Physiological Concentrations of Retinoic Acid Favor Myeloid Dendritic Cell Development over Granulocyte Development in Cultures of Bone Marrow Cells from Mice \(^1\),\(^2\)

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ABSTRACT

Differentiation of hematopoietic progenitors to dendritic cells (DCs) is a complex, poorly understood process regulated by cytokines, colony-stimulating factors, growth factor receptors, and transcription factors. However, nutritional factors may play an important role. Vitamin A is essential for proper immune function and is implicated in the development of myeloid lineage cells, especially granulocytes. We investigated the role of vitamin A in the differentiation of myeloid DCs. Cultures of bone marrow cells from mice stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF) in medium with reduced serum retinol demonstrated significantly decreased DC development compared with control cultures containing retinol. Surprisingly, granulocytes predominated in cultures stimulated with GM-CSF when retinol was depleted. The addition of all-trans or 9-cis retinoic acid to cultures depleted of retinol significantly restored DCs and inhibited granulocyte development. The DC-promoting effect of vitamin A was specific to myeloid lineage development stimulated by GM-CSF because vitamin A significantly inhibited DC development stimulated by flt-3 ligand. Vitamin A also affected DC major histocompatibility complex (MHC) class II and costimulatory molecule expression. In response to increasing concentrations of vitamin A, the expression of MHC class II decreased on the DC, whereas the expression of costimulatory molecules increased, especially CD86. Our data suggest that vitamin A favors the differentiation of myeloid progenitors to immature myeloid DC instead of granulocytes when dietary vitamin A is adequate, and that vitamin A deficiency may compromise adaptive immune responses that depend on myeloid DC antigen presentation.


KEY WORDS: • all-trans retinoic acid • dendritic cells • granulocytes • myelopoiesis

Vitamin A is essential for maintaining normal immune function \((1,2)\). Without this micronutrient, both the innate and adaptive immune systems are compromised, and the balance of the T helper 1 (Th1) \(^4\) and Th2 responses are biased toward Th1 (1). Research studies using animal models demonstrated that T lymphocyte– dependent antibody responses are markedly impaired in vitamin A deficiency \((3–5)\), due to inadequate stimulation of antibody-secreting B lymphocytes by Th cells \((6)\). Vitamin A deficiency was shown to decrease

naïve T-cell stimulation for Th2 development, and the antigen-presenting cells were the target of retinoic acid in the restoration of Th2 responses \((7)\). Vitamin A– deficient mice did not show a decrease in absolute numbers of B and T lymphocytes in spleen or lymph nodes \((8)\), but vitamin A– deficient rats did have significant decreases in the absolute numbers of B and T lymphocytes in peripheral blood and spleen \((9)\).

Retinoic acid and retinoic acid receptors (RARs) play a role in the regulation of myelopoiesis \((10)\), but disparate results exist concerning which mature myeloid lineage cell requires vitamin A for development. Several studies indicated that RARs (and in particular RAR\(\alpha\)) were important regulators necessary for granulocyte development. Alternatively, feeding studies with retinyl acetate and 13-cis retinoic acid at pharmacologic doses resulted in consistent enlargement of immune tissues due to an increase in macrophages and dendritic cells \((11,12)\). In contrast, it was shown that vitamin A– deficient rodents exhibited an expansion of granulocytes in the bone marrow, spleen, and peripheral blood \((9,13)\). Finally, it was also hypothesized that all-trans retinoic acid \((\text{atRA})\) might regulate the proliferation and differentiation of early myeloid precursors \((10)\).

Dendritic cells (DCs) are antigen-presenting cells that link the innate and adaptive immune systems by means of antigen

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\(^{2}\) Supported by Michigan State University Intramural Research Grant Program (K.A.H.) and Food, Nutrition, and Chronic Disease Award Fellowship from the Michigan State University Graduate School (established by an endowment from Pharmacia; L.M.H.).

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\(^{4}\) Abbreviations used: 9cRA, 9-cis retinoic acid; atRA, all-trans retinoic acid; CD, charcoal/dextran; CD-FBS, charcoal/dextran-treated FBS; CHR, characterized [FBS]; DC, dendritic cell; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; FL, flt-3 ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IMDM, Iscove’s modified Dulbecco’s medium; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; MHC-II, MHC class II; RAR, retinoic acid receptor; RXR, retinoid X receptor; Th, T helper; TNF, tumor necrosis factor.
presentation to naïve T cells [reviewed in (14)]. Immature DCs patrol peripheral tissues for pathogens and tissue damage. Contact with microbial products or inflammatory mediators initiates a maturation process involving migration to regional lymph nodes, antigen processing, upregulation of major histocompatibility complex (MHC) class II surface expression, induction of costimulatory molecule expression, and initiation of cytokine production. Antigen presentation relies on MHC presentation of peptide antigens in combination with costimulation via CD40, CD80, and CD86.

DCs are a heterogeneous mix of antigen-presenting cells that may derive from separate hematopoietic lineages (15). Certain surface markers delineate unique functional phenotypic subsets of DCs that have a propensity to cause different adaptive immune responses (16). A subset of DCs expressing CD8α controls the balance of CD4 + Th1 and Th2 cell responses through the preferential production of interleukin (IL-12), thereby promoting a Th1 response. In contrast, CD8α negative DCs appear to play a role in promoting Th2 responses, although the direct mechanism is currently unclear (17).

At present, there are 2 main models for DC development. One model suggests that there are discrete developmental pathways resulting in separate lineages, whereas the second model instead delineates unique developmental stages of DC on the basis of differential cell surface marker expression, especially CD8α (16). It was shown that the common lymphoid progenitor can give rise to T lymphocytes, B lymphocytes, natural killer cells, and CD8α + DCs (18). The common myeloid progenitor has the potential to generate both CD8α + and CD8α − DC subsets, challenging the dual lineage model (19). However, 2 separate laboratories identified at least 2 DC precursor stages that can be found in murine peripheral blood (20,21). The DC precursors are CD11c low–int and MHC class II negative and can develop into both CD8α + and CD8α − DC, as well as a population that resembles human plasmacytoid DC. Because these DC precursors express CD11c, a common myeloid lineage marker, and vitamin A regulates myeloid development, we hypothesized that vitamin A is essential for myeloid DC hematopoiesis. To test this hypothesis, we developed a culture system with reduced serum retinol. We controlled the amount and form of vitamin A in the cultures by adding different concentrations of atRA or 9-cis retinoic acid (9cRA) and then assessed the development of mouse bone marrow cells to DC either in response to granulocyte-macrophage colony-stimulating factor (GM-CSF) or flt-3 ligand (FL).

### MATERIALS AND METHODS

**Mice.** Male 3- to 4-week-old BALB/c mice were purchased from Jackson Laboratory. Animals were maintained according to institutional guidelines set by University Laboratory Animal Resources under a protocol approved by Michigan State University’s Institutional Care and Use Committee. The mice were fed commercial solid pellets (Harlan Teklad 22/5 Rodent Diet #8640) containing all essential macro- and micronutrients including 15.93 IU/g vitamin A acetate. The mice were killed at the age of 5–12 wk by carbon dioxide asphyxiation.

**Reagents.** Characterized fetal bovine serum (FBS) and charcoal/dextran-treated FBS (CD-FBS) were purchased from HyClone. Three separate lots of FBS used in the experiments contained 0.447–0.531 μmol/L retinol, whereas the single lot of CD-FBS contained 0.223 μmol/L retinol (HPLC analysis performed by Craft Technologies). The medium, complete Iscove’s modified Dulbecco’s medium (cIMDM), consisted of cIMDM (Bio-Whittaker) supplemented with 10% (v/v) serum (FBS or CD-FBS), 2 mmol/L L-glutamine, 100 U/L penicillin, 100 μg/L streptomycin (Bio-Whittaker) and 10 μmol/L β-mercaptoethanol (Sigma). Vitamin A, in the form of atRA or 9cRA (Sigma), was dissolved in HYBRI-MAX dimethyl sulfoxide (DMSO; Sigma) and stored in single-use aliquots at −70°C in the dark under an argon atmosphere. Purified recombinant GM-CSF was purchased from PeproTech. Alternately, GM-CSF containing supernatant was produced from the X-63 cell line transfected with GM-CSF cDNA (22). The GM-CSF concentration in the X-63 supernatant was determined by ELISA (BD Biosciences). Human fms-like tyrosine kinase 3 ligand also known as flt-3 ligand (FL) was purchased from PeproTech. The following mAb were used to label the cells: fluorescein isothiocyanate (FITC)-conjugated anti-CD8α (53–67 clone; Rat IgG2a, κ); FITC-conjugated anti-CD86 (GL1 clone; Rat IgG2a, κ); FITC-conjugated anti-CD40 (3/23 clone; Rat IgG2a, κ); FITC-conjugated anti-MHC class II (I-A^d^) (AMS-32.1 clone; Mouse IgG2a, κ); FITC-conjugated anti-CD80 (16–10A1 clone; Armenian Hamster IgG, κ); FITC-conjugated anti-CD11b (M1/70 clone; Rat IgG2a, κ); FITC- or allophycocyanin-conjugated anti-Gr-1 (RB6–SC5 clone; Rat IgG2a, κ); and R-PE-conjugated anti-CD11c (HL3 clone; Armenian Hamster IgG, κ). Antibodies and the appropriate isotype controls were purchased from BD Biosciences.

**GM-CSF cell cultures.** Bone marrow cells obtained from the femurs and tibias of mice were cultured in vitro with 20 ng/L GM-CSF using an adaptation of the protocol established by Inaba et al. (23). Cells were grown in 10 mL of cIMDM supplemented with either 10% FBS (positive control) or 10% CD-FBS (all other treatment groups). Cultures in medium containing CD-FBS were further divided into treatment groups (5 replicates/treatment) consisting of the DMSO vehicle control, 0.1, 1, or 10 nmol/L atRA, or 0.1, 1, or 10 nmol/L 9cRA. Cultures received one-half volume fresh medium containing GM-CSF ± atRA or 9cRA on d 3, 6, and 8. Cells were harvested on d 10 of culture. Cell count and viability were determined using trypan blue dye (Sigma) and a hemocytometer.

**FL cell cultures.** Bone marrow cells, obtained as described above, were cultured in vitro with 200 ng/L FL using a protocol adapted from Brasel et al. (24). Cells were grown in 5 mL of cIMDM supplemented with either 10% (v/v) FBS or 10% (v/v) CD-FBS. The CD-FBS cultures were divided into the same treatment groups used in the GM-CSF experiments. The cells were harvested on d 9 of culture. Two wells were pooled per replicate to obtain enough cells for characterization (5 replicates/treatment). The cell number per treatment was determined with trypan blue dye and a hemocytometer.

**Flow cytometry staining and analysis.** Cells (0.5–1.0 × 10^6^) were incubated with Fc block (BD Pharrmingen) or purified anti-FcγRIII/I/II from the 2.4G2 hybirdoma (ATCC) (1 μg/tube, 5 min, on ice, in the dark). Primary antibody or isotype control antibody directly conjugated to a fluorochrome was added and the cells were incubated again (1 μg/tube, 30 min, on ice, in the dark). The cells were washed with staining buffer (1% FBS, 0.1% sodium azide in PBS, sterile filtered, 4°C, pH 7.4) then resuspended in fresh staining buffer. Cell viability during flow cytometric analysis was determined by the addition of 4,6-diamidino-2-phenylindole (1 μg/tube; Sigma) to each sample 2–3 min before being run on the FACS Vantage flow cytometer. Alternatively, cells were fixed in 2% paraformaldehyde (Electron Microscopy Sciences) in PBS (pH 7.4) and stored at 4°C until analysis.

**Immunohistochemistry.** Immunohistochemistry staining was performed by the Michigan State University Histology Laboratory in the Department of Physiology; Division of Human Pathology. Thin Prep slides were prepared and stained according to established protocols(25). Primary antibody was rabbit anti-rat PMN antiserum (Inter-Cell Technologies, a gift from Dr. R. A. Roth, Michigan State University).

**Statistics.** Data were analyzed using Instat version 3.05 and figures were prepared using Prism version 3.02 (GraphPad Software). A Tukey-Kramer 1-way ANOVA was used to analyze the raw data with Bartlett’s post-test. The GM-CSF experiment was repeated 6 times and the FL experiment was completed 3 times. Data and statistics shown are for 1 representative experiment with 5 replicates per treatment, with the exception of the percentage of Gr-1 + cells shown in Figure 2, which had 3 replicates per treatment. Differences were considered significant at *P* ≤ 0.05.
RESULTS

Vitamin A requirement for myeloid DC development stimulated with GM-CSF. The cell yields and the percentage viability did not differ among treatments for bone marrow cells cultured with GM-CSF and various concentrations of retinoic acid (data not shown). Cell viability of all treatments was >90% (data not shown). Positive control cultures contained 62% DC, whereas negative control cultures contained only 12% DC (Fig. 1A). Dendritic cell development was significantly increased by the addition of either atRA or 9cRA, with the percentage of DC increasing 1- to 1.8-fold compared with the negative control. There was no consistent dose response at the concentrations of atRA and 9cRA tested.

We examined the effect of vitamin A on MHC class II (MHC-II) expression within the CD11c+ DC population. The positive control cultures contained the greatest percentage of MHC-II+ cells within the CD11c+ population and were significantly higher than all other treatments (Fig. 1B). The addition of higher concentrations of atRA (1 or 10 nmol/L) significantly increased the percentage of MHC-II+ cells compared with the negative control, but only the highest concentration of 9cRA (10 nmol/L) had this effect. Cultures containing 0.1 nmol/L atRA or 1 nmol/L 9cRA had fewer MHC-II+ DC than all other treatments.

The effect of vitamin A on expression of costimulatory molecules within the CD11c+ population was also analyzed. In the positive control cultures, the percentage of CD11c+ cells that expressed CD80 was significantly less than the negative control (Fig. 1C). However, because the percentage of DC in the positive control was 4-fold higher than in the negative control (Fig. 1A), the positive control contained more CD11c+CD80+ DC per culture (data not shown). Cultures containing the highest concentrations of atRA and 9cRA (10 nmol/L) had a significantly higher percentage of CD11c+ cells that coexpressed CD80 compared with all other treatments (Fig. 1C). The percentage of CD11c+CD86+ DC did not differ between the positive and negative controls (Fig. 1D). With the exception of 0.1 nmol/L 9cRA, all other retinoid treatments had significantly fewer CD11c+ cells expressing CD86 compared with the controls. All-trans retinoic acid and 9cRA had opposite effects on the percentage of CD86+ DC. Increasing the concentration of atRA increased the percentage of DC expressing CD86, whereas increasing the concentration of 9cRA decreased the percentage of cells expressing CD86.

Maturation and lineage markers were detected on only a minor percentage of the cells. The DC activation marker CD40 was present on <5% of the total population (data not shown). The DC lineage marker CD8 was present on <2% of the total population (data not shown). The groups did not differ in percentages with the marker for either CD40 or CD8 (data not shown).

Mean fluorescence intensity (MFI) is a relative measure of the presence of a molecule on the cell surface. The MFI for CD11c, MHC-II, CD80, and CD86 within the CD11c+ population was assessed (Table 1). Although the positive and the negative controls did not differ, the MFI-II- DC was affected by atRA or 9cRA treatment. As the concentration of atRA or 9cRA increased, the MHC-II expression on the DCs decreased significantly. All-trans retinoic acid had a more dramatic effect on MHC-II MFI than 9cRA at the 2 lower concentrations tested. In contrast, the costimulatory molecules CD80 and CD86 increased on the DC surface as the concentration of atRA, but not that of 9cRA increased. Only 10 nmol/L 9cRA increased the MFI of CD80 on the DC surface significantly.
relative to the negative control, and 9cRA had no direct effect on the level of expression of CD86 on DCs.

**Granulocytes develop in GM-CSF stimulated cultures in the absence of vitamin A.** Flow cytometric analysis of Gr-1 and immunohistochemical staining with anti-rat neutrophil antiserum were used to investigate the alternative cell population that developed in response to GM-CSF in the absence of vitamin A. Nearly 65% of the cells obtained in negative control cultures were Gr-1\(^+\) granulocytes, whereas 10% of the cells in the positive control cultures containing retinol were Gr-1\(^+\) (Fig. 2). The addition of atRA and 9cRA significantly decreased the percentage of granulocytes compared with the negative control culture, but atRA and 9cRA had opposite concentration-dependent effects, and as the concentration of 9cRA increased, the percentage of Gr-1\(^+\) cells decreased. As the concentration of atRA increased, the percentage of Gr-1\(^+\) cells increased, but remained <30% of the negative control. Immunohistochemical staining confirmed granulocyte development in the absence of vitamin A. The results indicated that 12% of cells in the positive control cultures were granulocytes, whereas 54% of the negative control cultures were granulocytes.

**Table 1**

<table>
<thead>
<tr>
<th>Culture treatments</th>
<th>CD11c</th>
<th>MHC class II</th>
<th>CD80</th>
<th>CD86</th>
</tr>
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<tbody>
<tr>
<td>Positive control</td>
<td>103 ± 8d</td>
<td>289 ± 28a</td>
<td>54 ± 2ab</td>
<td>44 ± 0.5e</td>
</tr>
<tr>
<td>Negative control</td>
<td>139 ± 11bc</td>
<td>286 ± 20a</td>
<td>38 ± 1d</td>
<td>87 ± 9a</td>
</tr>
<tr>
<td>+0.1 nmol/L atRA</td>
<td>170 ± 13a</td>
<td>203 ± 28b</td>
<td>33 ± 0.8e</td>
<td>63 ± 5d</td>
</tr>
<tr>
<td>+1 nmol/L atRA</td>
<td>149 ± 9b</td>
<td>112 ± 3c</td>
<td>50 ± 1bc</td>
<td>73 ± 5b</td>
</tr>
<tr>
<td>+10 nmol/L atRA</td>
<td>122 ± 8c</td>
<td>91 ± 15c</td>
<td>56 ± 4a</td>
<td>86 ± 3a</td>
</tr>
<tr>
<td>+0.1 nmol/L 9cRA</td>
<td>168 ± 8a</td>
<td>276 ± 43a</td>
<td>36 ± 0.9de</td>
<td>73 ± 4bc</td>
</tr>
<tr>
<td>+1 nmol/L 9cRA</td>
<td>173 ± 11a</td>
<td>250 ± 25ab</td>
<td>37 ± 0.6de</td>
<td>61 ± 3d</td>
</tr>
<tr>
<td>+10 nmol/L 9cRA</td>
<td>130 ± 5bc</td>
<td>88 ± 3c</td>
<td>49 ± 4c</td>
<td>68 ± 6bcd</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEM of 1 representative experiment of 6 (5 replicates per treatment). Means without a common letter differ, \(P < 0.05.\)

\(^2\) Arbitrary units.

**Vitamin A blocks DC development stimulated by FL.** The positive control and cultures treated with 1 and 10 nmol/L atRA and 1 and 10 nmol/L 9cRA had significantly lower mean cell yields than the negative control (Fig. 3A) for bone marrow cell cultures stimulated with FL. The effects of atRA and 9cRA were dose dependent. Although the total cell yield was lower in the positive control cultures than in the negative control, the raw percentage of CD11c\(^+\) DC in the
positive control cultures was 60% higher than in the negative control (Fig. 3B). The highest concentrations of atRA or 9cRA (10 nmol/L) significantly decreased the percentage of CD11c+ DC obtained in response to FL stimulation compared with all other treatments.

Within the CD11c+ population, the percentage of cells expressing MHC-II and CD11b (a myeloid lineage marker) were assessed. Because the cell yields for several treatments were significantly different from the negative control, the absolute numbers of DCs expressing these molecules were calculated (Fig. 4A and B). Addition of the 2 higher concentrations of vitamin A (1 or 10 nmol/L atRA, or 1 or 10 nmol/L 9cRA) dramatically reduced the absolute number of DCs expressing MHC-II, in a dose-dependent fashion, compared with both the positive and negative controls (Fig. 4A). This same trend was observed in the absolute number of DCs expressing CD11b, except that only cultures containing 10 nmol/L atRA or 10 nmol/L 9cRA were significantly lower than the negative control (Fig. 4B). The lowest concentrations of atRA and 9cRA (0.1 nmol/L) resulted in cultures with absolute numbers of CD11c+CD11b+ DC that were intermediate to the positive and negative controls.

The costimulatory molecules CD80 and CD86 were examined in the CD11c+ population. For both CD80 and CD86, the positive control cultures had a significantly greater absolute number of cells expressing the costimulatory molecules than the negative control (Fig. 4C and D). Neither atRA nor 9cRA treatment significantly increased the absolute number of DC expressing CD80 or CD86 compared with the negative control. Retinoic acid had minimal effects on MFI of CD11c, MHC-II, CD11b, CD80, and CD86 with no dose-dependent responses observed (data not shown).

**DISCUSSION**

Our data demonstrated that vitamin A is necessary for myeloid DC development from bone marrow hematopoietic progenitors. In the absence of serum retinol, the development of myeloid DC resulting from GM-CSF stimulation of primary bone marrow cells decreased 81%. However, the total cell yield in these cultures was not significantly different, indicating that some other cell fate occurred in the absence of vitamin A. Further investigation revealed that granulocytes comprised a substantial proportion of the final cell population when serum retinol was depleted. Both atRA and 9cRA significantly increased DC development and significantly inhibited granulocyte development. These data indicated that vitamin A is essential for DC commitment and crucial to the development of immature myeloid DCs.

The bulk of the literature concerning the role of retinoic acid in myeloid lineage development was generated from experiments that used human acute promyelocytic leukemia cell lines (10). The data published from these experiments suggested that retinoic acid plays a role in the development and maturation of granulocytes. Our data directly contradict these published reports because we showed that physiologic concentrations of retinoic acid promoted DC development over granulocyte development. The dramatic difference may be due to the cell populations studied and the difference in concentration of retinoic acid used in the experiments. The majority of published results used cell lines with defective RARα signaling due to various gene translocations, and required pharmacologic doses of retinoic acid (1 μmol/L) to drive granulocyte development. This model system may substantially alter the signaling mechanisms during development, which has led to the commonly held belief that vitamin A promotes granulo-
cyte development. However, our culture system used primary cells taken from normal animals cultured with physiologic concentrations of vitamin A, and therefore is not expected to yield results identical to those models that rely on cell lines.

Our findings that retinoic acid promotes dendritic cell development over granulocyte development are supported by recent publications by others studying immature myeloid cells that accumulate in chronic disease and during vitamin A deficiency. Several investigators demonstrated that immature myeloid cells that are CD11b+ Gr-1+ CD31+ accumulate in the periphery in tumor-bearing mice (26,27), mice with vitamin A deficiency (13), and mice treated with a pan-RAR antagonist (28). These immature mouse myeloid cells are likely comparable to the immature myeloid cells that accumulate in humans with cancer, who concurrently have a decrease in DC number and function (29). These immature myeloid cells (alternatively termed ImC or iMac) inhibit CD8+ T cell stimulation via MHC Class I (26,27); importantly, they can be induced to mature cell stimulation of DC by treatment with retinoic acid (27,29). More recent studies using adoptive transfer demonstrated that these immature myeloid cells can give rise to granulocytes, macrophages, and mature dendritic cells in vivo (30). In addition, Mohty et al. (31) recently showed that human monocytes cultured with GM-CSF and 1 pmol/L atRA develop into CD11a+ DC, similar to the positive control cultures containing GM-CSF + IL-4. Our data confirm and extend these published results by showing that physiologic concentrations of retinoic acid can modulate the ratio of myeloid DC and granulocytes that are derived from myeloid precursors after GM-CSF stimulation in favor of myeloid DC.

Although retinoic acid enhances myeloid dendritic cell differentiation, our data are the first to show that retinoic acid dramatically inhibits dendritic cell differentiation stimulated by FL. The generation of DCS by FL normally results in a mixture of lymphoid and myeloid DCS at a ratio of 2:1 (24). Retinoic acid significantly decreased the total cell yield, with the highest doses of vitamin A decreasing cell yield by 64% compared with the negative control. However, immature DC developed with FL do not express detectable CD8α until stimulated to mature (data not shown), we could not assess whether retinoic acid inhibited lymphoid or myeloid DC development equally. In addition, we did not investigate whether retinoic acid decreased cell yield by blocking progenitor cell proliferation in response to FL or whether it may have induced apoptosis.

The effect of RA on the expression of MHC-II and co-stimulatory molecules on DCS suggests that RA may favor development of a Th2 response. A previous study by Cantorna et al. (32) showed that RA downregulated the ability of antigen-presenting cells to stimulate IFN-γ secretion, although the target cells were not identified. We found that immature myeloid DCs exposed to increasing concentrations of vitamin A had a decreased level of expression of MHC-II on the cell. At the same time, these dendritic cells upregulated co-stimulatory molecules on their surface—especially CD86. This antigen-presenting cell phenotype is reminiscent of CD8α+ DC pulsed with the soluble egg antigen from *Schistosoma mansoni*, which induce a Th2 response (33).

Although we have not yet investigated the molecular mechanism of action of retinoic acid in promotion of myeloid DC development, it is likely that nuclear receptor modification of gene expression must be involved. Both atRA and 9cRA are able to act as ligands for RAR, whereas only 9cRA can act as a ligand for retinoid X receptors (RXR). Because atRA and 9cRA had similar, but not identical, effects on myeloid DC development, this suggests that RARs must be the primary target of vitamin A action in our culture system. Studies of acute promyelocytic leukemia clearly demonstrate that RARα is necessary for terminal myeloid differentiation, and the presence of a mutated RARα caused by gene translocation blocks cells in the immature promyelocyte stage. However, differences in the effects of atRA and 9cRA in our system may be due to RXR signaling effects of 9cRA because 9cRA can act as a ligand for RXR in addition to RAR, whereas atRA is a ligand only for RAR. RXR can bind certain DNA-responsive elements as a homodimer and can also form functional heterodimers with other nuclear hormone family members (such as the vitamin D receptor), which could modulate signaling through RAR/RXR heterodimers by competition for limited coactivator proteins.

Because GM-CSF upregulates RARα expression and signaling activity (34,35), our studies suggest that holo-RARα promotes final differentiation to myeloid DC in the presence of GM-CSF, whereas apo-RARα favors granulocyte development in the presence of GM-CSF. We developed a model hypothesizing that in the steady state in response to GM-CSF, retinoic acid would promote development of immature myeloid DCs that seed peripheral tissues and be permissive for maintenance of granulocytes as well (Fig. 5A). However, in prolonged vitamin A deficiency, the immature myeloid DC would not be produced, and these cells would be expected to be significantly reduced in peripheral tissues. Their absence would result in diminished antibody responses that require
Th2 cell help. This model is consistent with the general observation that vitamin A deficiency compromises T-lymphocyte dependent antibody responses.

ACKNOWLEDGMENTS

We thank the Michigan State University flow cytometry facility, especially P. Fraker and L. King for their technical advice and expertise. We thank T. Zal (The Scripps Research Institute, La Jolla, CA) and B. Stockinger (National Institute for Medical Research, London, UK) for supplying the X63-GM-CSF cell line. Finally, we thank R. A. Roth and the Histology Laboratory at Michigan State University for immunohistochemical analysis of cell culture cytokins for granulocytes.

LITERATURE CITED