Retinoic Acid Regulates CD1d Gene Expression at the Transcriptional Level in Human and Rodent Monocytic Cells

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CD1d belongs to a group of nonclassical antigen-presenting molecules that present glycolipid antigens and thereby activate natural killer T (NKT) cells, a subset of bifunctional T cells. Little is known so far regarding the expression and physiologic regulation of CD1d. Here we show that all-*trans*-retinoic acid (RA), the active metabolite of vitamin A, rapidly (1 hr after treatment) increases CD1d mRNA in human and rodent monocytic cells at a physiologic dose (10 n*M*). The induction is RA specific and RA receptor (RAR) dependent—RA and an RARα agonist, Am580, both had a pronounced positive effect, whereas the addition of RARα antagonist partially blocked the increase in CD1d mRNA induced by RA and Am580. The induction was also completely blocked by the presence of actinomycin D. A putative RA-response element was identified in the distal 5′ flanking region of the CD1d gene, which binds nuclear retinoid receptors and was responsive to RA in both gel mobility shift assay and transient transfection assay in THP-1 cells. These results further confirmed the transcriptional regulation of RA in CD1d gene expression. Moreover, RA significantly increased α-galactosylceramide-induced spleen cell proliferation. These studies together provide evidence for a previously unknown mechanism of CD1d gene expression regulation by RA and suggest that RA is a significant modulator of NKT cell activation.
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Key words: gene expression; promoter; α-galactosylceramide; retinoic acid response element

Introduction

CD1d, a major histocompatibility complex (MHC) class I–like molecule, is constitutively expressed by many cell types in the hematopoietic cell family (1). It is expressed by professional antigen-presenting cells such as macrophages, dendritic cells (DC), splenic B cells, and even T cells, and the cell surface expression of CD1d is subject to additional regulation by cytokines or microbial products, as observed for classical MHC molecules (2). CD1d is responsible for glycolipid antigen presentation, which activates a unique group of T cells, the natural killer T (NKT) cells (3), that are involved in the regulation of both innate and adaptive immune responses (4). Therefore, CD1d plays an important role in mediating the antibacterial, autoimmune, and antitumor responses of the host attributed to CD1d-antigen complexes (1, 3–5). Among the emergent spectrum of lipid antigens that can be presented by CD1d, a synthetic glycolipid antigen, α-galactosylceramide (α-GalCer), has been cocrystallized in the antigen-binding site of human and mouse CD1d (6, 7) and has been studied most intensively in experimental and clinical studies in the activation of invariant NKT (iNKT) cells (1, 8). Although the increased surface expression of CD1d has been shown to increase the efficacy of T-cell activation (4), the mechanisms regarding CD1d gene expression are poorly understood.

Retinoic acid (RA), the most active metabolite of vitamin A, is an important regulator of innate and acquired immunity, as documented in both animal experiments and clinical settings (9–13). RA has been shown to potentiate T cell–dependent antibody production (11, 13) and T-cell homing (14) *in vivo*, while at the cellular level RA is essential for maintaining T-cell, NKT-cell and B-cell functions (15–17). Because RA and other retinoids can induce the differentiation and maturation of monocytic cells, they are implicated in the regulation of antigen-presenting cell functions (18–20). The actions of RA and other bioactive retinoids are mediated by retinoic acid receptors (RARs) and retinoid X receptors (RXRs), members of the nuclear hormone receptor superfamily (21), which function as ligand-regulated transcription factors by binding, most often as heterodimers, to specific hormone response elements, designated RARE and RXRE, respectively, in

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the promoter/enhancer regions of target genes (21, 22). Through the activation of RAR and RXR, RA modulates the expression of a large number of genes, and many of their products are associated with the control of cell proliferation, differentiation, and tissue homeostasis (22–24).

Previous studies have shown that RA can induce THP-1 cells to differentiate toward monocytic cells (20, 25). To understand the underlying mechanisms, microarray studies were carried out to determine the gene expression profile regulated by RA. We observed and confirmed that RA is a potent regulator of CD1d gene expression. Identification of a functional RARE in the 5′ flanking region of the human CD1d gene further supported the transcriptional regulatory effect of RA on CD1d gene expression. Furthermore, the presence of RA also markedly enhanced the α-GalCer-induced splenocyte proliferation, suggesting an active role of RA in CD1d/α-GalCer-mediated iNKT-cell activation.

Materials and Methods

Cell Culture and Treatment. THP-1, HL-60, U937, and HepG2 cells (ATCC, Manassas, VA) were propagated in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 5×10^{-5} *M* β-mercaptoethanol (Invitrogen, Carlsbad, CA.). During experiments cells were cultured in medium with 3% FBS. All-*trans*-RA and 9-*cis*-RA (Sigma-Aldrich, St. Louis, MO) and Am580 were applied to cells at a final concentration of 10 nM. α-GalCer (Alexis Biochemicals, San Diego, CA) and β-GalCer (Sigma-Aldrich) were used at 100 n*M*.

Peritoneal macrophages were obtained from 3-month-old male and female Sprague-Dawley rats by lavage with 20 ml of cold PBS. These macrophages were washed and plated in medium (RPMI-1640 medium supplemented with 10% FBS and 5×10^{-5} *M* β-mercaptoethanol; Invitrogen) at 37°C for 4 hrs, the unattached cells were removed by washing with warm medium, and remaining cells were cultured and subjected to treatment.

Mouse spleen cells were obtained from male and female C57BL/6 mice $\geq$8 weeks. Briefly, the mouse spleen tissues were minced and passed through nylon mesh to acquire single cell suspensions, centrifuged in Ficoll (Sigma-Aldrich) to obtain mononuclear cells, and washed thoroughly prior to experimentation. RPMI-1640 medium supplemented with 10% FBS was used throughout experiments.

RNA Isolation and Microarray. THP-1 cells were cultured in 12-well plates (5×10^5 cells/ml/well) with RA (10 n*M*) for various times. Total cellular RNA was isolated at the time of harvesting (RNeasy Mini Kit; Qiagen, Valencia, CA). RNA was subjected to microarray analysis using Affymetrix U133–2 arrays (kindly provided by the NCI Microarray Facility, Frederick, MD). Data were analyzed with the median intensity set to 1 (0 on $\log_2$ scale), and all other values were expressed as $\log_2$ of intensity. Data were analyzed by one-way analysis of variance (ANOVA) using SuperAnova software (Abacus, Berkeley, CA).

Reverse Transcription and Real-Time Polymerase Chain Reaction (PCR). One μg of total RNA was isolated from cultured cells as described above and subjected to reverse transcription (Promega, Madison, WI) and real-time PCR analysis (Bio-Rad, Hercules, CA). Primers for CD1d were: forward 5′-CAGATCTCGTCC-TTCGCCAA-3′; reverse 5′-GCTCGGAGATACCATGAC-TC-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was amplified at the same time as internal controls for RNA integrity and reverse transcriptase (RT)-PCR amplification (25). The real-time PCR product was also electrophoresed on agarose gel to confirm the specificity of the amplification. PCR conditions were optimized as denaturing at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min for 35 cycles.

Gel Mobility Shift Assay. THP-1 cells were cultured and nuclear protein was isolated at the time of harvesting as described previously (25). Based on analysis by the Tess Transcription Element Search System (http://www.cbil.upenn.edu), a putative CD1d-RARE was located within the CD1d gene promoter, and a fragment was chosen and synthesized as a probe. The sequence of the probe was ggtgAGGTGAggagaAGGTCAagaa, which contained 2 core binding sites (indicated by capital letters) separated by 5 nucleotides. Several surrounding nucleotides at each end were kept to ensure necessary binding ability. A mutant fragment was also synthesized by modifying 2 nucleotides (underlined) in the core region, giving the sequence: ggtgAGAAGAg gagaAGAACAagaa. The wild-type fragment was labeled with [γ-^{32}P]dATP and incubated with the nuclear protein extract at room temperature for 15 mins. A commercially available RARE, which has 2 half-sites with 5 nucleotides in between (DR5; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and a respective mutant fragment were used as competitors. For the supershift assay, nuclear protein was incubated with antibodies for 15 mins and the probe was added to the reaction for a further 15 mins. The reaction was then separated on a 5% nondenatured acrylamide gel by electrophoresis.

Transient Transfection and Luciferase Assay. Promoter constructs were made by cloning the CD1d 5′ flanking region (amplified by PCR from THP-1 genomic DNA) into pGL3 plasmid (Promega). The inserted fragments contained the transcription initiation site and extended toward the 5′ end that would drive the transcription of firefly luciferase. A TK-Renilla luciferase plasmid (Promega) was used as an internal control for transfection. THP-1 cells were transfected by Nucleofactor (Amaxa Biosystems, Gaithersburg, MD) according to the manufacturer's instructions and then cultured for 14 hrs in the presence and absence of 10 n*M* RA. Cells were harvested, and cell viability was checked by trypan blue staining (Sigma-Aldrich) with viable cells higher than 90%.

Cell lysates were subjected to luciferase assay (Promega). The ratio of firefly versus renilla luciferase activity is presented as the promoter activity.

Thymidine Incorporation Assay. Mouse splenocytes (2×10^5/well) were plated in a 96-well plate with different treatments for a total of 72 hrs. [^{3}H]thymidine (0.2 μCi/well; Amersham Biosciences, Inc., Piscataway, NJ) was added to cells for the last 4 hrs of culture. Cells were then harvested onto glass-fiber filters with a cell harvester (Cambridge Technology Inc., Watertown, MA) to determine incorporation of [^{3}H]thymidine by liquid scintillation spectrometry.

Flow Cytometry Analysis. Mouse spleen cells were subjected to staining with anti-CD11b-PE (BD Biosciences, San Jose, CA) and anti-CD1d-Alexa 488 (Santa Cruz Biotechnology Inc.). After staining, cells were washed and subjected to flow cytometric analysis. The flow cytometric data were analyzed by FlowJo software (Tree Star, Ashland, OR).

Statistics. Values in charts are presented as mean ± SEM. Two-tailed students *t*-test was performed unless otherwise specified in figure legends, and $P < 0.05$ was considered significant.

Results

RA Induces CD1d Gene Expression in THP-1 Cells. The regulation of CD1d mRNA in THP-1 cells was first observed in microarray studies to identify cell differentiation–related genes, for which THP-1 cells were treated for varying times with a physiologic concentration of RA (10 n*M*). Gene expression profiling identified CD1d as a gene that was expressed at more than the median level of intensity in untreated THP-1 cells and as one that responded rapidly to RA. RA up-regulated the signal for CD1d by 10-fold in 2 independent studies (Fig. 1A). In contrast, most other genes involved in antigen presentation, such as genes of the MHC class I and II families and the nonclassical group 1 CD1 family members, CD1a, CD1b, CD1c, and CD1e, were expressed at very low or negligible levels and were not increased by RA. The MHC class II molecule HLA-DRA was detectable in THP-1 cells but was minimally regulated by RA ($P = 0.062$, data not shown).

Quantitative real-time RT-PCR was conducted using cells from independent experiments. This study confirmed the microarray results (Fig. 1B) and demonstrated that RA increased the level of CD1d mRNA more than 4-fold within 1 hr after addition of RA, with a peak expression around 6 hrs.

RA Induces CD1d Expression Selectively in Cell Types. We also tested whether CD1d expression is regulated by RA in other myeloid leukemic cell lines, using U937 and HL-60 cells, which have been shown to undergo cell differentiation in response to RA (26) and in primary rat peritoneal macrophages. A similar increase in CD1d mRNA by RA was observed for each of these monocytic cells,

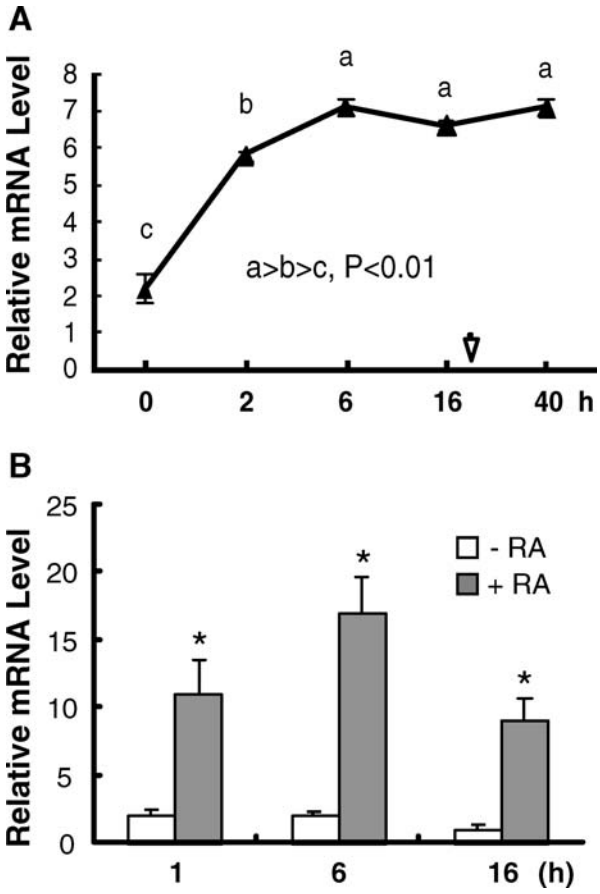

Figure 1. All-*trans*-RA induces CD1d gene expression analyzed by microarray and quantitative real-time RT-PCR. THP-1 cells were cultured in the presence and absence of RA (10 n*M*) for different times. (A) Total cellular RNA was isolated and subjected to microarray analysis or RT-PCR. RA increased CD1d gene expression in a kinetic study by microarray analysis. A second dose of RA (10 n*M*) was added to cells after 24 hrs as indicated by the arrow Data are expressed as $\log_2$ of signal intensity, with 0 equal to the median intensity for each array. $n = 3$–6/time. Results for one-way ANOVA followed by least significant difference test are shown; groups with different letters are significantly different ($P < 0.01$). (B) RA-induced CD1d mRNA expression was confirmed by real-time RT-RCR in THP-1 cells cultured in the presence and absence of RA for 1–16 hrs. GAPDH was measured alone with CD1d and used as an internal control. Data were derived from 2 independent experiments, $n = 6$. * represents the significant difference between with and without RA ($P < 0.01$).

although less than in THP-1 cells, showing that the induction of CD1d gene expression by RA is not limited to THP-1 cells (Fig. 2A). In contrast, CD1d was not up-regulated by RA in Jurkat lymphoblast cells, although Jurkat cells expressed a comparable basal level of CD1d mRNA; CD1d mRNA was below the limit of detection in HepG2 hepatocarcinoma cells (data not shown). Together, these data provide evidence that the basal level of CD1d gene expression and the responsiveness of the CD1d gene to RA are cell type specific.

CD1d mRNA Is Up-Regulated by RARα-Selective Retinoids in a Cycloheximide-Insensitive Manner. To examine the transcriptional regulation of CD1d by

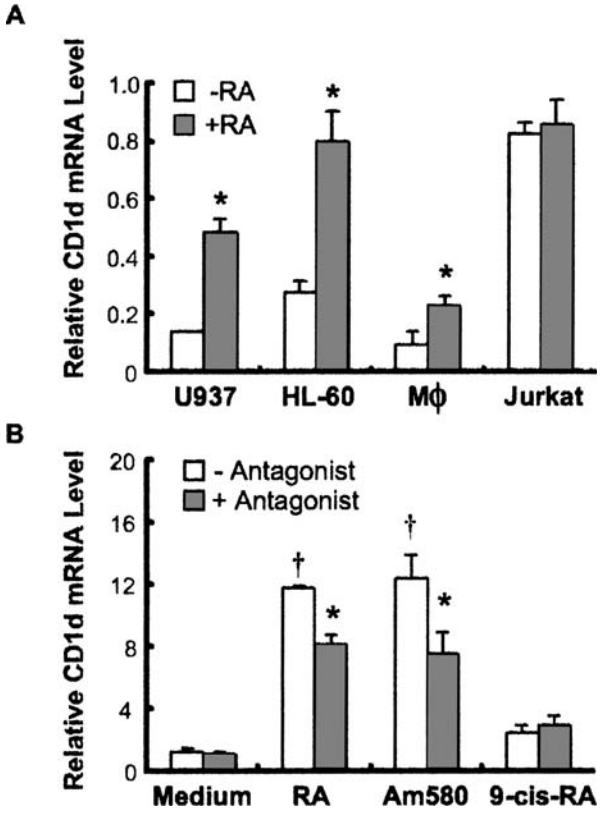

Figure 2. All-*trans*-RA and an RARα-selective retinoid rapidly induce CD1d mRNA in monocytic cells and rat peritoneal macrophages. THP-1 cells and other cells, such as U937, HL-60, Jurkat, and rat peritoneal macrophages (MΦ) were cultured in the presence and absence of RA (10 n*M*) for 4 hrs. Total cellular RNA was isolated and subjected to real-time RT-PCR for CD1d expression. GAPDH was measured alone as an internal control. (A) RA induced the expression of CD1d mRNA in other human myeloid leukemic cell lines and in normal rat peritoneal macrophages but not in Jurkat cells. * = significant increase by RA, $P < 0.01$. (B) RA and an RARα agonist, Am580, induced CD1d mRNA in THP-1 cells; the induction was partially blocked by a simultaneous addition of 10-fold excess of RARα antagonist (Ro 41–5253). THP-1 cells were treated with or without RA/Am580 and with or without Ro 41–5253 for 4 hrs. Total RNA was isolated for real-time RT-PCR analysis. * = significant reduction ($P < 0.05$) by antagonist; † = significant induction by RA or Am580 compared with medium ($P < 0.05$).

RA in more detail, real-time RT-PCR was conducted comparing the activity of natural and synthetic retinoids. CD1d expression was induced nearly equally by an RARα-selective ligand (RA and Am580). In contrast, *9-cis*-RA and retinoid agonists of RARβ, RARγ, and RXR were not as effective at the same concentration (Fig. 2B; data not shown for other ligands). Moreover, the induction of CD1d mRNA by all-*trans*-RA and Am580 was partially blocked by an RARα antagonist, indicating that RARα is likely to play a substantial if not dominant role in mediating the regulatory effect of RA. Besides the retinoids, we also tested the effect of vitamin D since it is reported to be involved in myeloid cell differentiation (27). No alteration was observed regarding the CD1d mRNA (data not shown). Taken together, these data suggested that the induction of CD1d expression is RA specific and involves the RAR family of receptors.

To confirm that the rapid induction of CD1d mRNA by RA is dependent on new RNA synthesis, an experiment was performed by adding actinomycin D (Act D), a transcription inhibitor, or cycloheximide (CHX), a protein synthesis inhibitor, 1 hour before the addition of RA. As shown in Figure 3A, Act D completely abolished the increase and reduced the basal level. The addition of CHX to inhibit new protein synthesis did not block the RA-mediated increase of CD1d mRNA, consistent with a direct mechanism of CD1d gene transcription by preexisting proteins.

The CD1d Promoter Possesses RAR-Binding Activity and Is Responsive to RA. Since RA rapidly and potently induced the level of CD1d transcripts and this induction was apparently direct, we tested to see if the induction was due to an effect of RA on transcription of the CD1d gene. About 1.5 kb of human CD1d 5′ untranslated region was analyzed by the Tess Transcription Element Search System. A putative RARE (direct hexanucleotide repeat with 5 nucleotides in between [DR5]) was identified in the distal region of the promoter (Fig. 3B). Comparison of the human sequence with the counterpart in mouse and rat revealed that all 3 species have the same putative RARE (except rat has a DR6 instead of DR5) in the similar region. A gel electrophoresis mobility shift assay (EMSA) was performed first to determine if specific binding complexes are formed on this region of the CD1d promoter. A human CD1d-RARE was synthesized and used as a probe. As shown in Figure 3C, the addition of RA to THP-1 cells resulted in a marked increase in complex formation on this probe, which could be completed by wild-type CD1d RARE, or an excess of a canonical RARE (DR5-W), but not by mutant oligonucleotides. A supershift assay showed that the complex was retarded by antibodies to RARα, RARγ, or RXRα.

To study the promoter activity under the influence of RA, promoter constructs were made as illustrated in Figure 3D. The vectors contained transcription initiation sites with differing lengths of the 5′ flanking region ligated to the coding region of the luciferase reporter gene. Construct p1.5 contained the CD1d 5′ flanking region with its putative RARE, and p1.3 contained most of the 5′ flanking region but with the RARE region deleted. Transient transfection assays with THP-1 cells showed that RA only increased the luciferase activity of p1.5, which contained the putative RARE. While the p1.3 promoter had a higher basal level of luciferase activity than the p1.5 promoter, which may be caused by the removal of an adjacent inhibitory element, it showed no regulation by RA. From this experiment, we inferred that CD1d-RARE is a functional element that is responsive to RA *in vivo*. Because THP-1 cells are known to be difficult to transfect, we shortened the incubation time after transfection to 14 hrs to improve cell viability, and thus the shorter time after transfection may explain the lower response (2-fold increase) of luciferase activity to RA, as

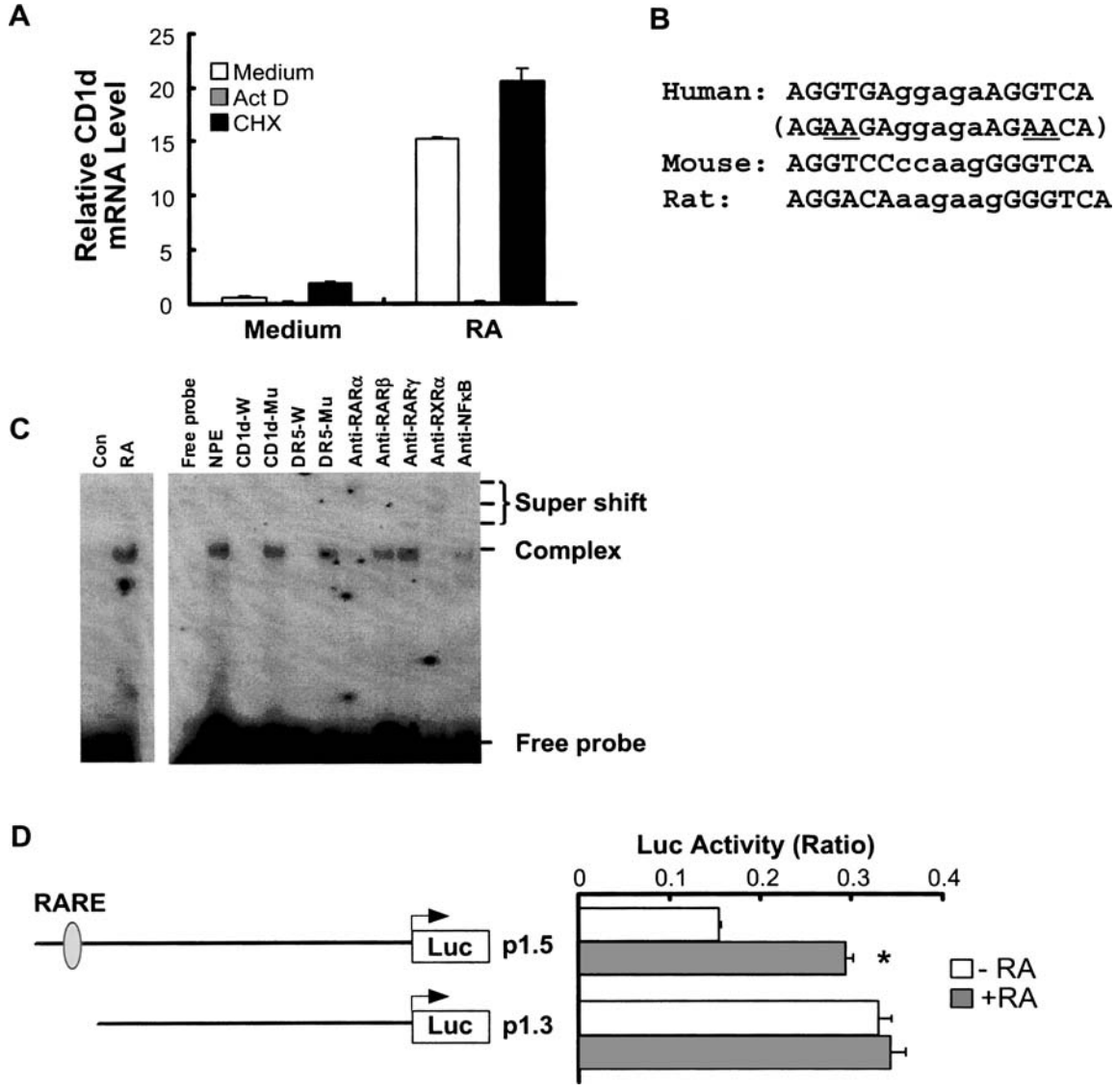

Figure 3. CD1d promoter possesses a functional RARE. (A) RA-induced CD1d mRNA expression was inhibited by addition of Act D 5 μg/ml ($P < 0.05$) but not by CHX 5 μg/ml for 1 hr, with RA then added to cells for another 4 hrs. (B) Sequence alignment of CD1d RARE at the distal region (–1.5 kb) of the promoters for human, mouse, and rat CD1d genes. Mutations for the human core binding sites are underlined. (C) A representative image of gel EMSA results with the putative CD1d RARE. Nuclear protein was extracted from THP-1 cells cultured with and without 10 n*M* RA for 6 hrs, and then 2 μg of protein was subjected to EMSA, using [γ^{32}P]-labeled CD1d-RARE as probe. The specificity of the binding was confirmed by using mutant CD1d-RARE, which has 2 nucleotides mutated in the core binding region, and a consensus RARE, wild-type DR5 (DR5-W) and mutant-type DR5 (DR5-Mu) as competitors. Supershift was performed by using antibodies against RARα, β, and γ and RXRα. An anti-NFκB antibody was used as a negative control. (D) RA increased the luciferase activity in p1.5-transfected cells. Two promoter constructs, p1.5 and p1.3, which differ by the presence or absence of the putative RARE, were used in the transient transfection assay. THP-1 cells were transfected with these constructs and treated with and without RA for 14 hrs. * = $P < 0.01$ due to RA.

compared with a stronger RA-induced increase in CD1d mRNA in nontransfected THP-1 cells.

RA Increases α-GalCer–Induced Mouse Spleen Cell Proliferation. It is known that α-GalCer is a specific antigen presented by CD1d to activate iNKT cells. Using mouse spleen cell cultures, we observed that α-GalCer induced a persistent spleen cell proliferation. Data for Day 3 are shown in Figure 4. RA alone did not increase the spleen cell proliferation—in contrast, it inhibited the proliferation of splenic B cells, which contribute about 55%–65% to the total spleen cell population (17). However, RA significantly enhanced α-GalCer–induced spleen cell proliferation (Fig. 4A), suggesting the positive role of RA in the regulation of α-GalCer-mediated iNKT cell activation. We also observed an increase in CD1d level in CD11b-positive spleen cells (13) by flow cytometric analysis (Fig. 4B), which in another system confirmed the positive regulatory effect of RA on CD1d expression.

Discussion

Although many studies have focused on CD1d-mediated NKT-cell activation, very little is known concern-

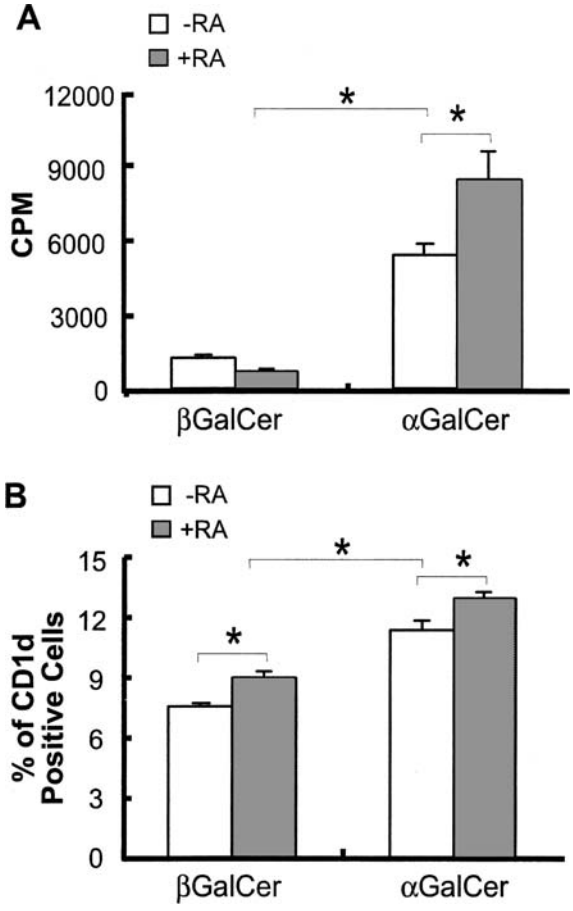

Figure 4. RA regulates CD1d/α-GalCer–mediated mouse spleen cell proliferation and CD1d expression. (A) Mouse spleen cells were cultured in the presence and absence of α-GalCer and/or RA for up to 3 days. Cell proliferation was determined each day by [^{3}H]thymidine incorporation, and the 3-day data are shown. * = $P < 0.05$ due to RA or α-GalCer. (B) Increase of CD1d expression on mouse splenic CD11b+ cells after 3 days of culture. Mouse spleen cells were stained with CD11b-PE and CD1d-Alexa 488. CD1d+ cells were gated on CD11b+ cells. * = $P < 0.05$.

ing the regulation of CD1d gene expression. Because the level of CD1d gene expression could directly regulate the activation of NKT cells (4), it is important to understand the regulation of CD1d as a factor in the activation and regulation of iNKT cells. Previously a few reports showed that CD1d expression in macrophages can be regulated by cytokines, such as IFNγ, and bacteria components (2). Although the 5′ untranslated region of the mouse and human CD1d gene has been studied in part and certain characteristics of the promoter have been defined, such as the involvement of more general transcription factors of the Sp-1 and Ets families (28, 29), the transcriptional regulation of the CD1d gene by specific regulatory factors has not been reported previously. Our current studies have identified a novel factor—the retinoid hormone all-*trans*-RA—which played a significant role in the regulation of CD1d expression and function.

The addition of RA to THP-1 cells, at a physiologic concentration similar to that in plasma (30), significantly up-regulated CD1d transcription activity by rapidly increasing the CD1d mRNA level (Figs. 1 and 2). This effect was stronger for THP-1 cells but was also evident in other human monocytic cell lines, such as U937 and HL-60 cells, as well as in rodent macrophages. Yet the regulation was cell type specific because CD1d was not up-regulated by RA in Jurkat cells, which expressed a detectable level of CD1d, or in HepG2 cells, which did not. The reasons for this specificity are not yet known but may be correlated with the previously studied ability of RA to induce cell differentiation in cells of the myeloid lineage (26). As it has been reported that the cell type–specific expression of CD1d greatly affects the functional development of NKT cells (31–33), our data may imply that RA-regulated CD1d expression on monocytic antigen-presenting cells may selectively affect the development and function of NKT cells, downstream of lipid antigen presentation by CD1d. Interestingly, no other member of the CD1 family was either highly expressed or regulated by RA in the microarray gene analysis of THP-1 cells, which led to our main study. It is worthy to mention that just recently Szatmari *et al*. (34) reported that PPARγ agonists can up-regulate CD1d gene expression by increasing RA synthesis in human dendritic cells, and the increased CD1d expression is correlated with NKT-cell expansion and cytokine production.

Analysis of the 5′ untranslated region of the CD1d gene revealed a putative RARE that is well conserved in human, mouse, and rat. This RARE contains the hexanucleotide direct repeat, AGGTG(C)A, spaced by 5 nucleotides, that is a hallmark of genes able to respond directly to RA though the binding of RAR/RXR heterodimers to this element (21). We confirmed by supershift assay that RARs and RXRs are contained within the protein complexes bound to the CD1d RARE. The use of a transient transfection assay further demonstrated that only the vector that had the RARE could response to RA treatment, implicating the functionality and specificity of the CD1d RARE (Fig. 3).

Alteration of CD1d expression on DC or other cells with antigen-presenting capabilities may be a mechanism that regulates NKT-cell activation (2). In mouse spleen cells, RA significantly enhanced α-GalCer–induced cell proliferation (Fig. 4). This outcome suggests that an increase in CD1d expression, induced by RA, may be one of the important ways explaining the well-recognized effect of RA in immune regulation, such as cytokine expression and antibody production (13, 35).

In conclusion, this study demonstrated that RA is a potent regulator of CD1d expression and function. RA can regulate CD1d mRNA directly through increased transcription of the CD1d gene. RA also regulates the α-GalCer–mediated activation of NKT cells by increasing cell proliferation. Our study provides new information on the regulation of CD1d expression and activity on which to base further studies to examine in detail CD1d protein expression, trafficking, and functionality under the regulation of RA, and to understand the biologic effect of RA on NKT

cell–mediated regulation of innate and adaptive immune responses.

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1. Brigl M, Brenner MB. CD1: antigen presentation and T cell function. Annu Rev Immunol 22:817–890, 2004.
2. Skold M, Xiong X, Illarionov PA, Besra GS, Behar SM. Interplay of cytokines and microbial signals in regulation of CD1d expression and NKT cell activation. J Immunol 175:3584–3593, 2005.
3. Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. J Clin Invest 114:1379–1388, 2004.
4. Skold M, Behar SM. Role of CD1d-restricted NKT cells in microbial immunity. Infect Immun 71:5447–5455, 2003.
5. Kinjo Y, Wu D, Kim G, Xing GW, Poles MA, Ho DD, Tsuji M, Kawahara K, Wong CH, Kronenberg M. Recognition of bacterial glycosphingolipids by natural killer T cells. Nature 434:520–525, 2005.
6. Koch M, Stronge VS, Shepherd D, Gadola SD, Mathew B, Ritter G, Fersht AR, Besra GS, Schmidt RR, Jones EY, Cerundolo V. The crystal structure of human CD1d with and without alpha-galactosylceramide. Nat Immunol 6:819–826, 2005.
7. Zajonc DM, Cantu C 3rd, Mattner J, Zhou D, Savage PB, Bendelac A, Wilson IA, Teyton L. Structure and function of a potent agonist for the semi-invariant natural killer T cell receptor. Nat Immunol 6:810–818, 2005.
8. Morita M, Motoki K, Akimoto K, Natori T, Sakai T, Sawa E, Yamaji K, Koezuka Y, Kobayashi E, Fukushima H. Structure-activity relationship of alpha-galactosylceramides against B16-bearing mice. J Med Chem 38:2176–2187, 1995.
9. Ross AC. Vitamin A, retinoids and immune responses. In: Livrea MA, Ed. Vitamin A and Retinoids: An update of biological aspects and clinical application. Basel, Switzerland: Birkauser Verlag, pp83–96, 2000.
10. Cui D, Moldoveanu Z, Stephensen CB. High-level dietary vitamin A enhances T-helper type 2 cytokine production and secretory immunoglobulin A response to influenza A virus infection in BALB/c mice. J Nutr 130:1132–1139, 2000.
11. DeCicco KL, Youngdahl JD, Ross AC. All-trans-retinoic acid and polyriboinosinic: polyribocytidylic acid in combination potentiate specific antibody production and cell-mediated immunity. Immunology 104:341–348, 2001.
12. Glasziou PP, Mackerras DE. Vitamin A supplementation in infectious diseases: a meta-analysis. BMJ 306:366–370, 1993.
13. Ma Y, Chen Q, Ross AC. Retinoic acid and polyriboinosinic: polyribocytidylic acid stimulate robust anti-tetanus antibody production while differentially regulating type 1/type 2 cytokines and lymphocyte populations. J Immunol 174:7961–7969, 2005.
14. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. Immunity 21: 527–538, 2004.
15. Seguin-Devaux C, Hanriot D, Dailloux M, Latger-Cannard V, Zannad F, Mertes PM, Longrois D, Devaux Y. Retinoic acid amplifies the host immune response to LPS through increased T lymphocytes number and LPS binding protein expression. Mol Cell Endocrinol 245:67–76, 2005.
16. Zhao Z, Ross AC. Retinoic acid repletion restores the number of leukocytes and their subsets and stimulates natural cytotoxicity in vitamin A-deficient rats. J Nutr 125:2064–2073, 1995.
17. Chen Q, Ross AC. Vitamin A and immune function: retinoic acid modulates population dynamics in antigen receptor and CD38-stimulated splenic B cells. Proc Natl Acad Sci U S A 102:14142–14149, 2005.
18. Estey EH, Giles FJ, Kantarjian H, O'Brien S, Cortes J, Freireich EJ, Lopez-Berestein G, Keating M. Molecular remissions induced by liposomal-encapsulated all-trans retinoic acid in newly diagnosed acute promyelocytic leukemia. Blood 94:2230–2235, 1999.
19. Gaboli M, Gandini D, Delva L, Wang ZG, Pandolfi PP. Acute promyelocytic leukemia as a model for cross-talk between interferon and retinoic acid pathways: from molecular biology to clinical applications. Leuk Lymphoma 30:11–22, 1998.
20. Chen Q, Ross AC. Retinoic acid regulates cell cycle progression and cell differentiation in human monocytic THP-1 cells. Exp Cell Res 297: 68–81, 2004.
21. Altucci L, Gronemeyer H. Retinoids and TRAIL: two cooperating actors to fight against cancer. Vitam Horm 67:319–345, 2004.
22. Mark M, Ghyselinck NB, Chambon P. Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis. Annu Rev Pharmacol Toxicol 46:451–480, 2006.
23. Evans TR, Kaye SB. Retinoids: present role and future potential. Br J Cancer 80:1–8, 1999.
24. Nagy L, Thomazy VA, Heyman RA, Davies PJ. Retinoid-induced apoptosis in normal and neoplastic tissues. Cell Death Differ 5:11–19, 1998.
25. Chen Q, Ma Y, Ross AC. Opposing cytokine-specific effects of all trans-retinoic acid on the activation and expression of signal transducer and activator of transcription (STAT)-1 in THP-1 cells. Immunology 107:199–208, 2002.
26. James SY, Williams MA, Newland AC, Colston KW. Leukemia cell differentiation: cellular and molecular interactions of retinoids and vitamin D. Gen Pharmacol 32:143–154, 1999.
27. Pramanik R, Asplin JR, Lindeman C, Favus MJ, Bai S, Coe FL. Lipopolysaccharide negatively modulates vitamin D action by down-regulating expression of vitamin D-induced VDR in human monocytic THP-1 cells. Cell Immunol 232:137–143, 2004.
28. Geng Y, Laslo P, Barton K, Wang CR. Transcriptional regulation of CD1D1 by Ets family transcription factors. J Immunol 175:1022–1029, 2005.
29. Chen QY, Jackson N. Human CD1D gene has TATA boxless dual promoters: an SP1-binding element determines the function of the proximal promoter. J Immunol 172:5512–5521, 2004.
30. Eckhoff C, Nau H. Identification and quantitation of all-trans- and 13-cis-retinoic acid and 13-cis-4-oxoretinoic acid in human plasma. J Lipid Res 31:1445–1454, 1990.
31. Forestier C, Park SH, Wei D, Benlagha K, Teyton L, Bendelac A. T cell development in mice expressing CD1d directed by a classical MHC class II promoter. J Immunol 171:4096–4104, 2003.
32. Xu H, Chun T, Colmone A, Nguyen H, Wang CR. Expression of CD1d under the control of a MHC class Ia promoter skews the development of NKT cells, but not CD8+ T cells. J Immunol 171:4105–4112, 2003.
33. Zimmer MI, Colmone A, Felio K, Xu H, Ma A, Wang CR. A cell-type specific CD1d expression program modulates invariant NKT cell development and function. J Immunol 176:1421–1430, 2006.
34. Szatmari I, Pap A, Ruhl R, Ma JX, Illarionov PA, Besra GS, Rajnavolgyi E, Dezso B, Nagy L. PPAR-gamma controls CD1d expression by turning on retinoic acid synthesis in developing human dendritic cells. J Exp Med 203:2351–2362, 2006.
35. Iwata M, Eshima Y, Kagechika H. Retinoic acids exert direct effects on T cells to suppress Th1 development and enhance Th2 development via retinoic acid receptors. Int Immunol 15:1017–1025, 2003.