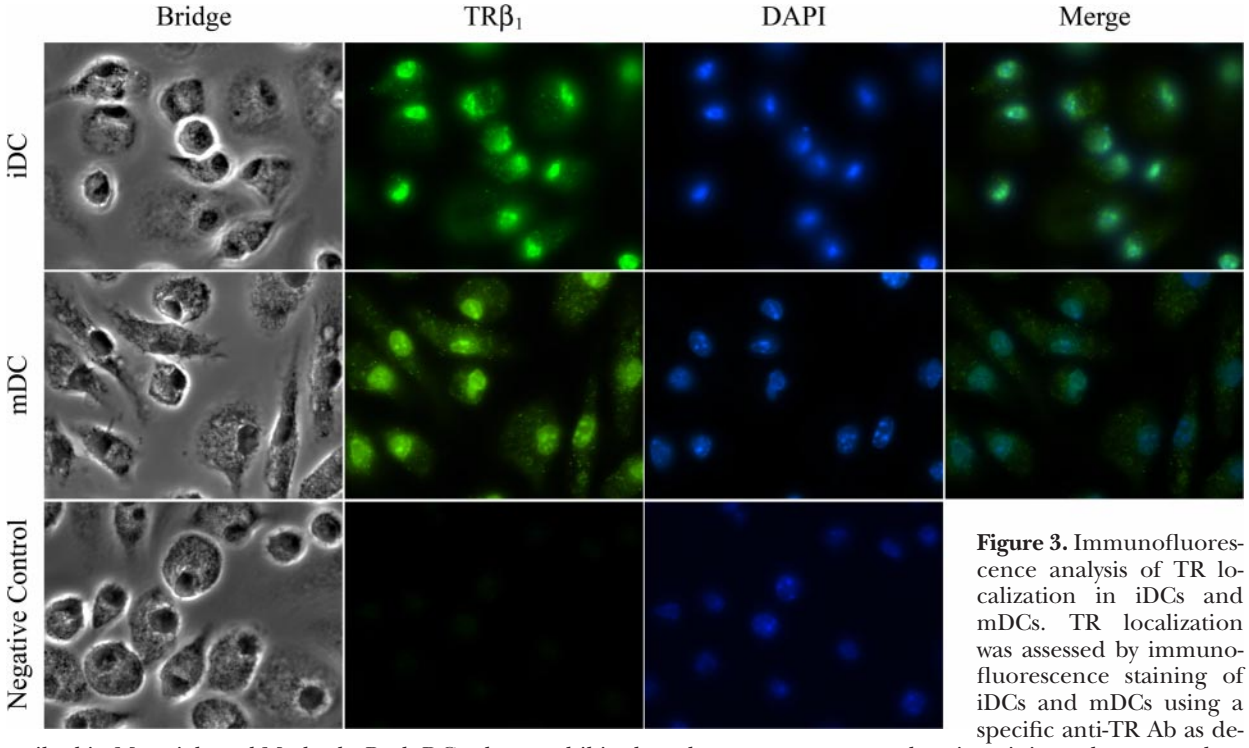

Figure 3. Immunofluorescence analysis of TR localization in iDCs and mDCs. TR localization was assessed by immunofluorescence staining of iDCs and mDCs using a specific anti-TR Ab as described in Materials and Methods. Both DC subsets exhibited moderate to strong cytoplasmic staining, whereas nuclear staining was scarce. Magnification ×1000.

type and functionality. Unstimulated DCs exhibited an immature phenotype characterized by marked expression of CD11c, but low levels of MHC II and the costimulatory molecules CD40, CD80, and CD86 (**Fig. 4**). As expected, LPS induced DC maturation, as demonstrated by the increased levels of MHC II,

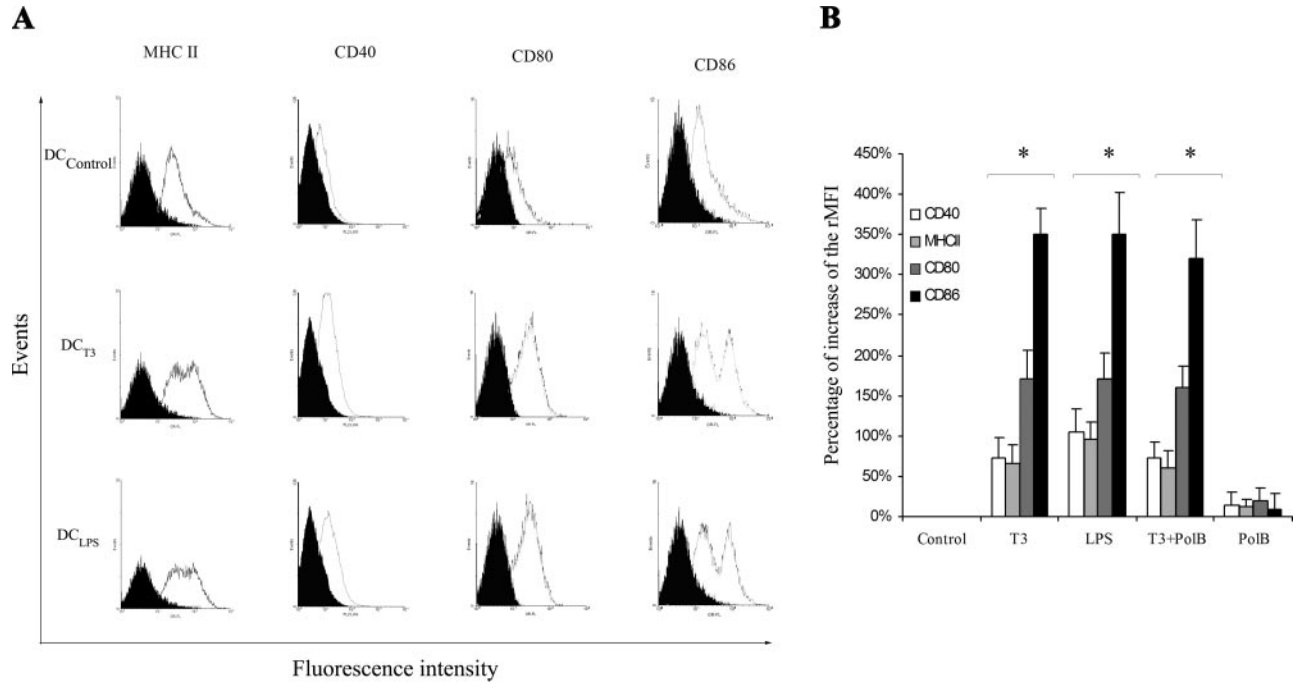

Figure 4. Effects of T3 on DC maturation. iDCs were incubated with T3 (5 nM) or LPS (100 ng/ml) for 18 h. *A*) Cell surface phenotype was analyzed by flow cytometric analysis of T3 or LPS-matured DCs using PE-conjugated anti-MHC II (IA/IE), anti-CD40, anti-CD80, and anti-CD86 mAbs. Polymyxin B (PolB) was added to DC cultures to check possible endotoxin contamination of T3 preparations. Representative histograms of eight independent experiments are shown. *B*) Results are expressed as the percentage of increase of the relative mean fluorescence intensity (rMFI). Data are the mean ± SD of eight independent experiments (*$P<0.001$ *vs.* control; Wilcoxon nonparametric test).

CD40, CD80, and CD86 on the surface of bone marrow-derived DCs. Remarkably, exposure to T3 resulted in a similar DC maturation phenotype (Fig. 4*A, B*). T3-induced DC maturation was found to be dose-dependent, showing an optimal T3 stimulating concentration of 5 nM. Interestingly, this concentration is close to that found in mice sera and was, therefore, used for further DC maturation assays. As a control, exposure of iDCs to T3 in the presence of polymyxin B did not abolish T3-induced DC maturation, disregarding the possibility of endotoxin contamination in the T3 preparation (Fig. 4*B*).

To investigate whether the maturation phenotype induced by T3 is associated with an increased functionality of these cells, we first examined the ability of T3 to modulate cytokine secretion by DCs. iDCs exposed to T3 showed a significant increase in IL-12 secretion (**Fig. 5*A***) ($P<0.05$), whereas the production of IL-10 was not modified (Fig. 5*B*). Consistently, treatment with T3 resulted in a significant increase in the frequency of IL-12-producing $CD11c^+$ DCs ($P<0.01$, Fig. 5*C, D*) with no changes in the frequency of IL-10-producing $CD11c^+$ cells (data not shown). Thus, T3-conditioned bone marrow-derived DCs exhibit a highly mature phenotype comparable to that induced by LPS.

T3 favors the generation of mDCs with increased T cell-stimulatory capacity

The mature cell surface phenotype and the increased IL-12 production induced by T3 treatment prompted us to investigate the T cell allostimulatory capacity of T3-conditioned DCs. Proliferation of BALB/c (H-2d) splenocytes was strongly enhanced in response to irradiated T3-conditioned DCs (C57BL/6) in a wide range of DC/splenocyte ratios at all T3 concentrations tested (**Fig. 6**). This effect was dose-dependent at concentrations ranging from 0.05 to 5 nM, reaching a plateau at a T3 dose of 5 nM. No differences were found between concentrations of 5 and 500 nM. Interestingly, DCs cultured in the presence of 5 and 500 nM T3 markedly enhanced the proliferation of responder splenocytes and were significantly more potent allostimulators than DCs matured in the presence of LPS ($P<0.01$).

The augmented allostimulatory capacity of T3-matured DCs was also reflected by the greatly enhanced production of the effector cytokine IFN-γ in culture supernatants of splenocytes stimulated with T3-conditioned DCs (DC/splenocyte ratio 1:15) (**Fig. 7*A***). Furthermore, T3 was able to potentiate the allostimulatory capacity of LPS-matured DCs, as shown by the increased IFN-γ production (Fig. 7*A*). However, IL-10 was undetectable in allogeneic T cell cultures stimulated with T3-conditioned DCs (Fig. 7*B*). To further explore the ability of T3-conditioned DCs to direct T cell differentiation toward a T1-type profile, naive allogeneic splenocytes were cultured with T3-stimulated DCs for 72 h at a cell ratio of 1:15 (DCs/splenocytes). Activated T cells were analyzed for intracellular IFN-γ and IL-5 production by flow cytometry. Remarkably, T3-stimulated DCs, but not control DCs significantly enhanced the frequency of IFN-γ-producing cells in both the $CD4^+$ and $CD4^-$ T cell compartments (**Fig. 8*A***) ($P<0.01$). As a positive control, LPS-treated DCs induced a significant increase in the proportion of IFN-γ-secreting $CD4^+$ T cells (Fig. 8*A*). However, no changes were observed in the percentage of IL-5-producing $CD4^+$ T cells after exposure to T3-conditioned DCs (Fig. 8*B*). Thus, T3 may instruct the generation of a DC1 phenotype with increased T cell stimulatory potential and the ability to direct the development of a dominant T1-type response.

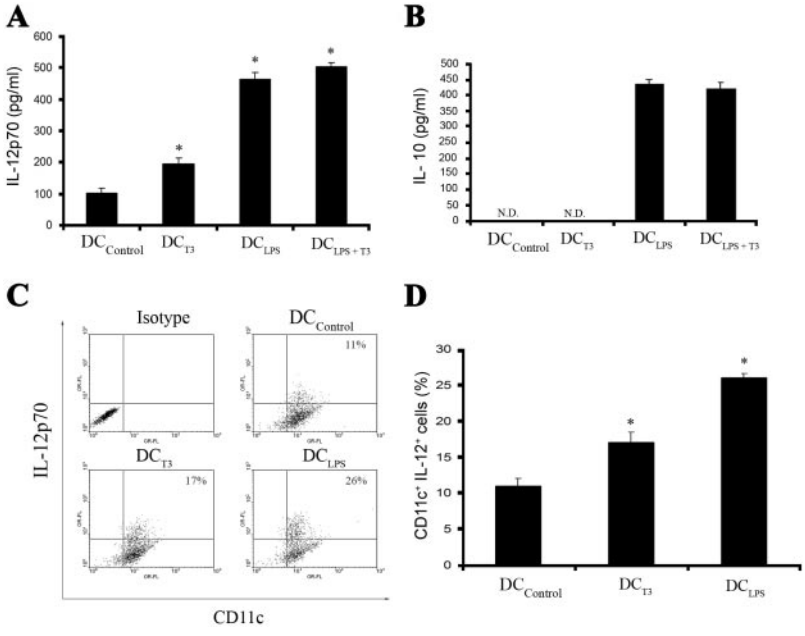

Figure 5. Effects of T3 in the modulation of the IL-12/IL-10 cytokine balance. *A, B*) DCs were stimulated with LPS (100 ng/ml), T3 (5 nM), or LPS plus T3 for 48 h. IL-12 (*A*) and IL-10 (*B*) production was determined in culture supernatants by ELISA. Data are expressed as mean ± SD (pg/ml) of three independent experiments. $*P < 0.05$, *vs.* control DCs. *C, D*) DCs were stimulated with T3 (5 nM) or LPS (100 ng/ml) for 18 h. For intracytoplasmic cytokine staining, cells were incubated with brefeldin A for 4 h, stained for CD11c, fixed, permeabilized, and then stained with anti-IL-12p70 Ab as described in Materials and Methods. The frequency of $CD11c^+$ $IL\text{-}12^+$ cells was determined by flow cytometry. Values are given as the percentage of total $CD11c^+$ IL-12-producing cells. *D*) Data are expressed as mean ± SD of three independent experiments. $*P < 0.01$, *vs.* control DCs.

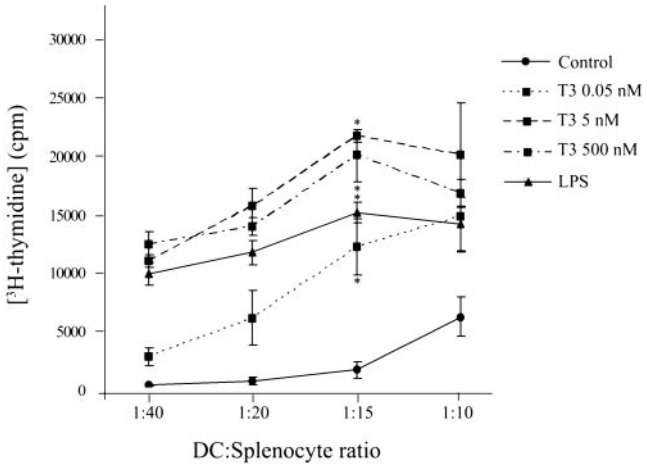

Figure 6. Impact of T3 on the allostimulatory capacity of DCs. Bone marrow-derived DCs were stimulated with T3 (0.05, 5, and 500 nM) or LPS (100 ng/ml). After 18 h, DCs were extensively washed, irradiated, and cultured with allogenic splenocytes (1×10^5 cells/well) for 3 days at different stimulator/responder ratios (1:10 to 1:40 DC/splenocyte). Proliferation of allogeneic splenocytes was measured by [^{3}H]thymidine incorporation. Data are expressed as mean ± SD (cpm) representative of six independent experiments. *$P <$ 0.01 *vs.* control DCs.

Control of DC maturation by T3 involves shuttling and nuclear translocation of NF-κB

The NF-κB pathway regulates different processes associated with DC maturation and function (13). This signaling pathway is activated by LPS or cytokines through phosphorylation of the NF-κB inhibitory protein kinases, which in turn phosphorylate the inhibitory protein IκBs that are bound to the NF-κB transcription factors in the cytoplasm. Phosphorylated IκBs are then degraded by the proteasome, allowing the NF-κB transcription factors to translocate to the nucleus and activate gene transcription (13). To gain insights into the mechanisms involved in T3-induced DC maturation, we investigated the potential role of NF-κB in this process. Analysis of the cytoplasmic-nuclear shuttling of this transcription factor revealed a substantial decrease in NF-κB/p65 in the cytoplasmic fractions of T3-treated compared with control DCs. In contrast, NF-κB/p65 was increased in the nuclear fractions of T3-treated cells (**Fig. 9**). Interestingly, T3-induced NF-κB translocation was even more pronounced than that observed in LPS-matured DCs (Fig. 9), suggesting involvement of the NF-κB signaling pathway in T3 effects. Thus, T3-induced DC maturation involves shuttling of NF-κB/p65 from the cytoplasmic compartment to the nucleus, a critical event in DC maturation and function.

DISCUSSION

Despite major advances in understanding of the interplay between distinct hormones and the immune cell network (11, 16, 18), the role of THs in the initiation of adaptive immunity still remains uncertain (20). Here we provide the first evidence of the expression of TRs on bone marrow-derived murine DCs and their striking localization in the cytoplasmic compartment of iDCs and mDCs. Furthermore, we demonstrate that THs contribute to DC maturation and IL-12 production and potentiate the T cell stimulatory capacity of these cells.

Until a few years ago, TH-mediated effects were thought to be primarily, if not solely, initiated by T3 binding to nuclear TRs attached to specific DNA sequences in the promoter region of target genes. However, extranuclear actions exerted mainly through a plasma membrane receptor for thyroxine (T4) (integrin $\alpha_V\beta_3$) were recently described (31). Moreover, effects mediated by the classical nuclear TRs with higher affinity for T3 than for T4, but exerted through cytosolic (32) and plasma membrane (33) mechanisms, were also reported. Albeit unusual, the preferential cytoplasmic compartmentalization of TRs at both stages of DC maturation was in accordance with TR localization in bone marrow mast cells (34), human hypothalamic and pituitary cells (35), human umbilical vein endothelial cells (36), rat hepatocytes (37), and rat thymic cells (38). In turn, several reports indicate that TRs may shuttle rapidly between the nuclear and the

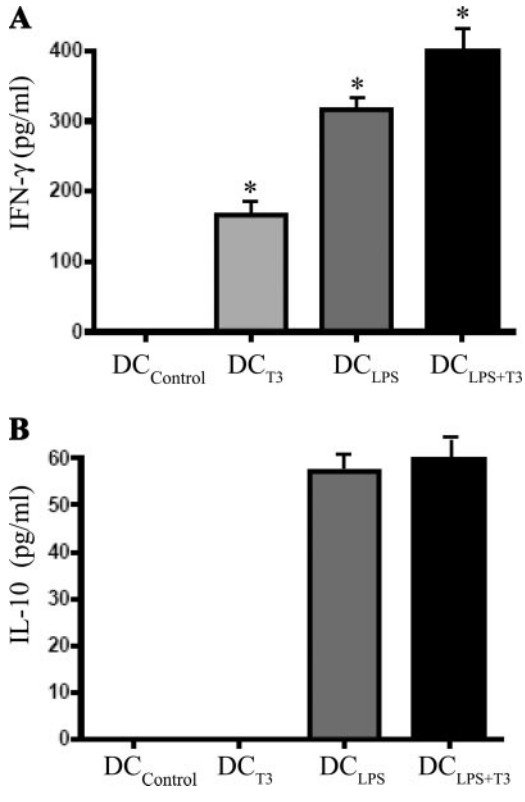

Figure 7. Influence of T3-conditioned DCs on cytokine secretion by splenocytes. DCs were stimulated with LPS (100 ng/ml), T3 (5 nM), or a combination of LPS and T3. After 18 h, DCs were extensively washed, irradiated, and cultured with allogeneic splenocytes (at an optimal DC/splenocyte ratio of 1:15) for 3 days. IFN-γ (*A*) and IL-10 (*B*) production were measured in culture supernatants by ELISA. Results are expressed as mean ± SD of three independent experiments. *$P <$ 0.01 *vs.* control DCs.

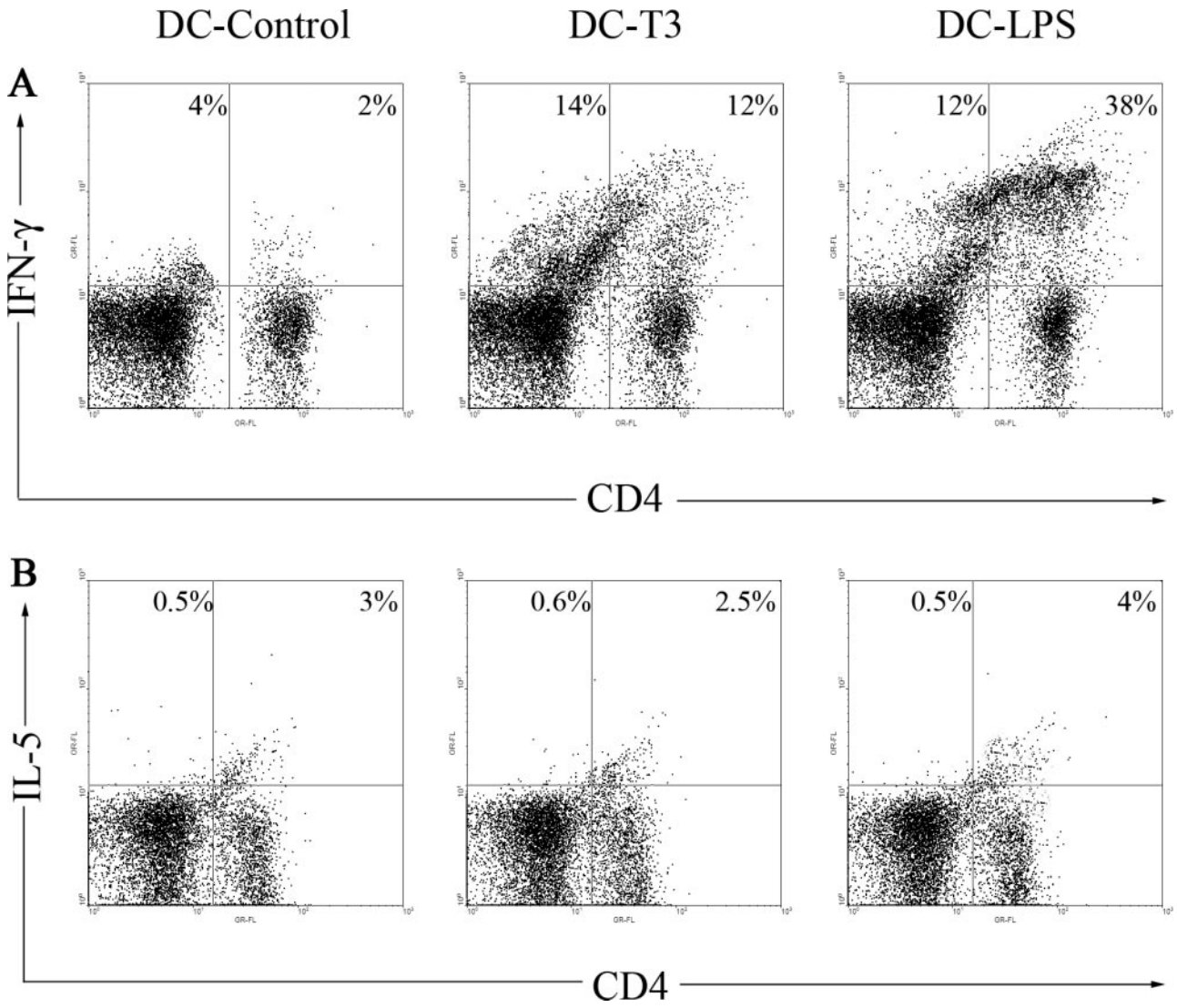

Figure 8. Modulation of Th1/Th2 cytokine production by T3-conditioned DCs. Bone marrow-derived DCs were stimulated with or without T3 (5 nM) or LPS (100 ng/mL) for 18 h. A total of 3.75×10^4 irradiated DCs were used to stimulate 1×10^6 allogeneic naive splenocytes. Brefeldin A (10 μg/ml) was added during the last 6 h of culture. Cells were stained with PE–conjugated anti-CD4- or FITC-conjugated anti-CD4 mAbs and then processed for intracellular cytokine staining using FITC-anti-IFN-γ (*A*) and PE-anti-IL-5 (*B*) mAb, respectively. Values in dot plots show the percentage of activated $CD4^+$ and $CD4^-$ T cells producing each cytokine. Results are representative of three independent experiments with similar results ($P<0.01$, T3-conditioned DCs *vs.* control DCs for IFN-γ; NS, T3-conditioned DCs *vs.* control DCs for IL-5).

cytoplasmic compartments (39–41). Furthermore, the dual cytoplasmic and nuclear localization appears to be a general feature of steroid hormone receptors (39, 42). In addition, the higher expression of $TR\beta_1$ compared with that of $TR\alpha_1$ in bone marrow-derived DCs is in consonance with the isoform distribution reported in other mouse immune cells including B and T lymphocytes (29, 30). However, the functional relevance of this particular subcellular distribution and the isoform prevalence of TRs in DC subsets still need to be fully elucidated.

The expression of TRs has recently been reported in other APCs such as macrophages as part of a functional nuclear receptor Atlas (43), although the effects of THs on these cells have not yet been described. Moreover, other members of the nuclear receptor superfamily have recently been identified in human and mice DCs, as the nonsteroid retinoic acid receptor (44), the peroxisome proliferator-activated receptors α, δ, γ1, and γ2 (45), and the steroid estrogen and glucocorticoid receptors (17, 46).

The endocrine system participates in regulating the differentiation and maturation of different DC subtypes, *e.g.*, thyroid-stimulating hormone induces a stimulatory effect on phagocytosis and cytokine production in murine DCs (47). In addition, mRNAs for estrogen receptor-α and -β have been demonstrated in $CD14^+$ monocytes, cultured immature $CD1a^+$ cells, and mature $CD83^+$ cells (48). In culture, bone marrow progenitors give rise to the generation of DCs (49), which can be influenced by the action of androgens and estrogens (48, 50). Moreover, glucocorticoids inhibit

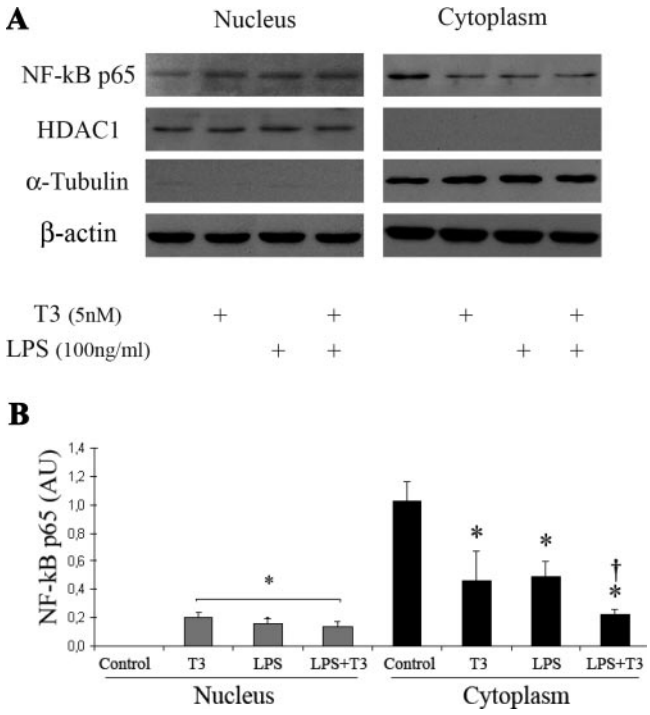

Figure 9. Control of the cytoplasmic-nuclear shuttling of NF-κB by T3. DCs were stimulated with T3 (5 nM), LPS (100 ng/ml), or a combination of T3 and LPS for 18 h. Nuclear and cytoplasmic DC extracts (40 μg) were used for immunodetection of NF-κB/p65. Anti-α-tubulin and HDAC1 Abs were used to control the purity of subcellular fractions. *A*) Representative Western blots of three independent experiments. *B*) Densitometric analysis of immunoreactive protein bands. Results are expressed as arbitrary units (AU) calculated from the densitometric profile of the NF-κB/p65 signal normalized to β-actin. Data are presented as mean ± SD of six independent experiments. $^{*}P < 0.01$ *vs.* control DCs; $^{\dagger}P < 0.05$ *vs.* T3-stimulated DCs and LPS-stimulated DCs.

the *in vitro* differentiation of DCs from their progenitors and impair their capacity to undergo terminal differentiation or generate proinflammatory cytokines (17). Our results indicating a positive role for T3 in triggering T cell-mediated immunity are in broad agreement with earlier observations showing a T3-mediated stimulatory effect on mitogen activation of T cells (51, 52). In addition, a differentiating effect of THs and other iodinated compounds was observed in the transition of human monocytes into veiled/DCs (20). Our study provides the first evidence of a stimulatory effect of T3 on DC maturation and function with critical implications in orchestrating protective immunity and/or inciting T helper 1 (Th1)-mediated immunopathology.

Similarly to LPS-induced DC maturation (53), exposure to T3 results in increased secretion of IL-12p70. In this regard, previous studies reported increased synthesis of IL-12 by DCs obtained from hyperthyroid mice (54), as well as increased amounts of IL-12 in sera from patients with Graves' disease (55). Although these situations cannot be directly extrapolated to the experimental conditions of our work (use of physiological concentrations of T3), further studies *in vitro* and *in vivo* are required to determine the different effects of increasing amounts of THs found in sera from patients with thyroid-related pathological conditions on the maturation and immunostimulatory capacity of DCs. As IL-12p70, a heterodimeric cytokine composed of the p40 and p35 subunits, is essential for the promotion and maintenance of Th1 differentiation (56), T3 might critically influence the development of T1-mediated immunity *in vivo*. Accordingly, our results reveal that T3 does not promote the secretion of IL-10 by DCs in contrast with the tolerogenic and regulatory responses induced by glucocorticoids (57), which share metabolic actions similar to those of THs in the context of other target tissues such as the pituitary (58) and the liver (59). In this regard, we found that T3-conditioned DCs are capable of directing the development of a T1-type cytokine response *in vitro*.

Circulating iDCs migrate within peripheral tissues, suggesting that at least part of the DCs found in the bloodstream might in fact represent a circulating pool of APCs available for immediate recruitment to sites of inflammation, where their antigen sampling and processing function is required (60). Unlike most other hormones, THs circulate at relatively constant levels throughout postnatal life in human and animal species (6). Therefore, the effects of circulating THs in the modulation of DC function *in vivo* is expected, given the stimulatory effects of physiological levels of T3 on DC maturation *in vitro*. In addition, DCs simultaneously exposed to LPS and T3 showed a greatly enhanced capacity to activate T cell responses, suggesting that T3 might potentiate LPS-induced initiation of adaptive immune responses during infectious processes. Studies are currently being conducted to address the role of T3 in the modulation of DC physiology *in vivo* and the effects of these hormones in inciting and perpetuating Th1-mediated immunopathology.

The transcription of proinflammatory cytokines including IL-12p70 is controlled, at least in part, by the transcription factor NF-κB (61). Accordingly, the increased cytoplasmic-nuclear shuttling of NF-κB after exposure of DCs to T3 may be associated with increased activity of the NF-κB pathway during T3-induced DC maturation and IL-12 production. In conclusion, our results provide the first evidence of the presence of TRs in immature and mature DCs and their preferential cytoplasmic localization. In addition, we demonstrate the effects of THs in DC differentiation and function, suggesting their possible role in controlling the initiation of adaptive immune responses. Our findings broaden our perspective of the interactions between the endocrine and immune systems, providing a novel link between THs and the initiation of T cell responses. Because thyroid-related pathological conditions are the most common endocrine dysfunctions, our observations may contribute to understanding the molecular bases of immune-mediated pathological conditions of the thyroid gland and the immunological consequences

of hypo- and hyperthyroid disorders. In addition, our findings provide a novel molecular target for manipulating the immunogenic potential of DCs to positively regulate the development of protective immunity or negatively control the generation of autoimmune thyroid inflammation. FJ

This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Ciencia y Técnica (FONCYT), Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba, and Third World Academy of Sciences and Fundación Sales/CONICET. I.D.M. is a research fellow of FONCYT. M.M.M., S.S., and J.M.I. are research fellows of CONICET. H.M.T., G.A.R., and C.G.P. are members of the research career of CONICET. The authors thank Dr. Mariana Matrajt, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT, USA, for excellent assistance in this work.

REFERENCES

1. Fabris, N., Mocchegiani, E., and Provinciali, M. (1995) Pituitary-thyroid axis and immune system: a reciprocal neuroendocrine-immune interaction. *Horm. Res.* **43,** 29–38
2. Yen, P. M. (2001) Physiological and molecular basis of thyroid hormone action. *Physiol. Rev.* **81,** 1097–1142
3. Foster, M. P., Jensen, E. R., Montecino-Rodriguez, E., Leathers, H., Horseman, N., and Dorshkind, K. (2000) Humoral and cell-mediated immunity in mice with genetic deficiencies of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormone. *Clin. Immunol.* **96,** 140–149
4. Dorshkind, K., and Horseman, N. D. (2001) Anterior pituitary hormones, stress, and immune system homeostasis. *Bioessays* **23,** 288–294
5. Klecha, A. J., Genaro, A. M., Gorelik, G., Barreiro Arcos, M. L., Magali Silberman, D., Schuman, M., Garcia, S. I., Pirola, C., and Cremaschi, G. A. (2006) Integrative study of hypothalamus-pituitary-thyroid-immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. *J. Endocrinol.* **189,** 45–55
6. Dorshkind, K., and Horseman, N. D. (2000) The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: insights from genetic models of hormone and hormone receptor deficiency. *Endocr. Rev.* **21,** 292–312
7. Wang, H. C., and Klein, J. R. (2001) Immune function of thyroid stimulating hormone and receptor. *Crit. Rev. Immunol.* **21,** 323–337
8. Davis, P. J., Davis, F. B., and Cody, V. (2005) Membrane receptors mediating thyroid hormone action. *Trends Endocrinol. Metab.* **16,** 429–435
9. Guermonprez, P., Valladeau, J., Zitvogel, L., Thery, C., and Amigorena, S. (2002) Antigen presentation and T cell stimulation by dendritic cells. *Annu. Rev. Immunol.* **20,** 621–667
10. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., and Palucka, K. (2000) Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18,** 767–811
11. Hewison, M., Freeman, L., Hughes, S. V., Evans, K. N., Bland, R., Eliopoulos, A. G., Kilby, M. D., Moss, P. A., and Chakraverty, R. (2003) Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. *J. Immunol.* **170,** 5382–5390
12. Rutella, S., Danese, S., and Leone, G. (2006) Tolerogenic dendritic cells: cytokine modulation comes of age. *Blood* **108,** 1435–1440
13. Bottero, V., Withoff, S., and Verma, I. M. (2006) NF-κB and the regulation of hematopoiesis. *Cell Death Differ.* **13,** 785–797
14. Bassett, J. H., Harvey, C. B., and Williams, G. R. (2003) Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. *Mol. Cell. Endocrinol.* **213,** 1–11
15. Yen, P. M., Ando, S., Feng, X., Liu, Y., Maruvada, P., and Xia, X. (2006) Thyroid hormone action at the cellular, genomic and target gene levels. *Mol. Cell. Endocrinol.* **246,** 121–127
16. D'Arezzo, S., Incerpi, S., Davis, F. B., Acconcia, F., Marino, M., Farias, R. N., and Davis, P. J. (2004) Rapid nongenomic effects of 3,5,3′-triiodo-L-thyronine on the intracellular pH of L-6 myoblasts are mediated by intracellular calcium mobilization and kinase pathways. *Endocrinology* **145,** 5694–5703
17. Freeman, L., Hewison, M., Hughes, S. V., Evans, K. N., Hardie, D., Means, T. K., and Chakraverty, R. (2005) Expression of 11β-hydroxysteroid dehydrogenase type 1 permits regulation of glucocorticoid bioavailability by human dendritic cells. *Blood* **106,** 2042–2049
18. Nahmod, K. A., Vermeulen, M. E., Raiden, S., Salamone, G., Gamberale, R., Fernandez-Calotti, P., Alvarez, A., Nahmod, V., Giordano, M., and Geffner, J. R. (2003) Control of dendritic cell differentiation by angiotensin II. *FASEB J.* **17,** 491–493
19. Delgado, M., Reduta, A., Sharma, V., and Ganea, D. (2004) VIP/PACAP oppositely affects immature and mature dendritic cell expression of CD80/CD86 and the stimulatory activity for $CD4^+$ T cells. *J. Leukoc. Biol.* **75,** 1122–1130
20. Mooij, P., Simons, P. J., de Haan-Meulman, M., de Wit, H. J., and Drexhage, H. A. (1994) Effect of thyroid hormones and other iodinated compounds on the transition of monocytes into veiled/dendritic cells: role of granulocyte-macrophage colony-stimulating factor, tumour-necrosis factor-α and interleukin-6. *J. Endocrinol.* **140,** 503–512
21. Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., and Steinman, R. M. (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* **176,** 1693–1702
22. Montesinos, M. M., Pellizas, C. G., Velez, M. L., Susperreguy, S., Masini-Repiso, A. M., and Coleoni, A. H. (2006) Thyroid hormone receptor β1 gene expression is increased by dexamethasone at transcriptional level in rat liver. *Life Sci.* **78,** 2584–2594
23. Ausubel, L. J., Kwan, C. K., Sette, A., Kuchroo, V., and Hafler, D. A. (1996) Complementary mutations in an antigenic peptide allow for cross-reactivity of autoreactive T-cell clones. *Proc. Natl. Acad. Sci. U. S. A.* **93,** 15317–15322
24. Chomczynski, P., and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159
25. Kreuzer, K. A., Lass, U., Landt, O., Nitsche, A., Laser, J., Ellerbrok, H., Pauli, G., Huhn, D., and Schmidt, C. A. (1999) Highly sensitive and specific fluorescence reverse transcription-PCR assay for the pseudogene-free detection of β-actin transcripts as quantitative reference. *Clin. Chem.* **45,** 297–300
26. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res.* **17,** 6419
27. Roelen, D. L., Schuurhuis, D. H., van den Boogaardt, D. E., Koekkoek, K., van Miert, P. P., van Schip, J. J., Laban, S., Rea, D., Melief, C. J., Offringa, R., Ossendorp, F., and Claas, F. H. (2003) Prolongation of skin graft survival by modulation of the alloimmune response with alternatively activated dendritic cells. *Transplantation* **76,** 1608–1615
28. Straw, A. D., MacDonald, A. S., Denkers, E. Y., and Pearce, E. J. (2003) CD154 plays a central role in regulating dendritic cell activation during infections that induce Th1 or Th2 responses. *J. Immunol.* **170,** 727–734
29. Villa-Verde, D. M., Defresne, M. P., Vannier-dos-Santos, M. A., Dussault, J. H., Boniver, J., and Savino, W. (1992) Identification of nuclear triiodothyronine receptors in the thymic epithelium. *Endocrinology* **131,** 1313–1320
30. Meier-Heusler, S., Pernin, A., Liang, H., Goumaz, M. O., Burger, A. G., and Meier, C. A. (1995) Quantitation of β1 triiodothyronine receptor mRNA in human tissues by competitive reverse transcription polymerase chain reaction. *J. Endocrinol. Invest.* **18,** 767–773
31. Bergh, J. J., Lin, H. Y., Lansing, L., Mohamed, S. N., Davis, F. B., Mousa, S., and Davis, P. J. (2005) Integrin αVβ3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* **146,** 2864–2871

32. Moeller, L. C., Cao, X., Dumitrescu, A. M., Seo, H., and Refetoff, S. (2006) Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor β through the phosphatidylinositol 3-kinase pathway. *Nucl. Recept. Signal.* **4,** e020
33. Storey, N. M., Gentile, S., Ullah, H., Russo, A., Muessel, M., Erxleben, C., and Armstrong, D. L. (2006) Rapid signaling at the plasma membrane by a nuclear receptor for thyroid hormone. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 5197–5201
34. Siebler, T., Robson, H., Bromley, M., Stevens, D. A., Shalet, S. M., and Williams, G. R. (2002) Thyroid status affects number and localization of thyroid hormone receptor expressing mast cells in bone marrow. *Bone* **30,** 259–266
35. Alkemade, A., Vuijst, C. L., Unmehopa, U. A., Bakker, O., Vennstrom, B., Wiersinga, W. M., Swaab, D. F., and Fliers, E. (2005) Thyroid hormone receptor expression in the human hypothalamus and anterior pituitary. *J. Clin. Endocrinol. Metab.* **90,** 904–912
36. Diekman, M. J., Zandieh Doulabi, B., Platvoet-Ter Schiphorst, M., Fliers, E., Bakker, O., and Wiersinga, W. M. (2001) The biological relevance of thyroid hormone receptors in immortalized human umbilical vein endothelial cells. *J. Endocrinol.* **168,** 427–433
37. Zandieh Doulabi, B., Platvoet-ter Schiphorst, M., van Beeren, H. C., Labruyere, W. T., Lamers, W. H., Fliers, E., Bakker, O., and Wiersinga, W. M. (2002) $TR\beta_1$ protein is preferentially expressed in the pericentral zone of rat liver and exhibits marked diurnal variation. *Endocrinology* **143,** 979–984
38. Csaba, G., Sudar, F., and Dobozy, O. (1977) Triiodothyronine receptors in lymphocytes of newborn and adult rats. *Horm. Metab. Res.* **9,** 499–501
39. Hager, G. L., Lim, C. S., Elbi, C., and Baumann, C. T. (2000) Trafficking of nuclear receptors in living cells. *J. Steroid Biochem. Mol. Biol.* **74,** 249–254
40. Zhu, X. G., Hanover, J. A., Hager, G. L., and Cheng, S. Y. (1998) Hormone-induced translocation of thyroid hormone receptors in living cells visualized using a receptor green fluorescent protein chimera. *J. Biol. Chem.* **273,** 27058–27063
41. Baumann, C. T., Maruvada, P., Hager, G. L., and Yen, P. M. (2001) Nuclear cytoplasmic shuttling by thyroid hormone receptors. multiple protein interactions are required for nuclear retention. *J. Biol. Chem.* **276,** 11237–11245
42. Maruvada, P., Baumann, C. T., Hager, G. L., and Yen, P. M. (2003) Dynamic shuttling and intranuclear mobility of nuclear hormone receptors. *J. Biol. Chem.* **278,** 12425–12432
43. Barish, G. D., Downes, M., Alaynick, W. A., Yu, R. T., Ocampo, C. B., Bookout, A. L., Mangelsdorf, D. J., and Evans, R. M. (2005) A nuclear receptor atlas: macrophage activation. *Mol. Endocrinol.* **19,** 2466–2477
44. Tao, Y., Yang, Y., and Wang, W. (2006) Effect of all-trans-retinoic acid on the differentiation, maturation and functions of dendritic cells derived from cord blood monocytes. *FEMS Immunol. Med. Microbiol.* **47,** 444–450
45. Jakobsen, M. A., Petersen, R. K., Kristiansen, K., Lange, M., and Lillevang, S. T. (2006) Peroxisome proliferator-activated receptor α, δ, γ1 and γ2 expressions are present in human monocyte-derived dendritic cells and modulate dendritic cell maturation by addition of subtype-specific ligands. *Scand. J. Immunol.* **63,** 330–337
46. Nalbandian, G., Paharkova-Vatchkova, V., Mao, A., Nale, S., and Kovats, S. (2005) The selective estrogen receptor modulators, tamoxifen and raloxifene, impair dendritic cell differentiation and activation. *J. Immunol.* **175,** 2666–2675
47. Bagriacik, E. U., and Klein, J. R. (2000) The thyrotropin (thyroid-stimulating hormone) receptor is expressed on murine dendritic cells and on a subset of CD45RBhigh lymph node T cells: functional role for thyroid-stimulating hormone during immune activation. *J. Immunol.* **164,** 6158–6165
48. Komi, J., and Lassila, O. (2000) Nonsteroidal anti-estrogens inhibit the functional differentiation of human monocyte-derived dendritic cells. *Blood* **95,** 2875–2882
49. Hagihara, M., Li, C., Gansuvd, B., Munkhbat, B., Inoue, H., Shimakura, Y., Tsuchiya, T., Ueda, Y., Oki, M., Ando, K., Kato, S., and Hotta, T. (2001) Extensive and long-term ex vivo production of dendritic cells from CD34 positive umbilical cord blood or bone marrow cells by novel culture system using mouse stroma. *J. Immunol. Methods* **253,** 45–55
50. Smithson, G., Couse, J. F., Lubahn, D. B., Korach, K. S., and Kincade, P. W. (1998) The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. *J. Immunol.* **161,** 27–34
51. Balazs, C., Leovey, A., Szabo, M., and Bako, G. (1980) Stimulating effect of triiodothyronine on cell-mediated immunity. *Eur. J. Clin. Pharmacol.* **17,** 19–23
52. Chatterjee, S., and Chandel, A. S. (1983) Immunomodulatory role of thyroid hormones: in vivo effect of thyroid hormones on the blastogenic response of lymphoid tissues. *Acta Endocrinol. (Copenh.)* **103,** 95–100
53. Lipscomb, M. F., and Masten, B. J. (2002) Dendritic cells: immune regulators in health and disease. *Physiol. Rev.* **82,** 97–130
54. Tamura, M., Matsuura, B., Miyauchi, S., and Onji, M. (1999) Dendritic cells produce interleukin-12 in hyperthyroid mice. *Eur. J. Endocrinol.* **141,** 625–629
55. Tamaru, M., Matsuura, B., and Onji, M. (1999) Increased levels of serum interleukin-12 in Graves' disease. *Eur. J. Endocrinol.* **141,** 111–116
56. Sundquist, M., Johansson, C., and Wick, M. J. (2003) Dendritic cells as inducers of antimicrobial immunity in vivo. *APMIS* **111,** 715–724
57. Gad, M., Claesson, M. H., and Pedersen, A. E. (2003) Dendritic cells in peripheral tolerance and immunity. *APMIS* **111,** 766–775
58. Liu, L., Dean, C. E., and Porter, T. E. (2003) Thyroid hormones interact with glucocorticoids to affect somatotroph abundance in chicken embryonic pituitary cells in vitro. *Endocrinology* **144,** 3836–3841
59. Yamaguchi, S., Murata, Y., Nagaya, T., Hayashi, Y., Ohmori, S., Nimura, Y., and Seo, H. (1999) Glucocorticoids increase retinoid-X receptor α (RXRα) expression and enhance thyroid hormone action in primary cultured rat hepatocytes. *J. Mol. Endocrinol.* **22,** 81–90
60. Bonasio, R., and von Andrian, U. H. (2006) Generation, migration and function of circulating dendritic cells. *Curr. Opin. Immunol.* **18,** 503–511
61. Laderach, D., Compagno, D., Danos, O., Vainchenker, W., and Galy, A. (2003) RNA interference shows critical requirement for NF-κB p50 in the production of IL-12 by human dendritic cells. *J. Immunol.* **171,** 1750–1757

Received for publication June 15, 2007.
Accepted for publication October 4, 2007.