# Retinoic Acid Enhances the T Helper 2 Cell Development That Is Essential for Robust Antibody Responses through Its Action on Antigen-Presenting Cells[1]

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**ABSTRACT Previously we reported that vitamin A–deficient (−A) mice had a profound reduction in T helper 2 (Th2) cells, accounting for their depressed T-dependent antibody responses. Providing vitamin A or its active metabolites reversed this defect. The current experiments utilized splenocytes from T cell receptor transgenic mice to investigate how all-*trans* retinoic acid (atRA) augments Th2 development. These cells were stimulated in vitro in the presence or absence of atRA, with or without exogenous cytokines driving Th1 or Th2 development. Without exogenous cytokines, atRA addition significantly inhibited the interferon (IFN)-$\gamma$ response but did not alter the interleukin (IL)-4 response. With Th1 polarizing cytokines, atRA enhanced the IFN-$\gamma$ response, with no effect on the IL-4 response. Most importantly, with the Th2 polarizing cytokine IL-4, atRA significantly increased the IL-4 secretion (fivefold) and also increased the Th2 cell frequency twofold. The striking Th2 enhancement was also observed when only antigen-presenting cells were treated with atRA before stimulation of untreated CD4$^+$ transgenic T cells, but not vice versa. Thus, atRA maximized Th2 cell development in an IL-4-dependent manner, through an effect on antigen-presenting cell function. J. Nutr. 132: 3736–3739, 2002.**

KEY WORDS: • T helper cell • vitamin A • all-*trans* retinoic acid • antigen-presenting cell.

Vitamin A deficiency markedly increases the risk of morbidity and mortality from infection, and, conversely, vitamin A supplementation substantially decreases this risk (1). The morbidity and mortality from infection is high in vitamin A–deficient (−A)[4] animals due to immunological defects that diminish antibody responses to T lymphocyte–dependent antigens [reviewed in (2)]. The poor antibody responses were not due to B lymphocyte defects in −A animals. Rather, they were due to dramatic decreases in the T helper 2 (Th2) cell frequency, which deprived B lymphocytes of essential signals to support antibody production (3). The most active vitamin A metabolite, all-*trans* retinoic acid (atRA), restored the Th2 cell frequency and the antibody responses (4,5) by a mechanism that was independent of atRA-mediated inhibition of interferon (IFN)-$\gamma$ synthesis (6). The present experiments were designed to investigate how atRA promotes Th2 development.

It is difficult to study Th development in normal mice because the frequency of T cells specific for any particular antigen is extremely low. Consequently, many Th cell differentiation studies have employed mice expressing transgenic T cell receptor (TCR) V$\alpha$ and V$\beta$ chains (7–9). These mice have a very high frequency of CD4$^+$ T cells specific for a particular antigen, and when stimulated, the transgenic T cells can give rise to undifferentiated T helper 0 (Th0) cells, or differentiated IFN-$\gamma$–secreting T helper 1 (Th1) or interleukin (IL)-4–secreting Th2 cells (10–12). Therefore, we used TCR-transgenic (TCR-Tg) CD4$^+$ T cells specific for myelin basic protein residues #1–11 [MBP#1–11 (13)] to study how atRA supports Th2 cell development. The experiments were similar to studies done using these TCR-Tg CD4$^+$ T cells to investigate how 1,25-dihyroxyvitamin $D_3$ influences Th development (14). We hypothesized that atRA might regulate the ability of antigen-presenting cells (APC) to stimulate Th2 development, the differentiation of uncommitted T cells to a Th2 fate and/or the clonal expansion of Th2-fated T cells.

## MATERIALS AND METHODS

***Mice.*** The B10.PL mice expressing TCR V$\alpha_{2.3}$ and V$\beta_{8.2}$ have been described (13). The B10.PL(73NS)/Sn mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were bred at the University of Wisconsin-Madison Department of Biochemistry animal facility under pathogen-free conditions. Protocols were approved by the Institutional Animal Care and Use Committee. Mice were maintained at 23°C with 40–60% humidity and 12-h light:dark cycles. Mice consumed a nonpurified diet with 15 IU/g vitamin A and 4.0 $\mu$g/g carotene (Formulab Diet 5008, PMI International, Brentwood, MO) ad libitum. They were killed by $CO_2$ asphyxiation.

***Antibodies and peptide.*** Dr. J. Bluestone (University of Chicago) provided the cells producing hamster monoclonal antibodies (mAb) to mouse CD3$\epsilon$ [145–2C11; (15)]; the mAb were purified from culture supernatants by protein G-Sepharose chromatography. Biotinylated rat mAb to mouse IL-4 (BVD6–24G2), fluorescein isothiocyanate (FITC) anti-mouse V$\alpha_2$ TCR (B20.1), CyChrome anti-

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[4] Abbreviations used: −A, vitamin A–deficient; APC, antigen-presenting cell; atRA, all-*trans* retinoic acid; 9cRA, 9-*cis* retinoic acid; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; mAb, monoclonal antibody; MBP#1–11, murine myelin basic protein amino acyl residues #1–11; PE, phycoerythrin; RAR, retinoic acid receptor; RXR, retinoid X receptor; TCR, T cell receptor; TCR-Tg, TCR-transgenic; Th, T helper.

mouse CD44 (IM7), phycoerythrin (PE) anti-mouse CD45RB (C363.16A), and FITC anti-mouse CD4 (GK1.5) were from BD PharMingen (San Diego, CA). R-PE labeled rat mAb to mouse CD62L (Mel-14) was from Caltag Laboratories (Burlingame, CA). MBP#1–11 peptide (acetyl-ASQKRPSQRSK) was synthesized by BioSynthesis (Lewisville, TX).

***Cell culture.*** Serum-free HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with 2-mercaptoethanol (50 μmol/L), L-glutamine (2 μmol/L), penicillin (10 kU/L) and streptomycin (10 mg/L) was used for all cell cultures. Primary 7-d cultures of splenocytes from 4- to 7-wk-old TCR-Tg mice were done exactly as described (14). Some primary cultures included IFN-γ and IL-12 to drive Th1 development, or IL-4 and neutralizing antibodies to IFN-γ to drive Th2 development (14). The atRA (Eastman Kodak, Rochester, NY) was dissolved in dimethyl sulfoxide (DMSO), stored under nitrogen at −20°C and diluted into the medium to yield 10 nmol/L atRA. Control medium contained an equal volume of DMSO without atRA. The DMSO concentration was 563 μmol/L medium. The cultured cells were collected and washed, and secondary cultures were established with B10.PL APC and peptide; the IL-4 and IFN-γ produced were quantified by ELISA as described (14,16). The medium used for the secondary cultures contained atRA in DMSO or DMSO only as above. For experiments requiring atRA-treated APC in the primary cultures, B10.PL splenocytes ($5 \times 10^9$ cells/L medium; 2 mL/well; 6-well plates) were cultured for 2 h (37°C in 7.5% $CO_2$), and the adherent APC (obtained by removing the nonadherent splenocytes) were then cultured for 24 h in medium with or without 10 nmol/L atRA. The atRA-treated APC were then washed, and purified T cells ($5 \times 10^5$) and peptide were added to establish primary cultures. Some cultures included supplements to drive Th1 or Th2 development as above, and they were split, restimulated and analyzed as detailed elsewhere (14). The T cells were purified by passage of TCR-Tg splenocytes through Cellect Plus Mouse CD4 Immunocolumns (Biotex Laboratories, Edmonton, Canada), or by flow-sorting the TCR-Tg splenocytes ($V\alpha_2$, $CD44^{lo}$, $CD45RB^{hi}$) on a FACStar-Plus (Becton Dickinson, San Jose, CA).

***Immunofluorescent staining and flow cytometric analysis.*** Cells from primary cultures were collected, washed, resuspended in 1 mL medium, layered over 5 mL Ficoll-Paque Plus (Pharmacia Biotech, Uppsala, Sweden) and centrifuged (1000 × *g*). The live cells were collected, washed and cultured ($10^9$ cells/L; 10 mL/100 mm plate) for 4 h at 37°C in medium plus monensin (2 μmol/L; Sigma), with and without plate-bound mAb to CD3ε (10 mg/L in PBS; 10 mL/dish). The T cells were washed in PBS with fetal calf serum (10 mL/L) and sodium azide (1 g/L) (staining buffer), fixed 20 min at 4°C in 100 μL of PBS with paraformaldehyde (40 g/L), washed with staining buffer and then incubated at 4°C for 30 min in 50 μL staining buffer containing saponin (1 g/L) and PE-labeled mAb to murine IL-4 (1 μg/$10^6$ cells) or PE-labeled isotype control mAb. The cells were washed with staining buffer containing saponin, and then with staining buffer. Cells were analyzed on an EPICS flow cytometer (Coulter Beckman, Miami, FL) at the Wisconsin State Laboratory of Hygiene.

***Statistical analysis.*** Cultures were done in triplicate, and experiments were repeated 3–6 times. For Figure 1, data from a representative experiment were analyzed by one-way ANOVA with Dunnett's post-test using GraphPad InStat version 3.05 for Windows 95/NT (GraphPad Software, San Diego, CA). For Figure 3, the means from five replicate experiments were combined to perform a two-tailed Student's *t* test assuming unequal variances. The minimum significance level used was $P \leq 0.05$.

## RESULTS

***Effect of atRA on Th development.*** Our goal was to investigate atRA control of Th1 or Th2 cell development from undifferentiated T cells. Spontaneous T cell differentiation can occur in TCR-Tg mice, because the activating antigen, MBP, is present in the spinal cord. To confine our analysis to undifferentiated T cells, each splenocyte sample was examined for CD62L, a marker of undifferentiated T cells, and only samples with high CD62L expression on the transgenic T cells were used. The splenocytes were cultured in serum-free medium that was devoid of vitamin A; thus, the splenocytes were rapidly depleted of vitamin A and its metabolites, unless exogenous atRA was added.

We first examined the effect of atRA on Th differentiation without adding exogenous cytokines. The atRA decreased IFN-γ secretion by 50% ($P < 0.01$) when it was added to the primary or secondary culture (**Fig. 1**A), and by 78% when it was added to both cultures ($P < 0.01$), but it did not enhance the IL-4 response under any conditions (Fig. 1*B*). Thus, atRA inhibition of IFN-γ synthesis is not sufficient to enhance the IL-4 response, and atRA does not increase the proportion of undifferentiated T cells following the Th2 cell fate. Next, we studied the effect of atRA when exogenous IFN-γ and IL-12 were added to promote Th1 development. Under these conditions, atRA enhanced IFN-γ synthesis when it was added to both cultures (Fig. 1C, $P < 0.05$), with no effect on IL-4 secretion (Fig. 1*D*, $P > 0.05$). Finally, we investigated the effect of atRA when IL-4 and mAb to IFN-γ were added to promote Th2 development. Under these conditions, atRA addition to primary cultures enhanced the IL-4 response fivefold (Fig. 1*F*, $P < 0.01$). However, atRA addition to secondary cultures only had no effect ($P > 0.05$), excluding atRA enhancement of IL-4 synthesis by differentiated Th2 cells.

***Effect of atRA on IL-4-secreting cell frequency and IL-4 yield.*** Because atRA increased the IL-4 response when exogenous IL-4 stimulated the Th2 cell fate, but did not promote this fate or stimulate IL-4 synthesis, we considered the possibility that atRA increased the clonal expansion of the Th2-

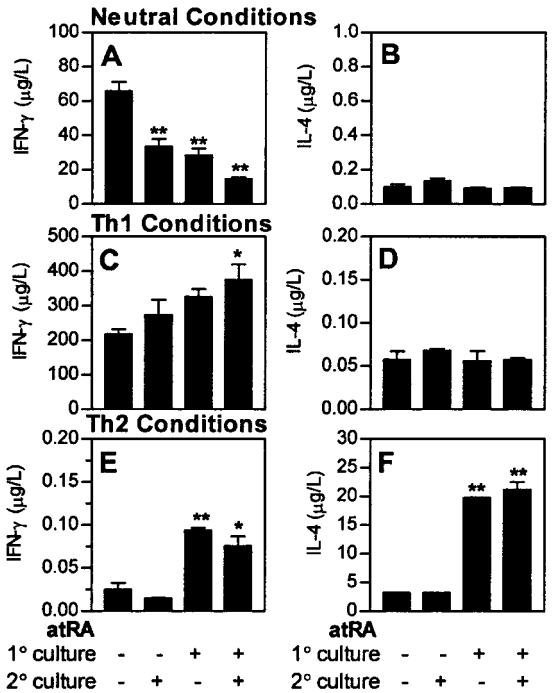


**FIGURE 1** Vitamin A effect on in vitro T helper lymphocyte development of splenocytes from B10.PL T cell receptor transgenic mice. Cells were cultured under neutral conditions (*A* and *B*), under T helper 1 (Th1) conditions (*C* and *D*), or under Th2 conditions (*E* and *F*). Cells were treated with all-*trans* retinoic acid (atRA) in 1° and/or 2° cultures, as indicated. Cultures without atRA contained dimethyl sulfoxide (DMSO) vehicle control. Panels represent interferon (IFN)-γ (*A*, *C*, and *E*) and interleukin (IL)-4 (*B*, *D*, and *F*) production after restimulation. Data shown are the mean ± SD for triplicate cultures/treatment from 1 representative experiment of 5–6 experiments. Asterisks indicate means that differ from DMSO control (−/−), $^*P < 0.05$ or $^{**}P < 0.01$.

fated cells. A flow cytometric analysis showed that atRA increased the IL-4$^+$ Th2 cell frequency twofold, when IL-4 was present to drive T cells toward the Th2 cell fate (**Fig. 2**). Also, the mean fluorescence intensity of the IL-4$^+$ cells increased 100% when the cultures contained atRA, suggesting that the IL-4 yield per cell was greater in these cultures, although a direct effect of atRA on IL-4 gene expression was ruled out.

***The APC as the atRA target cell.*** To determine whether atRA acted on the APC, the T cell or both to enhance the clonal expansion and IL-4 yield of the Th2-fated cells, the APC or the T cells were pretreated with atRA before culturing together under Th2-driving conditions. The flow-sorted T cells used in these experiments were ≥97% pure. When the APC were atRA-treated, the T cell yield (data not shown) and the IL-4 secretion rate per T cell (atRA cultures, 74 ng/$10^6$ cells; controls, 35 ng/$10^6$ cells) each increased >100%, giving a 4.8-fold increase in IL-4 production compared with the controls (**Fig. 3**; $P < 0.001$). In contrast, the cultures with atRA-treated T cells were not different from the controls (data not shown). We conclude that atRA-treated APC are very strong stimulators of Th2 cell development during primary antigen stimulation.

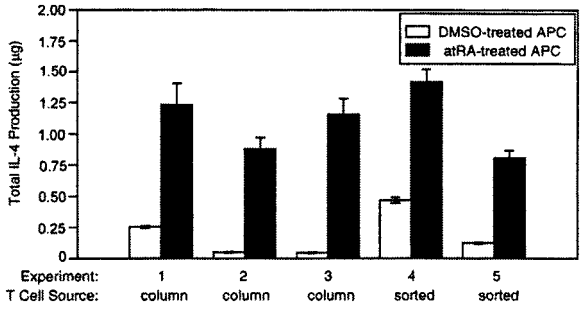


**FIGURE 3** T helper 2 (Th2) development of CD4$^+$ murine B10.PL T cell receptor transgenic T cells stimulated with B10.PL antigen-presenting cells (APC) pretreated with dimethyl sulfoxide (DMSO) vehicle or all-*trans* retinoic acid (atRA). T cells were either CD4 immunocolumn-enriched (column) or sorted by flow cytometry (sorted), as indicated. Total interleukin (IL)-4 production (μg) = [(Total d 7 cell yield)/(# restimulated cells/L)] × [IL-4 secreted (μg/L)]. Data shown are the mean ± SD derived from triplicate restimulation cultures of 5 separate experiments. The atRA-treated APC cultures differed ($P < 0.001$) from those treated with DMSO. (Data from all 5 experiments were combined.)

## DISCUSSION

Our long-term objective is to understand the cellular and molecular mechanisms that explain why adequate vitamin A nutrition is essential for robust antibody responses to infections, and conversely, why high morbidity and mortality from infection is characteristic of vitamin A deficiency (reviewed in 2). We investigated how the active vitamin A metabolite, atRA, supported robust antibody responses by promoting Th2 cell development in vivo and in vitro (4,5). No effect of atRA on fully differentiated Th2 cells was observed, ruling out a direct enhancing effect of atRA on IL-4 gene expression. The atRA inhibited IFN-γ synthesis, as we reported previously (6,17), but this effect was not sufficient to enhance Th2 development. The most important finding was that through an action on the APC, atRA increased the clonal expansion and IL-4 yield of the Th2 cells only if exogenous IL-4 was present, although atRA alone did not promote commitment to the Th2 cell phenotype.

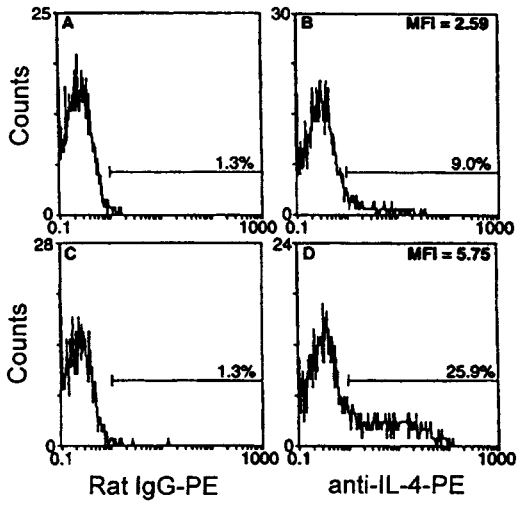


**FIGURE 2** Intracellular interleukin (IL)-4 staining of murine B10.PL T cell receptor transgenic splenocytes cultured under T helper 2 (Th2) conditions with and without all-*trans* retinoic acid (atRA). Panels represent cultures containing dimethyl sulfoxide vehicle (*A* and *B*) or atRA (*C* and *D*). Data shown are from cells labeled intracellularly with isotype control rat immunoglobulin (Ig)G-phycoerythrin [Rat IgG-PE (*A* and *C*)] or anti-mouse IL-4-PE (*B* and *D*). Mean fluorescence intensity (MFI) of the anti-IL-4-PE staining is shown. One experiment of 2 is shown.

The cytokines present during antigen stimulation are the strongest determinants of the T cell differentiation fate, although many other factors influence this process [reviewed in (18,19)]. IL-4 and IL-12 are the most potent cytokines for promoting Th2 and Th1 development, respectively. Accordingly, we interpret our finding that IL-4 was required for the atRA to support Th2 development as indicating that the IL-4 determined the Th2 cell fate choice, and through an action on the APC, atRA supported the growth and productivity of the Th2-fated T cells.

Other investigators recently reported that 9-*cis* retinoic acid (9cRA) but not atRA acted directly on T cells to enhance Th2 development in vitro (20). These investigators did not study a possible effect of 9cRA or atRA on APC. We do not know why they found that atRA was inactive. Our results showing that this vitamin A metabolite is a potent stimulator of Th2 development are consistent with our previous studies showing that atRA was the most active retinoid for restoring immunoglobulin $G_1$ antibody responses and Th2 cells in −A mice (4). It is also not clear why they observed a direct effect of 9cRA on T cells. A possible difference could be the T cell preparation method. Our flow-sorted T cell preparations were >97% pure, and the atRA had no effect on these cells. On the other hand, other T cell enrichment methods can yield lower purity, and APC may still be present. Other possible differences between their system and ours include the TCR-Tg mouse strain and the culture conditions. These differences may also have contributed to the inconsistent results. Further experiments will be required to resolve the discrepancies.

We do not know how atRA improved the Th2-stimulating function of the APC. The APC express several constitutive and inducible costimulatory molecules, specifically CD80 and CD86, CD40 and OX40-L, that facilitate Th2 development (21). For example, OX40-L on APC binding to OX40 on CD4$^+$ T cells inhibited T cell IFN-γ synthesis (22) and also promoted Th2 development in BALB/c mice infected with *Leishmania major* (23). Decreased expression of one or more

costimulatory molecules might occur in vitamin A deficiency, and conversely, atRA might act to increase one or more of these costimulatory molecules. Th2 development is more dependent on costimulation than Th1 development (24); thus, this hypothesis is consistent with our observation that Th1 responses are unimpaired or enhanced in vitamin A deficiency, whereas Th2 responses are dramatically diminished (4,5,17).

The atRA is a transcriptional regulator of gene expression [reviewed in (25)]. The retinoic acid isomers bind to nuclear receptors from two distinct classes, the retinoic acid receptors (RAR) and the retinoid X receptors (RXR). RAR binds 9cRA and atRA, whereas RXR binds only 9cRA. RXR forms functional heterodimers with many members of the steroid receptor family including RAR. We speculate that atRA binding to one of the RAR-RXR receptor complexes enhances transcription of one or more genes encoding costimulatory molecules on the APC. To test this hypothesis, experiments are currently underway to examine possible effects of atRA on APC costimulatory molecule expression.

The results presented here are directly relevant to the problem of vitamin A malnutrition. Vitamin A malnutrition causes a dysfunction of antibody-mediated immunity, which predisposes individuals to infections, and the infections further deplete the vitamin A supplies [reviewed in (2)]. Our results suggest that the APC from malnourished individuals would likely retain the capacity to promote Th1 development in response to stimulation by the antigens of infectious organisms, but would lack the capacity to promote Th2 development. This underlying defect could decrease the individual's capacity to respond to infection with robust antibody production, and furthermore, could undermine the ability of vaccines to establish a memory antibody response. These concerns are particularly significant for Africa, where a high prevalence of vitamin A deficiency coincides with a high prevalence of infectious diseases such as human immunodeficiency virus (HIV) (26). For example, HIV-infected Ugandan infants with low plasma carotenoid concentrations had a significantly increased risk of death from infection compared with those with high carotenoid concentrations (27). Addressing the malnutrition in HIV-infected individuals is likely to improve resistance to opportunistic infections in these individuals, and improve vaccine efficacy when one becomes available.

## LITERATURE CITED


1. Sommer, A. (1993) Vitamin A, infectious disease, and childhood mortality: a 2 cent solution? J. Infect. Dis. 167: 1003–1007.
2. Hayes, C. E., Nashold, F. E., Gomez, F. E. & Hoag, K. A. (1999) Retinoids and immunity. In: Handbook of Experimental Pharmacology: Retinoids (Nau, H. & Blaner, W. S., eds.), pp. 589–610. Springer-Verlag, New York, NY.
3. Carman, J. A., Smith, S. M. & Hayes, C. E. (1989) Characterization of a helper T lymphocyte defect in vitamin A-deficient mice. J. Immunol. 142: 388–393.
4. Chun, T. Y., Carman, J. A. & Hayes, C. E. (1992) Retinoid repletion of vitamin A–deficient mice restores IgG responses. J. Nutr. 122: 1062–1069.
5. Cantorna, M. T., Nashold, F. E. & Hayes, C. E. (1994) In vitamin A deficiency multiple mechanisms establish a regulatory T helper cell imbalance with excess Th1 and insufficient Th2 function. J. Immunol. 152: 1515–1522.
6. Cantorna, M. T., Nashold, F. E. & Hayes, C. E. (1995) Vitamin A deficiency results in a priming environment conducive for Th1 cell development. Eur. J. Immunol. 25: 1673–1679.
7. Hsieh, C.-S., Hiemberger, A. B., Gold, J. S. & O'Garra, A. (1992) Differential regulation of T helper phenotype development by interleukins 4 and 10 in an $\alpha\beta$ T-cell-receptor transgenic system. Proc. Natl. Acad. Sci. U.S.A. 89: 6065–6069.
8. Seder, R. A., Paul, W. E., Davis, M. M. & Fazekas de St. Groth, B. (1992) The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of $CD4^+$ T cells from T cell receptor transgenic mice. J. Exp. Med. 176: 1091–1098.
9. Hsieh, C.-S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A. & Murphy, K. M. (1993) Development of $T_H1$ $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. Science (Washington, DC) 260: 547–549.
10. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136: 2348–2357.
11. Street, N. E., Schumacher, J. H., Fong, T.A.T., Bass, H., Fiorentino, D. F., Leverah, J. A. & Mosmann, T. R. (1990) Heterogeneity of mouse helper T cells. Evidence from bulk cultures and limiting dilution cloning for precursors of Th1 and Th2 cells. J. Immunol. 144: 1629–1639.
12. Seder, R. A. & Paul, W. E. (1994) Acquisition of lymphokine-producing phenotype by $CD4^+$ T cells. Annu. Rev. Immunol. 12: 635–673.
13. Goverman, J., Woods, A., Larson, L., Weiner, L. P., Hood, L. & Zaller, D. M. (1993) Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. Cell 72: 551–560.
14. Nashold, F. E., Hoag, K. A., Goverman, J. & Hayes, C. E. (2001) *Rag*-1-dependent cells are necessary for 1,25-dihydroxyvitamin $D_3$ prevention of experimental autoimmune encephalitis. J. Neuroimmunol. 119:16–29.
15. Leo, O., Foo, M., Sachs, D. H., Samelson, L. E. & Bluestone, J. A. (1987) Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc. Natl. Acad. Sci. U.S.A. 84: 1374–1378.
16. Mosmann, T. R. & Fong, T. A. (1989) Specific assays for cytokine production by T cells. J. Immunol. Methods 116: 151–158.
17. Carman, J. A. & Hayes, C. E. (1991) Abnormal regulation of IFN-$\gamma$ secretion in vitamin A deficiency. J. Immunol. 147: 1247–1252.
18. O'Garra, A. (1998) Cytokines induce the development of functionally heterogeneous T helper cell subsets. Immunity 8: 275–283.
19. Ho, I.-C. & Glimcher, L. H. (2002) Transcription: tantalizing times for T cells. Cell 109: S109–S120.
20. Stephensen, C. B., Rasooly, R., Jiang, X., Ceddia, M. A., Weaver, C. T., Chandraratna, R.A.S. & Bucy, R. P. (2002) Vitamin A enhances in vitro Th2 development via retinoid X receptor pathway. J. Immunol. 168: 4495–4503.
21. Banchereau, J., Briere, F., Caux, C., Davost, J., Lebecque, S., Liu, Y.-J., Pulendran, B. & Palucka, K. (2000) Immunobiology of dendritic cells. Annu. Rev. Immunol. 18: 767–811.
22. Flynn, S., Toellner, K.-M., Raykundalia, C., Goodall, M. & Lane, P. (1998) CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. J. Exp. Med. 188: 297–304.
23. Akiba, H., Miyahira, Y., Atsuta, M., Takeda, K., Nohara, C., Futagawa, T., Matsuda, H., Aoki, T., Yagita, H. & Okumura, K. (2000) Critical contribution of OX40 ligand to T helper cell type 2 differentiation in experimental leishmaniasis. J. Exp. Med. 191: 375–380.
24. Jankovic, D., Liu, Z. & Gause, W. C. (2001) Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. Trends Immunol. 22: 450–457.
25. Piedrafita, F. J. & Phahl, M. (1999) Nuclear retinoid receptors and mechanisms of action. In: Handbook of Experimental Pharmacology: Retinoids (Nau, H. & Blaner, W. S., eds.), pp. 153–184, Springer-Verlag, New York, NY.
26. Semba, R. D. (1997) Vitamin A and human immunodeficiency virus infection. Proc. Nutr. Soc. 56: 459–469.
27. Melikian, G., Mmiro, F., Ndugwa, C., Perry, R., Jackson, J. B., Garrett, E., Tielsch, J. & Semba, R. D. (2001) Relation of vitamin A and carotenoid status to growth failure and mortality among Ugandan infants with human immunodeficiency virus. Nutrition 17: 567–572.


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